Lafutidine-Induced Increase in Intracellular Ca\(^{2+}\) Concentrations in PC12 and Endothelial Cells

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**Abstract.** Lafutidine, a histamine H\(_2\) receptor antagonist, exerts gastroprotective effects in addition to gastric antisecretory activity. The gastrointestinal protective effects of lafutidine are mediated by capsaicin-sensitive neurons, where capsaicin excites neurons by opening a member of the transient receptor potential channel family (TRPV1). Since the effect of lafutidine on the intracellular Ca\(^{2+}\) concentration ([Ca\(^{2+}\)]\(_i\)) in cells has not been elucidated, we investigated the lafutidine response to [Ca\(^{2+}\)]\(_i\) in rat pheochromocytoma PC12 and human endothelial cells. Lafutidine at pharmacological concentrations greater than 1 mM induced a sustained increase in [Ca\(^{2+}\)]\(_i\) in the presence of extracellular CaCl\(_2\) in PC12 cells, while capsaicin showed dual effects on [Ca\(^{2+}\)]\(_i\) in PC12 cells, where it activated TRPV1 and inhibited store-operated Ca\(^{2+}\) entry. The thapsigargin (an activator of store-operated Ca\(^{2+}\) entry)-induced increase in [Ca\(^{2+}\)]\(_i\) in PC12 cells was inhibited by capsaicin and SKF96365, an inhibitor of store-operated Ca\(^{2+}\) entry, and the lafutidine response was inhibited by capsaicin but not by SKF96365. In endothelial cells, lafutidine induced an increase in [Ca\(^{2+}\)]\(_i\) in a SKF96365-insensitive manner. These results suggest that lafutidine stimulates Ca\(^{2+}\) entry via the capsaicin-sensitive pathway but not the SKF96365-sensitive pathway. The possible role of store-operated Ca\(^{2+}\) entry induced by lafutidine on gastrointestinal function is also discussed.

**Keywords:** lafutidine, intracellular Ca\(^{2+}\) concentration, PC12 cell, human umbilical vein endothelial cell, store-operated Ca\(^{2+}\) entry

**Introduction**

Lafutidine, ((±)-2-(furfurylsulfinyl)-N-[4-[4-(piperidinomethyl)-2-pyridyloxy](Z)-2-butenyl]acetamide), is a novel antiulcer drug that exhibits long-lasting gastric antisecretory effects due to blockade of the histamine H\(_2\) receptor (1, 2). In addition, lafutidine exerts gastroprotective effects independent of its antisecretory action (3, 4). These gastroprotective effects (3, 4, 5–7) and intestinal protective effects (8, 9) of lafutidine are due to the activation of capsaicin-sensitive neurons. Nishihara et al. (10) reported that lafutidine, which alone shows no effect, enhances the capsaicin-induced release of calcitonin gene-related peptide (CGRP) from neurons in the rat stomach. Also, recently we showed that

lafutidine at concentrations ranging from 0.1 to 1 \(\mu\)M, which alone has no effect, enhances ileal contraction induced by capsaicin in the isolated guinea pig ileum (11). Since the effects of capsaicin with and without lafutidine are completely inhibited by tetrodotoxin, lafutidine appears to activate myenteric neurons in the ileum. However, the mechanism(s) responsible for lafutidine activation by capsaicin-sensitive neurons has not been clarified.

The intracellular free Ca\(^{2+}\) concentration ([Ca\(^{2+}\)]\(_i\)) is strictly regulated, since Ca\(^{2+}\) is an important trigger of numerous neuronal functions including neurotransmitter release. In addition, capsaicin stimulates Ca\(^{2+}\) entry through the vanilloid VR1 receptor/TRPV1 (a member of transient receptor potential channel family), a receptor for capsaicin (12, 13), and then induces the release of neurotransmitters including CGRP. In the present study,
we focused on the effects of capsaicin and lafutidine on [Ca\(^{2+}\)] in cells.

Rat pheochromocytoma cell line PC12 is a well-established model used for the investigation of signaling mechanisms in neurons. Previously, we reported that capsaicin stimulates arachidonic acid release and prostaglandin formation in PC12 cells (14). The existence of TRPV1 mRNA in PC12 cells was recently observed (15), and we found that TRPV1 protein is expressed and capsaicin induces Ca\(^{2+}\) entry via TRPV1 in PC12 cells (16). In addition, Choi and Kim (17) reported that capsaicin acts as an inhibitor of store-operated Ca\(^{2+}\) entry in PC12 cells. Thus, PC12 cells are a useful model for studying Ca\(^{2+}\) regulation by capsaicin and/or lafutidine. In the present study, we show that lafutidine induces an increase in [Ca\(^{2+}\)] by Ca\(^{2+}\) influx from extracellular spaces in PC12 cells. Since lafutidine causes an increase in gastric mucosal blood flow (GMBF) in the gastric microvasculature (4, 18), the lafutidine response on [Ca\(^{2+}\)] was also examined in human umbilical vein endothelial cells (HUVECs). Lafutidine, in addition to being an antagonist of histamine H\(_2\) receptors, may act on store-operated Ca\(^{2+}\) entry in cells. The possible role of increase in [Ca\(^{2+}\)] induced by lafutidine on lafutidine-induced pharmacological effects such as gastrointestinal protective effects and the modification of ileum contraction is also discussed.

Materials and Methods

Chemicals

Capsaicin, capsazepine, and famotidine were purchased from Sigma (St. Louis, MO, USA). Adenosine-5’-O-(3-thiotriphosphate) (ATP\(_7\)) was purchased from Boehringer-Mannheim (Mannheim, Germany). Thapsigargin and 1-[β-(3-(4-methoxyphenyl)propoxy)-4-methoxyphenyl]-1H-imidazole hydrochloride (SKF96365) were purchased from Wako (Osaka). Lafutidine was supplied from Tokushima Research Center of Taiho Pharmaceutical Co. Ltd. (Tokushima). These reagents were diluted with deionized water or a minimum volume of dimethyl sulfoxide. The final concentrations of capsaicin, thapsigargin, lafutidine, ATP\(_7\), and SKF96365 in the assay mixture were selected based on previous studies (16, 17, 19, 20). The final concentration of dimethyl sulfoxide in the assay mixtures was less than 1%, and the pHs of the assay mixtures containing reagents were 7.3 – 7.5. The vehicles did not show any significant effects on [Ca\(^{2+}\)].

Cells and measurement of [Ca\(^{2+}\)]

PC12 cells were cultured on collagen-coated dishes in Dulbecco’s modified Eagle’s medium (DMEM) supplemented with 5% fetal bovine serum (FBS) and 5% horse serum at 37°C under 5% CO\(_2\) as previously described (16), while HUVECs were cultured on collagen-coated dishes in DMEM/Ham F12 (1:1) supplemented with 10% FBS and 10 ng/mL human basic-fibroblast growth factor (PeproTech, Rocky Hill, NJ, USA) as previously reported (21). [Ca\(^{2+}\)] in PC12 cells and endothelial cells were measured as previously described (16) with minor modifications. In brief, cells on dishes were loaded with 3 μM Fura 2-acetoxymethyl ester (Dojindo, Kumamoto) for 30 min at 37°C in normal Tyrode’s buffer (135 mM NaCl, 5.4 mM KCl, 1.8 mM CaCl\(_2\), 1.8 mM MgCl\(_2\), 5 mM HEPES, 10 mM glucose, pH 7.4) containing 0.05% cremophor EL (Nacalal Tesque, Kyoto). The cells were then washed and detached from the dishes under a gentle stream of buffer. Detached cells were next washed by centrifugation (800 × g, 30 s) at 4°C, and resuspended. In some experiments, extracellular CaCl\(_2\) was omitted from the assay mixture. Finally, fluorescence readings were taken with a Hitachi F-2500 spectrophotometer (Hitachi, Tokyo).

Cell viability

Cell viability was estimated by the leakage of lactate dehydrogenase as previously described (22), where leakage (%) was defined as the ratio of lactate dehydrogenase activity in the buffer to total activity [% = (extracellular activity) / (extracellular activity and remaining cellular activity) × 100] per tube.

Statistics

Data were presented as means ± S.E.M. for three independent experiments performed in duplicate. The experiments, in which a batch of cells were used, were performed on different days, and the difference in duplicate determinations for an experiment was within 10%. The results were reproducible when different batches of cells were used. Statistical analyses were performed using the two-tailed Student’s t-test for comparison of two groups and by one-way analysis of variance followed by Dunnett’s multiple comparison test for comparison of more than three groups. Values of P<0.05 were regarded as being statistically significant.

Results

Effect of lafutidine on [Ca\(^{2+}\)] in PC12 cells

Lafutidine (1 – 10 mM) induced an increase in [Ca\(^{2+}\)], in PC12 cells in a concentration-dependent manner (Fig. 1, Panel A). The lafutidine (3 mM)-induced response reached a maximum within 30 s after addition.
and was sustained for at least 3 min. In some experiments, the lafutidine response slowly and slightly decreased 1 or 2 min after addition. The concentration-dependence of lafutidine concerning the maximal increase in $[\text{Ca}^{2+}]$, is shown in Panel B. Although the lafutidine response was not saturated under the tested conditions, its effects at concentrations above 10 mM could not be determined because of its poor solubility. Lafutidine (3 mM) did not induce an increase in $[\text{Ca}^{2+}]$, when the extracellular CaCl$_2$ was omitted (Panel C). However, subsequent addition of CaCl$_2$ to the extracellular solution evoked a sustained increase in $[\text{Ca}^{2+}]$, to a level above the control. Since lafutidine is proposed to interact with capsaicin-sensitive neurons, we investigated the effect of capsazepine, an antagonist of TRPV1, on the lafutidine-induced increase in $[\text{Ca}^{2+}]$. Treatment with capsazepine marginally but not significantly induced a sustained increased in $[\text{Ca}^{2+}]$, where the levels 2 min after vehicle and 100 μM capsazepine addition were 127 ± 27 nM (n = 3) and 207 ± 37 nM (n = 3), respectively. The $[\text{Ca}^{2+}]$, level induced by 1 mM lafutidine in the presence of 100 μM capsazepine was 273 ± 38 nM (n = 3), and the net increase induced by lafutidine was almost the same as that in the control cells. The addition of 3 mM lafutidine caused a sustained increase in $[\text{Ca}^{2+}]$, in the presence of 100 μM capsazepine, where the net increases were 150 – 200 nM for two experiments. Also, the addition of 100 μM histamine and 3 mM famotidine, another antagonist of the histamine H$_2$ receptor, showed no effect on $[\text{Ca}^{2+}]$, in PC12 cells (data not shown).

The addition of 300 μM ATP$_7$S evoked a transient increase in $[\text{Ca}^{2+}]$, and the peak value (approximately 500 nM) was observed 5 – 10 s after addition in PC12 cells (Panel D) as previously reported (19). Lafutidine at 3 mM still induced a sustained increase in $[\text{Ca}^{2+}]$, in the ATP$_7$S-treated cells. Furthermore, the addition of 300 μM ATP$_7$S still induced an increase in $[\text{Ca}^{2+}]$, in 3 mM (Panel E) and 10 mM (data not shown) lafutidine-treated cells, although the peak value induced for the

Fig. 1. The effect of lafutidine on $[\text{Ca}^{2+}]$, in PC12 cells. PC12 cells were incubated with the indicated concentrations of lafutidine (LAF) in the presence of 1.8 mM CaCl$_2$ (Panel A). Each individually performed recording is presented on the same panel. Panel B shows quantitative analyses of lafutidine on $[\text{Ca}^{2+}]$, in PC12 cells in the presence (open circle) or absence (closed circle) of extracellular CaCl$_2$. Each value represents the mean ± S.E.M. of three independent experiments. *P<0.05, significantly different from the value without lafutidine. In Panel C, cells were stimulated with 3 mM lafutidine in the absence of extracellular CaCl$_2$, and CaCl$_2$ (1.8 mM at final concentration) was subsequently added to the assay mixture as indicated by the horizontal bar. PC12 cells were incubated with 300 μM ATP$_7$S (Panel D) or 3 mM lafutidine (Panel E) and then further stimulated with 3 mM lafutidine (Panel D) or 300 μM ATP$_7$S (Panel E) in the presence of CaCl$_2$. Each Panel (A, C, D, and E) shows typical recordings made more than three times.
treated cells was lower than that for the control cells. Lafutidine at 3 and 10 mM did not stimulate lactate dehydrogenase leakage for at least 30 min. For this, the values of lactate dehydrogenase leakage in the vehicle-treated, lafutidine (10 mM)-treated, and control PC12 cells were under 5% of the total activity.

**Effects of SKF96365 and capsaicin on the lafutidine-induced increase in \([\text{Ca}^{2+}]\)**

Thapsigargin, an inhibitor of \(\text{Ca}^{2+}\)-ATPase found in intracellular \(\text{Ca}^{2+}\) stores, was used to activate store-operated \(\text{Ca}^{2+}\) entry by inducing \(\text{Ca}^{2+}\) store depletion independently of receptor activation. Addition of 5 mM thapsigargin induced a sustained increase in \([\text{Ca}^{2+}]\) in PC12 cells (Fig. 2, Panel A) as previously reported (17, 23). The magnitude and time course of the thapsigargin response were similar to those of the 3 mM lafutidine response. Next, we investigated the effects of inhibitors of store-operated \(\text{Ca}^{2+}\) entry on the lafutidine- and the thapsigargin-induced responses. SKF96365 is an inhibitor of store-operated \(\text{Ca}^{2+}\) channels (17, 20). The sustained increase in \([\text{Ca}^{2+}]\), induced by thapsigargin was reduced by half and significantly inhibited by 30 \(\mu\)M SKF96365 (Panel A), but the lafutidine response was not affected (Panel B). The effect of 10 \(\mu\)M SKF96365 on the thapsigargin response was found to be limited and/or marginal (data not shown).

Capsaicin alone evoked a transient increase in \([\text{Ca}^{2+}]\) in PC12 cells; a peak increase was observed after 10 – 20 s and the \([\text{Ca}^{2+}]\) levels decreased within 30 s after capsaicin addition (Panel C) as previously reported (16). The increase induced by capsaicin was completely inhibited by an antagonist of TRPV1 (16). In addition to the effect on TRPV1, capsaicin inhibits store-operated \(\text{Ca}^{2+}\) entry in PC12 cells (17). We previously reported that the sustained increase in \([\text{Ca}^{2+}]\), induced by 5 \(\mu\)M thapsigargin is inhibited by 300 \(\mu\)M capsaicin and that pretreatment with capsaicin abolishes the thapsigargin response (16). Similar to the thapsigargin response (Panel D), the sustained increase in \([\text{Ca}^{2+}]\), induced by 3 mM lafutidine was inhibited by 300 \(\mu\)M capsaicin (Panel E). Quantitative analyses of the capsaicin

![Fig. 2. The effects of capsaicin and SKF96365 on the lafutidine- or thapsigargin-induced increase in \([\text{Ca}^{2+}]\).](image-url)
responses are shown in Panel F and Table 1. The increase in \([\text{Ca}^{2+}]_i\) induced by lafutidine tended to decrease slightly faster compared with the thapsigargin response in PC12 cells. Also, pretreatment with 300 \(\mu\text{M}\) capsaicin for 2 min markedly inhibited the 3 mM lafutidine response (Panel C) and almost completely abolished the thapsigargin response (Table 1). Pretreatment with 30 \(\mu\text{M}\) SKF96365 for 2 min markedly inhibited the thapsigargin response (70 – 80% inhibition), but only slightly affected the lafutidine response (20 – 30% inhibition).

### Effect of lafutidine on \([\text{Ca}^{2+}]_i\) in endothelial cells

Next, we examined whether lafutidine induces an increase in \([\text{Ca}^{2+}]_i\) in endothelial cells (HUVECs). Similar to PC12 cells, lafutidine induced a sustained increase in \([\text{Ca}^{2+}]_i\) in the endothelial cells (Fig. 3, Panel A). The lafutidine response was concentration-dependent and the 10 mM lafutidine response in endothelial cells was greater than that in PC12 cells. However, famotidine at 3 and 10 mM had no effect (data not shown). The lafutidine response in endothelial cells was significantly inhibited by 300 \(\mu\text{M}\) capsaicin, but not by 30 \(\mu\text{M}\) SKF96365 (Table 1), and pretreatment of endothelial cells with 300 \(\mu\text{M}\) capsaicin for 2 min also inhibited the increase in \([\text{Ca}^{2+}]_i\), induced by following challenge with 3 mM lafutidine. The sustained increase in

### Table 1. The effects of capsaicin and SKF96365 on the lafutidine-induced increase in \([\text{Ca}^{2+}]_i\), in PC12 cells and HUVECs

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Stimuli</th>
<th>PC12 cells</th>
<th>HUVECs</th>
</tr>
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<tbody>
<tr>
<td>I (1 min)</td>
<td>Thapsigargin</td>
<td>95.2 ± 6.3</td>
<td>45.4 ± 5.5*</td>
</tr>
<tr>
<td></td>
<td>Lafutidine</td>
<td>83.2 ± 8.9</td>
<td>40.2 ± 4.2*</td>
</tr>
<tr>
<td></td>
<td>SKF96365</td>
<td>40.1 ± 1.5*</td>
<td>80.3 ± 3.3</td>
</tr>
<tr>
<td>II (Pretreatment)</td>
<td>Capsaicin</td>
<td>10.1 ± 1.5*</td>
<td>40.2 ± 2.9*</td>
</tr>
<tr>
<td></td>
<td>SKF96365</td>
<td>40.1 ± 1.5*</td>
<td>80.4 ± 4.8</td>
</tr>
</tbody>
</table>

In Experiment I, cells were incubated with 5 \(\mu\text{M}\) thapsigargin or 3 mM lafutidine for 2 min as a first stimulus; and then they were further incubated with vehicle, 300 \(\mu\text{M}\) capsaicin, or 30 \(\mu\text{M}\) SKF96365 as a second stimulus. Each value was measured 1 min after the second stimuli and is shown as the percentage of a net increase in \([\text{Ca}^{2+}]_i\) measured just before the second stimuli. In Experiment II, cells were incubated with 300 \(\mu\text{M}\) capsaicin for 2 min and then stimulated with 5 \(\mu\text{M}\) thapsigargin or 3 mM lafutidine. The data is shown as percentages of the net increase in \([\text{Ca}^{2+}]_i\) in the control cells. The absolute increases in \([\text{Ca}^{2+}]_i\), induced by thapsigargin and lafutidine are shown in Figs. 1 – 3. Each data point indicates the mean ± S.E.M. of three independent experiments. *\(P<0.01\), significantly different from the vehicle-treated group. \(^bP<0.01\) and \(^cP<0.001\), significantly different from the control group.

Fig. 3. The effect of lafutidine on \([\text{Ca}^{2+}]_i\) in HUVECs. In Panel A, HUVECs were stimulated with the indicated concentrations of lafutidine (LAF) in the presence of extracellular CaCl₂. Each individually performed recording is presented on the same panel. Panel B shows quantitative analyses of the effects of lafutidine on \([\text{Ca}^{2+}]_i\) in HUVECs, and the values represent the means ± S.E.M. of three independent experiments. \(^aP<0.05\), significantly different from the value without lafutidine.
[Ca$^{2+}$], induced by 5 μM thapsigargin in endothelial cells was inhibited by capsaicin and SKF96365 (data not shown). Although 100 μM histamine caused a rapid increase in [Ca$^{2+}$], in HUVECs as previously reported (24), the histamine response was not inhibited by 1 and 3 mM lafutidine and famotidine (data not shown).

Addition of 300 μM capsaicin alone caused a transient increase in [Ca$^{2+}$], in HUVECs (data not shown), like in PC12 cells. However, we could not detect TRPV1 protein in endothelial cells by immunoblotting using anti-TRPV1 antibody (sc-12498, Santa Cruz Biotech) against human TRPV1 under our conditions. It should be determined whether TRPV1 is expressed in HUVECs or not.

**Discussion**

The present study showed that lafutidine and not famotidine at pharmacological concentrations above 1 mM induces a sustained increase in [Ca$^{2+}$]), in PC12 cells, a neuronal model cell line, and HUVECs, an endothelial model cell line. The stimulatory effects of 3 and 10 mM lafutidine were not due to cell toxicity because i) receptor stimulation using ATPγS still induced an increase in [Ca$^{2+}$] in lafutidine-treated PC12 cells, and ii) lafutidine showed little and/or marginal effects on lactate dehydrogenase leakage from PC12 cells for at least 30 min. These results suggest that lafutidine increases [Ca$^{2+}$] in cells without inducing cell toxicity.

**Non-involvement of TRPV1 and histamine receptors on the lafutidine-induced increase in [Ca$^{2+}$].**

As previously mentioned, PC12 cells express TRPV1 mRNA and its protein (15, 16). Thus, lafutidine may act on TRPV1 and therefore stimulate Ca$^{2+}$ influx in PC12 cells. Capsaicin elicited a transient increase in [Ca$^{2+}$], in PC12 cells, but the lafutidine response was long-lasting similar to that of the thapsigargin response (Figs. 1 and 2). Capsazepine is known to be an effective antagonist of TRPV1, although capsazepine is somewhat nonspecific. Recently, Phillips et al. (25) reported that mutation of rat TRPV1 at several positions decreased the IC$\text{50}$ values for capsazepine inhibition of the TRPV1-mediated response. Although capsazepine almost completely inhibited the capsaicin response (16), the inhibitory effect of capsazepine on the lafutidine-induced increase in [Ca$^{2+}$], was limited in PC12 cells. Resiniferatoxin (a potent agonist of TRPV1, Ref. 13) at 10 μM did not inhibit the thapsigargin response and capsazepine inhibited the response in the presence of 10 μM ruthenium red (an antagonist of TRPV1, Ref. 13) in PC12 cells (data not shown), as previously reported (17). Lafutidine caused a sustained increase in [Ca$^{2+}$] in HUVECs. The pharmacological characteristic of its response in HUVECs was similar to that in PC12 cells. Nishihara et al. (10) showed that capsaicin but not lafutidine inhibits the specific binding of [$^3$H]resiniferatoxin in neuronal cells. Mimaki et al. (18) showed that lafutidine alone at 0.1 mM does not increase [Ca$^{2+}$], in human embryo kidney cells that express TRPV1. These findings and reports suggest that lafutidine activates Ca$^{2+}$ entry in PC12 and endothelial cells in a TRPV1-independent manner.

Lafutidine is an antagonist of histamine H2 receptors (1, 2). In some cases, a receptor antagonist may act as a partial agonist. The activation of histamine H2 receptors stimulates cyclic AMP accumulation, but the change in [Ca$^{2+}$], induced by histamine is small or negative in PC12 cells (26). In the present study, lafutidine caused an increase in [Ca$^{2+}$], in PC12 cells, but histamine and famotidine had no effect. In HUVECs, histamine induces a rapid increase in [Ca$^{2+}$], and regulates the expression of various molecules via histamine H2 but not H1 receptors (21, 24). However, lafutidine did not change the Ca$^{2+}$ response induced by histamine in endothelial cells. Thus, a sustained increase in [Ca$^{2+}$], induced by lafutidine in cells does not appear to be mediated by histamine H1 and H2 receptors.

**Role of store-operated Ca$^{2+}$ entry on the lafutidine-induced increase in [Ca$^{2+}$].**

Ca$^{2+}$ channels that are activated by intracellular store-depletion and/or by thapsigargin were recently classified as belonging to the TRP classic (TRPC) subfamily and as members of TRP ion channel family (27, 28). mRNAs encoding TRPC1-6 have also been detected in PC12 cells (20). As with thapsigargin, lafutidine elicited a long-lasting increase in [Ca$^{2+}$], in PC12 cells. Similar to this, lafutidine causes a sustained increase in [Ca$^{2+}$], in endothelial cells, where mRNA and/or the proteins of some TRPC are expressed (29, 30). In addition to its action on TRPV1, capsaicin acts as an inhibitor of store-operated Ca$^{2+}$ entry induced by thapsigargin in PC12 cells (16, 17) and inhibits store-operated Ca$^{2+}$ entry in human leukemia HL-60 cells (31) and Jurkat T cells (32). The sustained increase in [Ca$^{2+}$], induced by lafutidine was significantly inhibited by capsazepin in PC12 and endothelial cells. Furthermore, pretreatment with capsazepin inhibited the thapsigargin- and lafutidine-induced responses in PC12 cells and the lafutidine response in endothelial cells. These results suggest the possible involvement of store-operated Ca$^{2+}$ entry in the lafutidine-induced increase in [Ca$^{2+}$], in cells.

Treatment with SKF96365 significantly inhibited the thapsigargin responses, but barely affected the lafutidine responses in PC12 and endothelial cells. In PC12 cells,
Ca\(^{2+}\) Entry by Lafutidine

Several studies have suggested the involvement of endogenous nitric oxide (NO) in the protective action of lafutidine (9, 10). In the present study, we found that lafutidine causes a sustained increase in \([\text{Ca}^{2+}]\), in endothelial cells. Store-operated \([\text{Ca}^{2+}]\) entry probably via TRPC and not a transient \([\text{Ca}^{2+}]\) influx is required for NO release in endothelial cells and vasodilation (35). Thus, lafutidine-induced increase in \([\text{Ca}^{2+}]\) in endothelial cells may contribute to the gastroprotective effect of lafutidine via NO production. However, lafutidine alone does not affect NO production in the rat gastric mucosa (10). Lafutidine given intraperitoneally hardly increases GMBF under basal conditions, although lafutidine augmented the increase in GMBF induced by mucosal acidification (18). The intragastric administration of lafutidine causes a sustained increase in GMBF, but this effect is totally attenuated by ablation of capsaicin-sensitive neurons (4). Thus, the lafutidine effect on \([\text{Ca}^{2+}]\) in endothelial cells does not appear to be involved directly in the gastroprotective effect of lafutidine. Lafutidine enhances CGRP release from afferent neurons in response to capsaicin in the rat stomach (10). CGRP stimulates NO production in endothelial cells, and this NO then participates in the regulation of GMBF through vasodilation in the gastric microvasculature (36, 37). An increase in \([\text{Ca}^{2+}]\), induced by lafutidine in endothelial cells may enhance NO production induced by stimuli such as CGRP and resulting vasodilation in endothelial cells. The effects of lafutidine on \([\text{Ca}^{2+}]\), levels in neuronal and endothelial cells in gastrointestinal tissues should be examined in the future.

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