# Omeprazole Suppresses Endothelial Calcium Response and eNOS Ser1177 Phosphorylation in Porcine Aortic Endothelial Cells

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Abstract

*Background* Although high doses of proton pump inhibitors can elicit an anticancer effect, this strategy may impair vascular biology. In particular, their effects on endothelial $Ca^{2+}$ signaling and production of endothelium-derived relaxing factor (EDRF) are unknown. To this end, we investigated the effects of high dosages of omeprazole on endothelial $Ca^{2+}$ responses and EDRF production in primary cultured porcine aortic endothelial cells.

*Methods and Results* Omeprazole (10–1000 μM) suppressed both bradykinin (BK)- and thapsigargin-induced endothelial $Ca^{2+}$ response in a dose-dependent manner. Furthermore, omeprazole slightly attenuated $Ca^{2+}$ mobilization from the endoplasmic reticulum, whereas no inhibitory effects on endoplasmic reticulum $Ca^{2+}$-ATPase were observed. Omeprazole decreased BK-induced phosphorylation of endothelial nitric oxide synthase (eNOS) at Ser1177 and tended to decrease BK-induced nitric oxide production. Production of prostaglandin $I_2$ metabolites, especially 6-keto-prostaglandin 1α, also tended to be reduced by omeprazole.

*Conclusion* Our results are the first to indicate that high doses of omeprazole may suppress both store-operated $Ca^{2+}$ channels and partially the G protein-coupled receptor/phospholipase C/inositol 1,4,5-triphosphate pathway, and decreased BK-induced, $Ca^{2+}$-dependent phosphorylation of eNOS(Ser1177). Thus, high dosages of

omeprazole impaired EDRF production by attenuating intracellular $Ca^{2+}$ signaling.

Keywords

omeprazole; nitric oxide synthase; endothelial cells; calcium; endothelium-dependent relaxing factors

Declarations

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Conflict of interest

The authors declare no conflicts of interest.

Availability of data and material

Data available on request.

Code availability

Not applicable.

## Authors' contribution

KO, CK, NI and HW constructed the concept of whole investigation. CK, RS, and AH performed the experiment. CK and KO validated the data and carried on statistical analysis. CK and KO wrote the original manuscript. NI and HW edited and revised the manuscript. All authors had read the manuscript and approved the publication of this manuscript.

## Ethics approval

This study conformed to the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH publication, 8th edition, 2011). All experiments were performed in accordance with the regulations of the Animal Research Committee of Hamamatsu University School of Medicine. Ethics approval was not required for this investigation.

## Consent to participate

Not applicable.

Consent for publication

Not applicable.

## Introduction

Proton pump inhibitors (PPIs) are widely used for treatment of gastroesophageal reflux disease (GERD). Although PPIs have a favorable gastric acid suppressing effect for GERD, several studies have reported a potential association between use of PPIs and increased risks of cardiovascular, chronic kidney, and end-stage renal diseases [1-3]. Despite the possibility of these adverse effects, the pleiotropic effects of PPIs, such as inhibition of cancer cell invasion and metastasis, anti-inflammatory actions, and suppression of fibrosis, have been under intense investigation for the past two decades [4-7]. In particular, the anti-cancer effects PPIs exert through apoptosis induction and anti-inflammatory actions have received a lot of attention [8]; however, the dosages of such PPIs used in basic and clinical research to investigate anti-cancer effects are higher than those used for treatment of GERD in clinical settings [9, 10]. Although a few basic research studies suggested that high dosages of PPIs could accelerate endothelial senescence and dysfunction, and impair vascular biology [11, 12], no previous studies

observed the effect of PPIs on endothelial calcium signaling or production of endothelium-derived relaxing factor (EDRF). Thus, we investigated the effect of high dosages of omeprazole on endothelial $Ca^{2+}$ response and EDRF production in primary-cultured porcine aortic endothelial cells (PAECs).

## Materials and Methods

### *Cell culture*

PAECs were isolated from ten descending aortas of pigs from a slaughterhouse by gently scraping the intima of the descending part of the porcine aorta, and cultured as previously described[13, 14]. Briefly, a PAEC pellet was purified from the generated suspension by centrifugation at 1400 × g for 15 min in Medium 199 (Gibco Life Technologies, Staley Rd, NY, USA) (M-199). After resuspension in M-199 medium containing Earle's salts, 100 IU/mL penicillin G, 100 µg/mL streptomycin (Gibco Life Technologies, Staley Rd, NY), and 20% newborn calf serum (Gibco Life Technologies, Auckland, New Zealand), cells were seeded onto culture dishes or silicone dishes fixed on glass coverslips, and incubated at 37 °C in 5% $CO_2$. Investigational protocols conformed to the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health [15]. All experiments were performed in accordance

with requirements of the Animal Research Committee of Hamamatsu University School of Medicine.

*Measurement of intracellular $Ca^{2+}$ concentration*

Intracellular calcium concentration ($[Ca^{2+}]_i$) was measured in individual PAECs using the acetoxymethyl ester of Fura-2 (Fura-2/AM; Dojindo Molecular Technologies, Kumamoto, Japan), as previously described [14]. PAECs were incubated for 45 min in modified Tyrode's solution composed of (in mM): 150 NaCl, 2.7 KCl, 1.2 $KH_2PO_4$, 1.2 $MgSO_4$, 1.0 $CaCl_2$, and 10 N-2-hydroxyethylpiperazine-N-2-ethanesulfonic acid (pH 7.4 at 25°C) with Fura-2/AM (2.5 μM). Fluorescent images of Fura-2 were acquired and quantified every 30 s from individual cells with a fluorescence analyzer (Aqua-Cosmos, Hamamatsu Photonics K.K., Hamamatsu, Japan) using an ultra-high sensitivity camera. The quantitative evaluation of $[Ca^{2+}]_i$ was not performed, because we did not calibrate Fura-2/AM fluorescence. Changes in the fluorescence ratio (F340/F380) of Fura-2 were used to express changes in $[Ca^{2+}]_i$.

*Measurement of nitric oxide and prostaglandin $I_2$*

Nitric oxide (NO) decomposes rapidly into its stable metabolites, nitrite ($NO_2$) and

nitrate ($NO_3$) [16]; thus, we measured the total amount of $NO_2^-$ and $NO_3^-$. The amount of prostaglandin $I_2$ ($PGI_2$) released from PAECs was measured as the concentration of its stable metabolite, 6-keto-prostaglandin 1 alpha (6-keto-$PGF_{1\alpha}$), as previously described [17]. PAECs were preincubated for 10 min in modified Tyrode's solution with omeprazole (100 μM; Sigma-Aldrich, St. Louis, MO). Next, bradykinin (BK, 10 nM; Sigma-Aldrich) was added and the incubation was continued for 7 min. Concentrations of $NO_2^-$ plus $NO_3^-$ in cell media were measured using a $NO_2/NO_3$ Assay Kit-C II (Fluorometric) according to the manufacturer's protocol (Dojindo Molecular Technologies). Concentrations of 6-keto-$PGF_{1\alpha}$ were determined using an enzyme-linked immunoassay, according to the manufacturer's protocol (Enzo Life Sciences, Farmingdale, NY).

*Western blotting*

PAECs were preincubated for 10 min in modified Tyrode's solution with omeprazole (100 μM). Next, BK (10 nM) was added, and the incubation was continued for 5 min. Then, cells were washed with ice-cold $Ca^{2+}$-containing phosphate-buffered saline, treated with lysis buffer containing protease inhibitors and phosphatase inhibitors (Ez RIPA Lysis Kit™; ATTO, Tokyo, Japan) for 15 min at 4°C, and then scraped and

harvested. Samples containing equal amounts (10 μg) of total cellular protein were loaded and separated by electrophoresis on a 10% sodium dodecyl sulfate-polyacrylamide gel at 20 mA for 80 min. The gel was then transferred onto a polyvinylidene difluoride membrane at 153 mA for 30 min. Blocking was carried out at room temperature for 1 h using a blocking agent (EzBlock Chemi™; ATTO). The membrane was stained with primary antibodies against endothelial nitric oxide synthesis (eNOS; 1:2000 dilution) and β-actin (1:2000 dilution) overnight at 4°C. Phosphorylated or unphosphorylated proteins bound to each primary antibody were probed with a horseradish peroxidase-conjugated goat anti-rabbit IgG (sc-2004; Santa Cruz Biotechnology, Dallas, TX) for 1 h at room temperature (1:10000 dilution). Protein bands were visualized with a ChemiDoc Touch system (Bio-Rad Laboratories, Hercules, CA).

*Statistical analysis*

Data are presented as mean ± standard deviation, and numbers of cells or experiments are shown as n. Statistical analysis was performed by two-way factorial analysis of variance (ANOVA) followed by the Bonferroni test, or one-way ANOVA followed by Tukey's honest significance test or paired t-test. $p < 0.05$ was accepted as statistically

significant. Analysis was performed using SPSS Statistics version 24.0 (IBM Corporation, Armonk, NY).

## Results

*Omeprazole attenuated bradykinin- or thapsigargin-induced cytosolic $Ca^{2+}$ responses of PAECs*

Because BK is a stimulator to increase intracellular $Ca^{2+}$ in endothelial cells, we first examined the effect of BK on cytosolic $Ca^{2+}$ responses in primary cultured PAECs. In the presence of extracellular $Ca^{2+}$ (1 mM), BK (10 nM) rapidly increased the fluorescence ratio of Fura-2 to 5.62 ± 1.71-fold of baseline at 90 s ($p < 0.0001$), which was followed by a sustained increase (2.00 ± 0.88-fold of baseline at 420 s). Pretreatment with omeprazole (10–1000 μM) inhibited BK-induced increases in $[Ca^{2+}]_i$ in a dose-dependent manner at 90 s, as indicated by fluorescence ratios of 5.11 ± 1.55-fold at 10 μM, 3.23 ± 1.81-fold at 100 μM, and 1.02 ± 0.11-fold at 1 mM (p for trend < 0.0001; Figure 1A and B).

Thapsigargin (TG)-induced endoplasmic reticulum (ER) $Ca^{2+}$ depletion is also considered to be a trigger of increase in intracellular $Ca^{2+}$ via influx of $Ca^{2+}$ from extracellular space in research settings. As shown in Figure 1C and 1D, TG (1 μM) also

increased $[Ca^{2+}]_{i,}$ to 6.51 ± 1.74-fold of baseline at 300 s ($p < 0.0001$), and this effect was also attenuated by omeprazole in a dose-dependent manner at 300 s, with observed fluorescence ratios of 6.69 ± 1.08-fold at 10 μM, 4.65 ± 0.97-fold at 100 μM, and 3.31 ± 1.03-fold at 1 mM (p for trend < 0.0001). These results indicate that omeprazole attenuated both BK- and TG-induced $Ca^{2+}$ responses in PAECs.

*Omeprazole partially inhibited bradykinin- or thapsigaigin-induced cytosolic $Ca^{2+}$ increase by reducing $Ca^{2+}$ release from endoplasmic reticulum*

Next, we investigated the effect of omeprazole on BK-induced $Ca^{2+}$ release from the ER. In the absence of extracellular $Ca^{2+}$, BK (10 nM) caused only a small and transient increase in the Fura-2 fluorescence ratio to a maximum of 3.97 ± 1.50-fold at 90 s ($p < 0.0001$), which was attenuated by 100 μM omeprazole [2.68 ± 1.27-fold at 90 s ($p < 0.0001$); Figure 2A and B]. We also investigated the effect of omeprazole on TG-induced $[Ca^{2+}]_i$ increase, which reflects inhibition of $Ca^{2+}$ re-uptake via ER-$Ca^{2+}$ATPase. TG (1 μM) caused only a small and transient increase to a maximum of 1.85 ± 0.27-fold of baseline at 150 s ($p < 0.0001$); however, omeprazole did not show any effects on TG-induced $[Ca^{2+}]_i$ increases in the absence of extracellular $Ca^{2+}$ concentration (Figure 2C and D). Taken together, these findings suggest that

omeprazole might partially affect $Ca^{2+}$ influx from extracellular space and the G protein-coupled receptor/phospholipase C/inositol 1,4,5-triphosphate (GPCR/PLC/$IP_3$) pathway.

*Effects of omeprazole on nitric oxide and prostaglandin $I_2$ production*

We next investigated whether omeprazole attenuated NO and $PGI_2$ production by evaluating its effect on phosphorylation of eNOS at serine residue 1177 (Ser1177), one of the most thoroughly studied activation sites [18, 19]. eNOS(Ser1177) phosphorylation was decreased by 27.5% ± 8.3 % following pretreatment with omeprazole (100 μM, p = 0.010; Figure 3A and B). BK (10 nM) increased NO production approximately 1.32-fold (control, 0.042 ± 0.032 μM/$10^6$ cells; BK, 0.053 ± 0.034 μM/$10^6$ cells; p=0.042). Omeprazole tended to decrease BK (10 nM)-induced NO production, although this was not significant (BK, 0.053 ± 0.035 μM/$10^6$ cells; omeprazole, 0.037 ± 0.025 μM/$10^6$ cells; p = 0.122; Figure 3C).

Finally, we evaluated whether omeprazole attenuated $PGI_2$ production. BK (10nM) increased $PGI_2$ production approximately 1.24-fold (control; 784.46 ± 212.45 pg/mL /$10^6$ cells, BK; 927.81 ± 283.48 pg/mL/$10^6$ cells, p=0.042). As with the effect on NO production, omeprazole (100 μM) tended to decrease 6-keto-$PGF_{1\alpha}$ production, but this

effect was not significant (omeprazole, 763.47 ± 257.6 pg/mL/$10^6$ cells; p = 0.160; Figure 4).

## Discussion

Omeprazole, an inhibitor of hydrogen-potassium adenosine triphosphatase ($H^+/K^+$-ATPase), strongly inhibits gastric acid secretion in gastric parietal cells [20]. The inhibitory effect of omeprazole on $H^+/K^+$-ATPase purified from gastric mucosa is time- and pH-dependent, and exhibits high potency (inhibitory concentrations ≥ 3 μM) [21]. Recent progress in basic research has identified the pleiotropic effects of PPIs. Fako *et al.* identified PPIs as effective inhibitors of thioesterase activity of human fatty acid synthase, which contributes to cancer cell survival, drug resistance, and poor prognosis. Indeed, omeprazole inhibited thioesterase activity with a half-maximal inhibitory concentration of 29.6 μM, while Bx3PC-3 cell survival occurred at a half-maximal concentration of 14.8 μM [22]. Thus, the anticancer effects of high-dosage PPIs (i.e., 100 μM omeprazole) has been evaluated in recent basic and clinical research, although the peak plasma concentration of omeprazole measured during clinical use is approximately 1–2 μM.

Endothelial cells play an indispensable role in vascular homeostasis, including blood

coagulation, vascular permeability, and EDRF production. Various endothelial functions depend on changes in $[Ca^{2+}]_i$. SOCE, an important $[Ca^{2+}]_i$ regulatory mechanism in endothelial cells, is characterized by mobilization of $Ca^{2+}$ from the ER and subsequent $Ca^{2+}$ influx from the extracellular space [23]. Although several basic studies suggested that PPIs could impair vascular biology [11, 12], few have focused on the effect of PPIs on endothelial calcium signaling.

Bradykinin is an agonist of bradykinin receptor B2, a GPCR expressed on the surface of endothelial cells. Once BK stimulates the B2 receptor, the GPCR/PLC/$IP_3$ pathway is activated, resulting in increased $Ca^{2+}$ release from the ER, followed by activation of store-operated $Ca^{2+}$ channels (SOCC) [24, 25]. TG also stimulates SOCE by blocking ER $Ca^{2+}$-ATPase and passively depleting ER $Ca^{2+}$ contents [26, 27]. In line with recent research showing that 100 μM omeprazole inhibited TG-induced SOCE in RBL-1 cells [28], we confirmed that omeprazole suppressed TG-induced SOCE in PAECs. Furthermore, although 100 μM omeprazole might partially attenuate BK-induced $Ca^{2+}$ release from the ER, it did not exert any pharmacological effects on ER $Ca^{2+}$-ATPase. Thus, it is possible that omeprazole suppressed the intracellular $Ca^{2+}$ response through two parallel pathways: SOCC-related proteins and the GPCR-PLC-$IP_3$ pathway. Previous reports ascribed the inhibitory properties of several SOCE inhibitors to an

interaction between such inhibitors and calcium release-activated calcium modulator 1 (Orai1) protein [28-30]. However, no published reports indicated whether omeprazole or other PPIs affect the GPCR-PLC-$IP_3$ pathway. Thus, further studies are necessary to clarify the precise inhibitory properties of omeprazole.

Our findings show that omeprazole decreased BK-induced phosphorylation of eNOS(Ser1177). These results indicate that PPIs decrease NO availability, likely by a previously proposed mechanism (e.g. rise an intracellular asymmetrical dimethylarginine level or decrease in eNOS expression) [1, 12, 31]; however, few studies have assessed the effect of PPIs on eNOS phosphorylation, with the exception of a preeclampsia animal model [32]. eNOS activity is modulated by various extracellular stimuli, such as shear stress, vascular endothelial growth factor, estrogen, insulin, sphingosine 1-phosphate, and BK [33, 34]. Insulin, estrogen, and vascular endothelial growth factor primarily phosphorylate eNOS(Ser1177) via protein kinase B (Akt) in a $[Ca^{2+}]_i$-independent manner [35, 36], whereas BK-induced eNOS(Ser1177) phosphorylation is mediated by calmodulin-dependent protein kinase II in a $[Ca^{2+}]_i$-dependent manner [37]. These previous reports support our finding that omeprazole suppressed BK-activated intracellular $Ca^{2+}$ signaling, leading to decreased phosphorylation of eNOS(Ser1177).

One remaining consideration is why the effect of omeprazole on BK-induced NO production did not reach statistical significance, although omeprazole significantly inhibited Ser1177 phosphorylation. One possibility is the reciprocal phosphorylation of activator (Ser1177) and inhibitor (Thr495) sites that regulate eNOS activity in endothelial cells [33]. Thr495 is constitutively phosphorylated, leading to dampened eNOS activity by inhibition of calmodulin binding. BK stimulation increased binding of calmodulin to eNOS when Thr495 was dephosphorylated by phosphatase 1 [37]. A recent report indicated that BK did not show any effect on Thr495 phosphorylation [38]. Furthermore, Ser1177 phosphorylation elicits only a moderate increase in enzyme activity (less than two-fold) [35, 39]. Although we did not assess the effect of omeprazole on Thr495 phosphorylation, the phosphorylation balance between Ser1177 and Thr495 following treatment with omeprazole might influence BK-induced NO production. $PGI_2$ is an important vasodilator whose production is regulated by increases in $[Ca^{2+}]_i$ [40, 41]. In endothelial cells, SOCE is required to activate calcium-dependent phospholipase A2, which converts membrane phospholipids to arachidonic acids, the precursor of proteinoids [42].

In accordance with previous reports showing calcium-dependent synthesis of prostaglandin [40, 43, 44], our findings indicated that omeprazole tended to decrease 6-

keto-$PGF_{1\alpha}$ production. Both NO and $PGI_2$ continuously synthesized in endothelial cells. NO and $PGI_2$ production without BK or omeprazole (control) were 0.042 ± 0.032 $\mu M/10^6$ cells and 784.46 ± 212.45 $pg/mL/10^6$ cells, respectively, indicating that both NO and $PGI_2$ were produced at steady state. The additional effect of BK on these EDRF production were approximately 1.32-fold for NO and 1.24-fold for $PGI_2$. Omeprazole attenuated the additional effect of BK on EDRF production (approximately 0.96-fold for NO and 1.03-fold for $PGI_2$, compared with control), whereas the inhibitory effect of omeprazole did not reached statistically significance. Finally, our sample size might be small, which could affect the power of statistical tests and increased the risk that a type II error could have occurred.

**Conclusion**

Our results are the first to indicate that 100 μM omeprazole suppressed $Ca^{2+}$ influx from extracellular space and partially inhibited the GPCR-PLC-$IP_3$ pathway, and decreased BK-induced, $Ca^{2+}$-dependent phosphorylation of eNOS(Ser1177) (Figure 5). Thus, high dosages of omeprazole may mildly impair EDRF production via attenuation of intracellular $Ca^{2+}$ signaling.

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**Figure legends**

Figure 1

Bradykinin (BK) or thapsigargin (TG) increased cytosolic $Ca^{2+}$ concentration in modified Tyrode's solution containing 1 mM $Ca^{2+}$. (A) Time course of changes in Fura-2 fluorescence ratio (F340/F380). Cells were incubated for 5 min with modified Tyrode's solution (1 mM $Ca^{2+}$) in the absence (CTL; open circle, n = 30) or presence of

omeprazole (open triangle: 10 μM, n = 54; open square: 100 μM, n = 53; open diamond: 1 mM, n = 102) and then BK (10 nM) was applied. (B) Summarized data of the Fura-2 fluorescence ratio (F340/F380) at 90 s. (C) Time course of changes in Fura-2 fluorescence ratio (F340/F380). Cells were incubated for 5 min with 1 mM $Ca^{2+}$ modified Tyrode's solution in the absence (CTL; open circle, n = 81) or presence of omeprazole (open triangle: 10 μM, n = 80; open square: 100 μM, n = 98; open diamond: 1 mM, n = 135) and then TG (1 μM) was applied. (D) Summarized data of the Fura-2 fluorescence ratio (F340/F380) at 90 s. Data are expressed as mean ± standard deviation of three independent experiments in separate cell culture wells.

Figure 2

Bradykinin (BK) or thapsigargin (TG) increased cytosolic $Ca^{2+}$ concentration in $Ca^{2+}$-free modified Tyrode's solution. (A) Time course of changes in the fluorescence ratio (F340/F380) of Fura-2. Cells were incubated for 5 min with $Ca^{2+}$-free modified Tyrode's solution, in the absence (open circle, n = 113) or presence of omeprazole (open square; 100 μM, n = 118) and then BK (10 nM) was applied. (B) Summarized data of the Fura-2 fluorescence ratio (F340/F380) at 150 s. (C) Time course of changes in Fura-2 fluorescence ratio (F340/F380). Cells were incubated for 5 min with $Ca^{2+}$-free

modified Tyrode's solution in the absence (open circle, n = 109) or presence of omeprazole (open triangle; 100 μM, n = 117) and then TG (1 μM) was applied. (E) Summarized data of the Fura-2 fluorescence ratio (F340/F380) at 150 sec. Data are expressed as mean ± standard deviation of three independent experiments in separate cell culture wells.

Figure 3

Effect of omeprazole on BK-induced phosphorylation of eNOS(Ser1177) and NO production. (A) Omeprazole (100 μM) suppressed BK (10 nM)-induced phosphorylation of eNOS(Ser1177) (n = 4). (B) Representative western blot of p-eNOS (S1177). (C) BK (10 nM) increased NO production approximately 1.32-fold compared with control. Omeprazole mildly, but not significantly, reduced BK-induced nitric oxide production (n = 7 per group). Data are expressed as mean ± standard deviation. #p = 0.010 vs BK. *p = 0.016 vs control. BK, bradykinin; eNOS, endothelial nitric oxide synthase; n.s., not significant; p-eNOS(Ser1177); phosphorylated eNOS at Ser1177.

Figure 4

BK (10nM) increased $PGI_2$ production approximately 1.24-fold compared with control.

Omeprazole tended to decrease 6-keto-$PGF_{1\alpha}$ production (n = 7 per group), although this result was not significant. Data are expressed as mean ± standard deviation. *p = 0.016 vs control. BK, bradykinin; $PGF_1\alpha$, prostaglandin 1 alpha.

Figure 5

Expected inhibitory mechanisms of omeprazole on SOCE in PAECs. (A) BK evokes SOCE via activating the GPCR-PLC-$IP_3$ pathway. (B) omeprazole suppressed SOCC and partially inhibited the GPCR-PLC-$IP_3$ pathway, and mildly impair EDRF production via attenuation of intracellular $Ca^{2+}$ signaling.

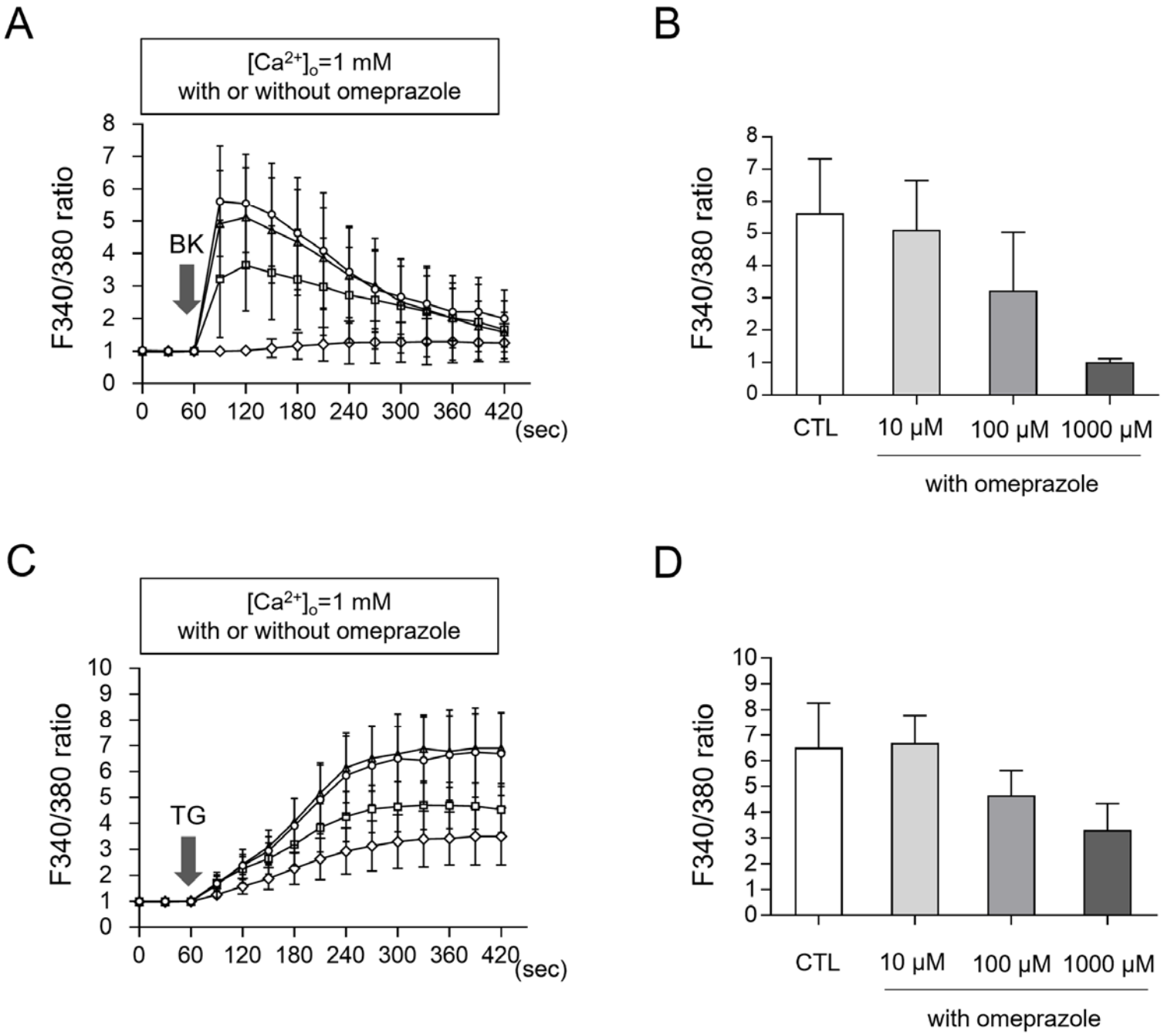
A
[Ca2+]o=1 mM
with or without omeprazole
F340/380 ratio
BK
0 60 120 180 240 300 360 420 (sec)
B
F340/380 ratio
CTL
10 μM
100 μM
1000 μM
with omeprazole
C
[Ca2+]o=1 mM
with or without omeprazole
F340/380 ratio
TG
0 60 120 180 240 300 360 420 (sec)
D
F340/380 ratio
CTL
10 μM
100 μM
1000 μM
with omeprazole

Figure 1

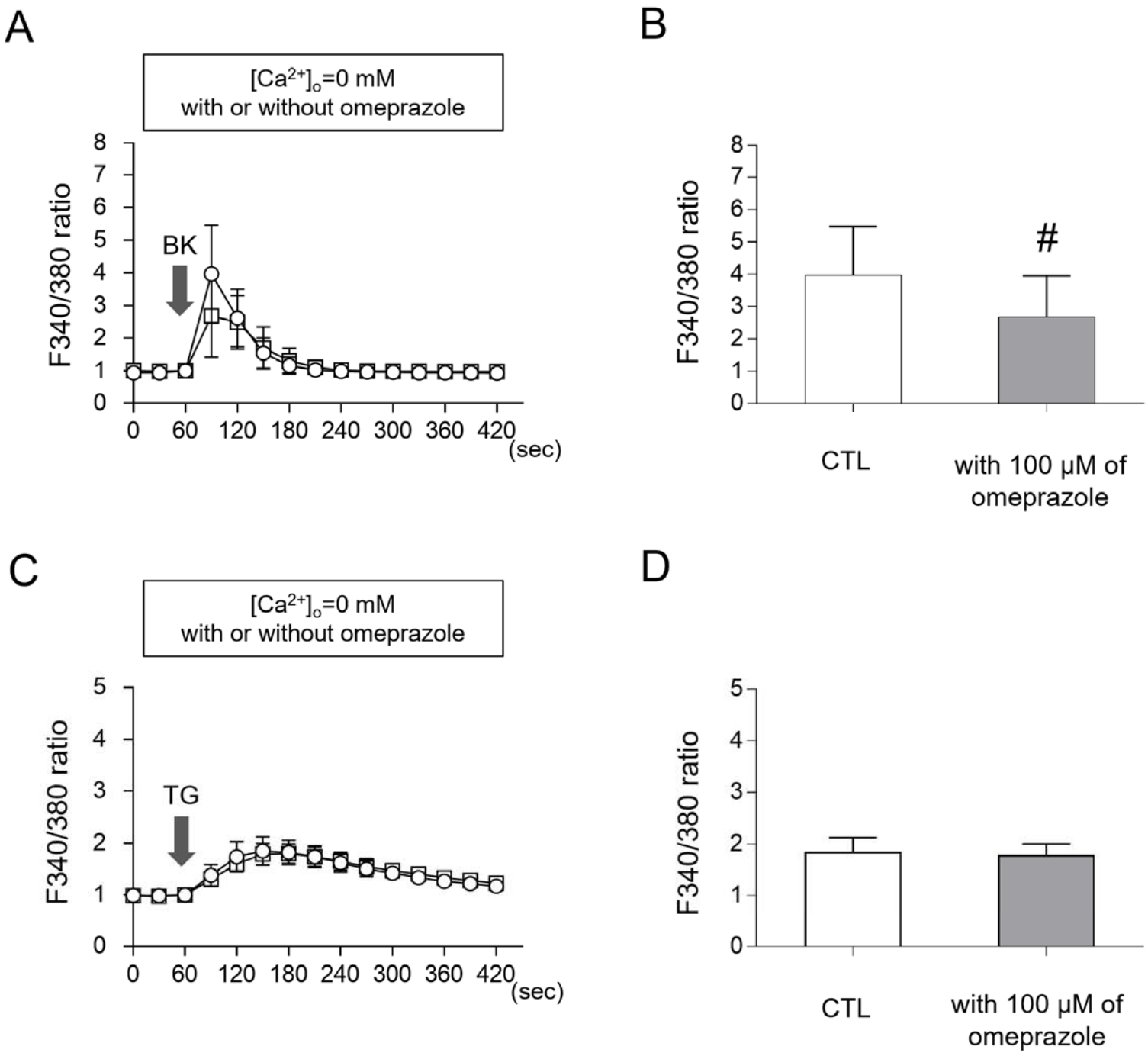
A
[Ca2+]o=0 mM
with or without omeprazole
F340/380 ratio
BK
0 60 120 180 240 300 360 420
(sec)
B
F340/380 ratio
#
CTL
with 100 μM of
omeprazole
C
[Ca2+]o=0 mM
with or without omeprazole
F340/380 ratio
TG
0 60 120 180 240 300 360 420
(sec)
D
F340/380 ratio
CTL
with 100 μM of
omeprazole

Figure 2

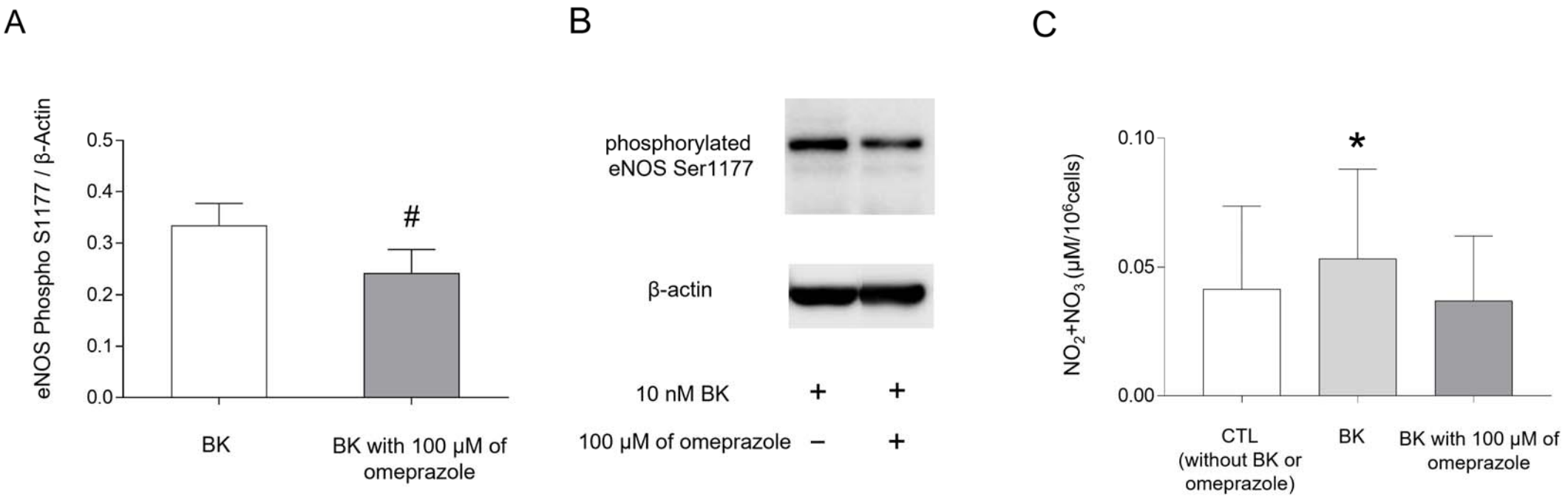
A
eNOS Phospho S1177 / β-Actin
0.5
0.4
0.3
0.2
0.1
0.0
#
BK
BK with 100 µM of omeprazole
B
phosphorylated eNOS Ser1177
β-actin
10 nM BK + +
100 µM of omeprazole – +
C
NO2+NO3 (µM/10^6cells)
0.10
0.05
0.00
*
CTL (without BK or omeprazole)
BK
BK with 100 µM of omeprazole

Figure 3

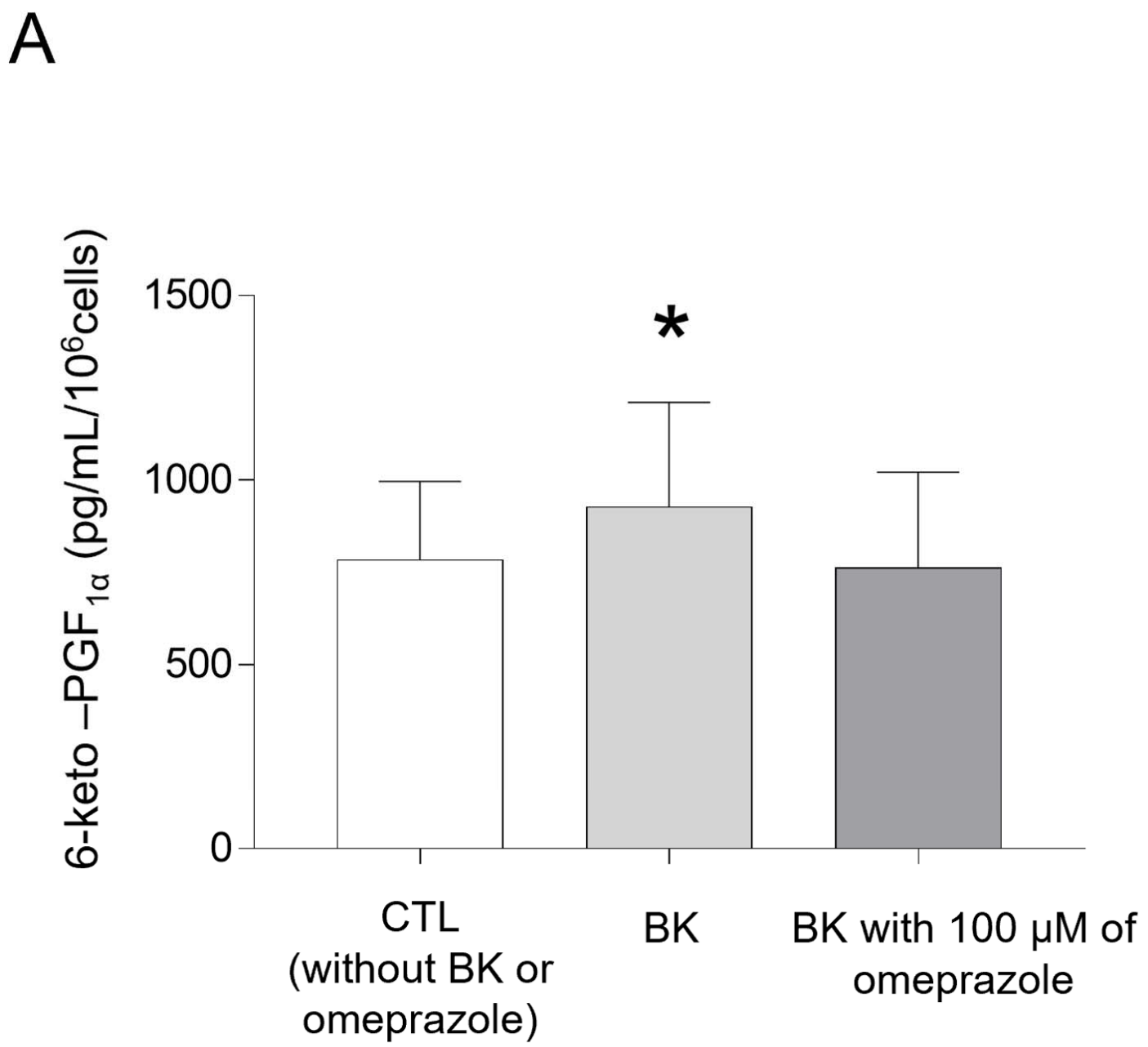
A
6-keto –PGF1α (pg/mL/10^6cells)
1500
1000
500
0
*
CTL (without BK or omeprazole)
BK
BK with 100 µM of omeprazole

Figure 4

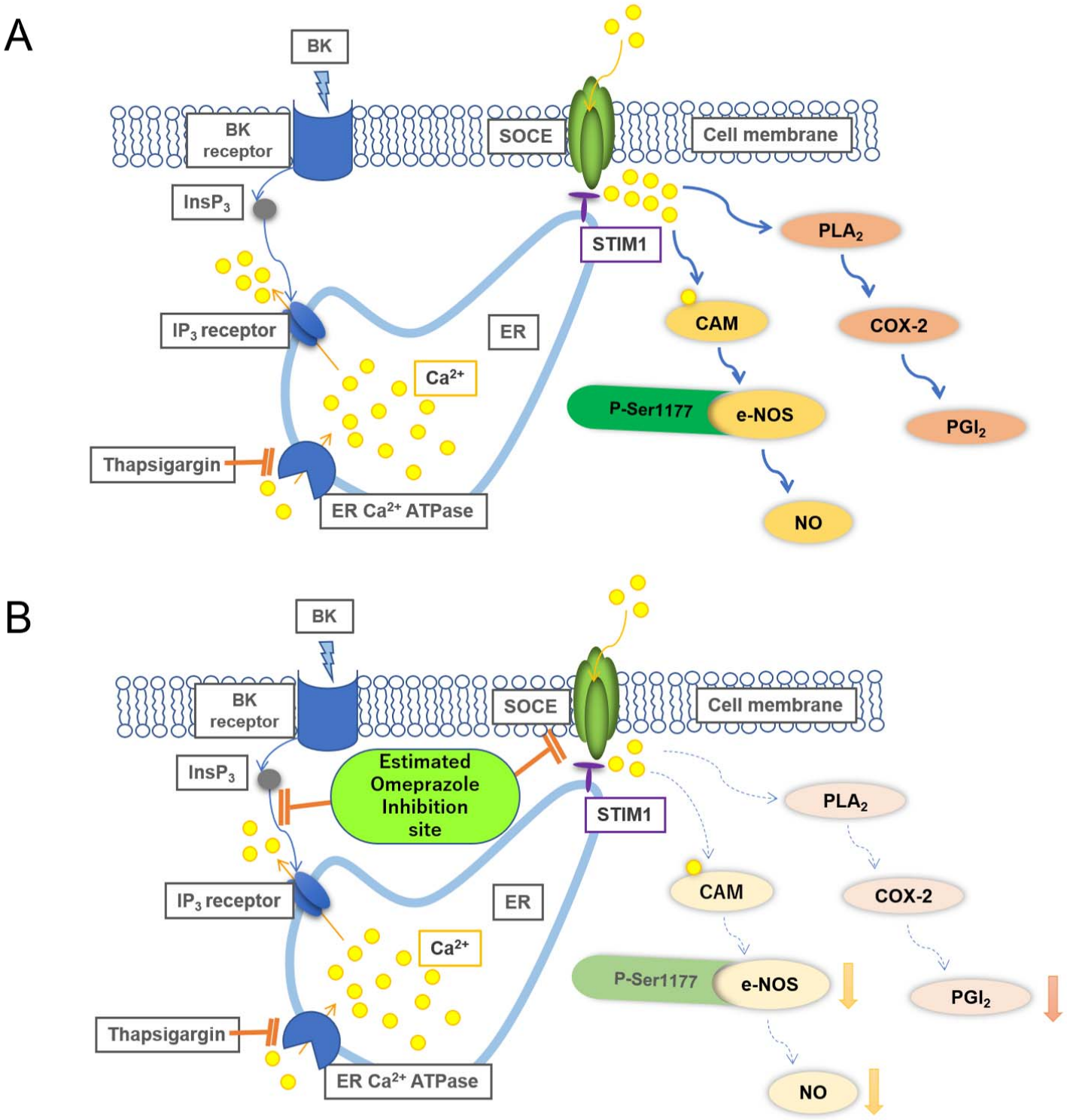
A
BK
BK receptor
SOCE
Cell membrane
InsP3
STIM1
PLA2
IP3 receptor
ER
CAM
COX-2
Ca2+
P-Ser1177
e-NOS
PGI2
Thapsigargin
ER Ca2+ ATPase
NO
B
BK
BK receptor
SOCE
Cell membrane
InsP3
Estimated Omeprazole Inhibition site
STIM1
PLA2
IP3 receptor
ER
CAM
COX-2
Ca2+
P-Ser1177
e-NOS
PGI2
Thapsigargin
ER Ca2+ ATPase
NO

Figure 5