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Ghrelin receptor is activated by naringin and naringenin, constituents of a prokinetic agent *Poncirus fructus*

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ABSTRACT

Ethnopharmacological relevance: *Poncirus fructus* (PF), also known as a dried immature fruit of *Poncirus trifoliata* (L.) Raf. (Rutaceae), has long been traditionally used for the various gastrointestinal disorders in Eastern Asia.
Aim of study: The aqueous extract of PF (PF-W) has the strong prokinetic effect, yet the underlying mechanism is still elusive. The present study investigated whether PF-W has any effect on motilin receptor or ghrelin receptor, since these receptors enhance intestinal motility when activated.
Materials and methods: The effect of PF-W and its components on motilin or ghrelin receptor was determined by calcium imaging and whole-cell patch clamp methods.
Results: PF-W activates the ghrelin receptor, but not the motilin receptor, resulting in a transient increase of intracellular calcium levels. Furthermore, among various constituents of PF, only naringin and naringenin evoked the intracellular calcium augmentation via the ghrelin receptor. Moreover, cortistatin-8 – a ghrelin receptor inhibitor – specifically blocked naringin- and naringenin-induced calcium increases. In addition, naringin and naringenin induced inward currents in ghrelin receptor-expressing cells under whole-cell patch clamp configuration.
Conclusion: PF-W activates the ghrelin receptor, and naringin and naringenin are key constituents responsible for the activation of ghrelin receptor. Therefore, the present study suggests that the ghrelin receptor is a molecular entity responsible for the strong prokinetic activity of PF-W.
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1. Introduction

The dried, immature fruit of *Poncirus trifoliata* (L.) Raf. (Rutaceae), also called *Poncirus fructus* (PF), has long been used as a traditional remedy for various gastrointestinal (GI) disorders, especially in Eastern Asia (Benavente-Garcia and Castillo, 2008). Particularly, its aqueous extract form (PF-W) increased GI motor function in both human and rodent, by accelerating the transit of intestinal contents (Lee et al., 2005b). Moreover, PF-W also improved the intestinal transit rate in animal models with GI motility dysfunction (Lee et al., 2005a). Recently, the underlying prokinetic mechanism of PF-W has been investigated, and serotonin receptor subtype 4 (5-HT4R) seems to play a role in this mechanism (Shim et al., 2010). Indeed, activation of 5-HT4R induces changes in GI motility including accelerated gastric emptying, enhanced colonic contractions and transitions (Manabe et al., 2010). In fact, many strong prokinetic agents such as cisapride and mosapride are agonists of 5-HT4R.

However, the 5-HT4R pathway is not the exclusive mechanism that governs GI motility. Motilin and ghrelin – hormones released from cells lining the stomach, pancreas, and small intestine – are well-known endogenous molecules that stimulate GI motilities through their distinctive receptors (Peeters, 2006). In detail, the motilin receptor (MLNR) and the ghrelin receptor (also known as growth hormone secretagogue receptor, GHSR) are typical G protein coupled receptors (GPCR) coupled with the $G\alpha_{q/11}$ pathway (Cruz and Smith, 2008). These receptors release Ca^{2+} from endoplasmic reticulum, stimulating inositol (1,4,5) triphosphate (IP_3) cleavage from phosphatidylinositol 4,5-biphosphate (PIP_2) by phospholipase C (Sato et al., 2012).

Recent advances in this field have revealed motilin/MLNR and ghrelin/GHSR are essentially involved in the intestinal motility (Peeters, 2006). Motilin is considered to be an appetite-regulating hormone following the interdigestive period. The cyclical plasma levels of motilin are synchronized to strong peristaltic contractions initiated from the stomach and migrating to the small intestine,

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modulating interdigestive migrating contractions in the GI tract (Ohno et al., 2010). Similar to motilin, peripheral administration of ghrelin expedites gastric emptying in rodents, enhancing coordinated motor pattern between the antrum and pylorus (antro-pyloric coordination) (Ariga et al., 2008). Ghrelin, when administered intraperitoneally, alleviates symptoms of dyspepsia in relevant animal models (Liu et al., 2006). Moreover, ghrelin regulates the contractions of migrating motor complex which is originating in the stomach and propagating along the small intestine (De Smet et al., 2009). Ghrelin also induced contractions of smooth muscle strip from fundus and antrum by directly activating cholinergic excitatory neurons (Dass et al., 2003). In fact, many GHSR and MLNR agonists, such as TZP-101, TZP-102, mitemcinal (GM-611), and PF-04548043 (KOS-2187), are currently being developed as potential prokinetic agents for the treatment of various GI disorders (Sanger, 2008).

Since PF-W is a mixture of various natural compounds, it is assumable that PF-W may possess a fraction that activates MLNR and/or GHSR. Therefore, the present study investigated whether PF-W has an ability to activate MLNR and/or GHSR. Specifically, it was examined whether the increment of intracellular Ca^{2+} ($[Ca^{2+}]_i$) can be induced by PF-W or its constituents, by calcium imaging in cells expressing respective receptors. Moreover, the effects of active compounds were further confirmed by the whole-cell patch clamp technique, and their involvement in 5-HT4R was verified.

2. Materials and methods

2.1. Plant material

Poncirus fructus was obtained from a certified oriental drug store located in the Gyungdong Market (Seoul, Korea). Reference specimens (no. EA239) were deposited at the Herbarium of the College of Pharmacy, Ewha Woman's University (Seoul, Korea).

2.2. Sample preparation

PF-W was prepared with previously described methods (Lee et al., 2005a). 2 kg of PF was boiled in 30 L of distilled water at 100 °C for 2 h, and the aqueous extract was filtered, concentrated *in vacuo*, and lyophilized. The yield was approximately 24.4% (w/w). In order to assure consistent quality, the hesperidin content was determined in each PF-W sample using HPLC. The hesperidin content was calculated to be $0.93 \pm 0.01\%$ (w/w, $n=3$), suggesting a fairly stable content in PF-W.

2.3. Gene cloning

Human full-length MLNR (NM_001507) and GHSR (NM_198407) cDNAs were kindly provided by LG Life Science Ltd. Rat 5-HT4R genes were cloned as mentioned in a previous article (Shim et al., 2010). All sequences were confirmed to be 100% identical to sequences in the Genbank database.

2.4. Cell culture and gene transfection

HEK293T cells were maintained in DMEM supplemented with 10% fetal bovine serum (FBS) and 100 units/ml penicillin/streptomycin at 37 °C in a humidified atmosphere of 5% CO_2. HEK293T cells were transfected with genes (pcDNA, MLNR, GHSR, 5-HT4R) using a FuGENE® HD transfection reagent (Promega, Wisconsin, USA) according to the manufacturer's protocol. One day after transfection, transfected cells were used for various experiments.

2.5. Measurement of intracellular Ca^{2+}

HEK293T cells were cultured and were transfected with pcDNA, motilin or ghrelin receptors in the lysine coated culture chamber (Lab-Tek™, Thermo Scientific). One day after transfection, the culture media were replaced with normal bath solution [140 mM NaCl, 5.0 mM KCl, 2 mM $CaCl_2$, 0.5 mM $MgCl_2$, 10 mM glucose, and 5.5 mM HEPES (pH 7.4)] containing Fluo-3 AM (2 μM, Invitrogen) and 0.1% Pluronic F-127 (Invitrogen), and were then incubated for 40 min at 37 °C. After incubation, the buffer solution containing Fluo-3 AM was washed out with normal bath solution. Analysis of the fluorescent images was performed as described previously (Jang et al., 2012a). F/F_0 indicates a relative ratio of the fluorescent intensity (F) divided by the first fluorescent intensity of the respective cell (F_0). For measuring changes in $[Ca^{2+}]_i$ evoked by PF-W and its constituents, we transfected GHSR or MLNR plasmids in the HEK293T cells, After 1 day of transfection, the HEK293T cells were incubated with Fluo-3 AM. After washing out the medium containing Fluo-3 AM, we confirmed the functional activity of overexpressed-HEK293T cells using GHSR agonists (10 μM L-692,585) or MLNR agonist (10 μM erythromycin). For blocking the receptors, we pre-treated the GHSR antagonist (2 μM cotistatin-8) in the HEK293T cells before applying agonists or test components.

2.6. Measurement of intracellular cAMP

Cells transfected with rat 5-HT4R were washed twice, and 1 mL of 5 mM theophylline was added and incubated for 15 min at 37 °C to inhibit 3′,5′-cyclic-AMP phosphodiesterases. Afterward, a final concentration of 10 μM naringin or naringenin were added and incubated for another 15 min at 37 °C. Media were then quickly removed and the cells were washed twice. Cell lysates were obtained by applying 0.1 N HCl, followed by 20 min incubation at room temperature. The cells were then scraped, put into microtubes, and were centrifuged for 10 min at 1000*g*. After centrifugation, supernatants were obtained and cAMP measurement was performed using a cyclic-AMP EIA kit (Cayman Chemical, Ann. Arbor, MI) following the manufacturer's protocol. Specifically, cAMP levels were obtained by the acetylation method and were normalized by the total protein amount, which was measured by the standard BCA assay method.

2.7. Patch-clamp recording

HEK293T cells were used to record membrane currents and potentials. Resistance of glass pipettes was about 3 MΩ. After forming a whole cell by gentle suction, transient currents were recorded using a patch-clamp amplifier and were filtered at 2 kHz. The output of the amplifier was digitized using a Digidata 1440 (Molecular Devices). The pipette solution contained 118.5 mM KCl, 10 mM HEPES, 2 mM $MgCl_2$. The bath solution contained 140 mM NaCl, 10 mM HEPES, 5 mM KCl, 2 mM $MgCl_2$, and 2 mM $CaCl_2$.

2.8. Statistical analysis

All data are presented as the mean ± standard error (SEM). Significances of differences were determined by the Student *t*-test or one-way ANOVA with a Tukey's post hoc test. Differences were regarded as significant when the *p*-values were less than 0.05.

3. Results

3.1. PF-W activates ghrelin receptor (GHSR), but not motilin receptor (MLNR)

To investigate whether PF-W activates motilin or ghrelin receptors, HEK293T cells expressing these receptors were prepared, and changes of $[Ca^{2+}]_i$ were measured after application of appropriate agonists. The treatment of erythromycin (10 μM) – an agonist for motilin receptor (MLNR) – markedly increased $[Ca^{2+}]_i$ in MLNR-expressing HEK293T cells (Fig. 1A). As expected, the erythromycin-induced responses were not observed in mock-transfected cells (Fig. 1C). Likewise, treatment of PF-W (1 mg/ml) on MLNR-expressing cells did not evoke any increase in $[Ca^{2+}]_i$ (Fig. 1B), indicating that there are no active components in PF-W that can stimulate MLNR. Therefore, it was concluded that PF-W does not include any constituent that can activate MLNR.

A similar approach has been made with the ghrelin receptor (GHSR) as well. The activity of GHSR was clearly verified, as a GHSR agonist (L-692,585; 10 μM) markedly increased $[Ca^{2+}]_i$ in GHSR-expressing HEK293T cells (data not shown). Unlike cases of MLNR, however, PF-W also induced a strong increase of $[Ca^{2+}]_i$ in GHSR-expressing cells (Fig. 2A), while mock-transfected cells lacked any response to PF-W (Fig. 2A). To further verify if the observed response was indeed mediated by GHSR, cortistatin-8 (CST-8), a synthetic octapeptide that acts as a specific GHSR antagonist, was used to pretreat in the GHSR-expressing HEK293T cells and PF-W was applied thereafter (Sibilia et al., 2006). As shown in Fig. 2B, CST-8 (2 μM) pretreatment dramatically inhibited the stimulating effect of PF-W, suggesting that the PF-W-induced $[Ca^{2+}]_i$ responses were indeed mediated by GHSR. Notably, CST-8 pretreatment alone did not evoke any $[Ca^{2+}]_i$ changes

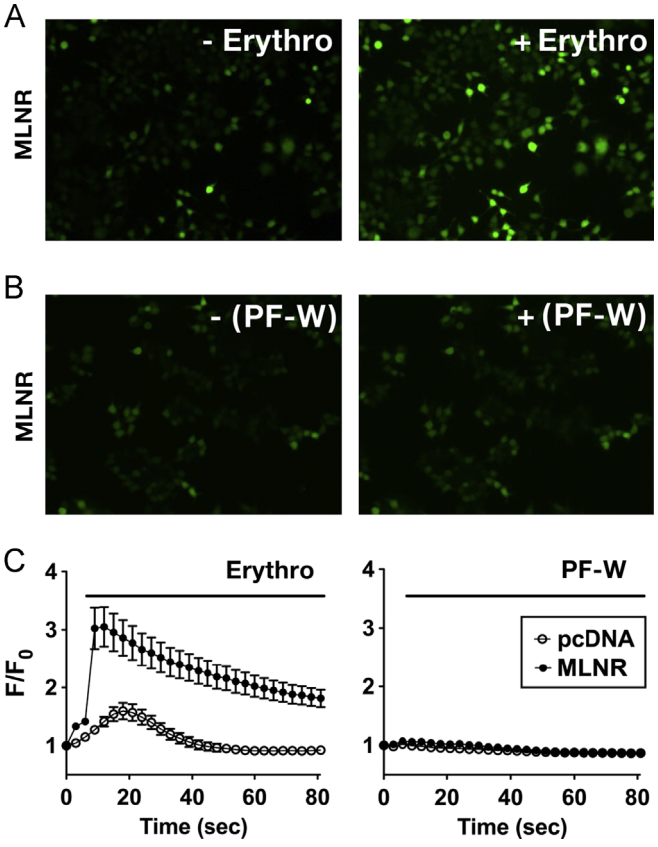


Fig. 1. PF-W does not activate MLNR. (A) Representative Ca^{2+}-imaging data from MLNR-expressing cells. The addition of an MLNR agonist erythromycin (right, "+Erythro", 10 μM) successfully induced Ca^{2+} influx when compared to the state before erythromycin treatment (left, "−Erythro"). (B) The application of PF-W (right, "+(PF-W)", 1 mg/ml) on MLNR-expressing cells did not evoke any change compared with the state before PF-W treatment (left, "−(PF-W)") (C) Summary of Ca^{2+}-imaging on MLNR-expressing cells. Notice that the erythromycin-induced response was not found in mock-transfected (pcDNA) cells (left, empty circle, $n=20$) unlike in MLNR-expressing cells (left, filled circle, $n=20$). The PF-W-induced responses were not observed both in MLNR-expressing (right, filled circle, $n=20$) or mock-transfected (pcDNA) cells (left, empty circle, $n=20$).

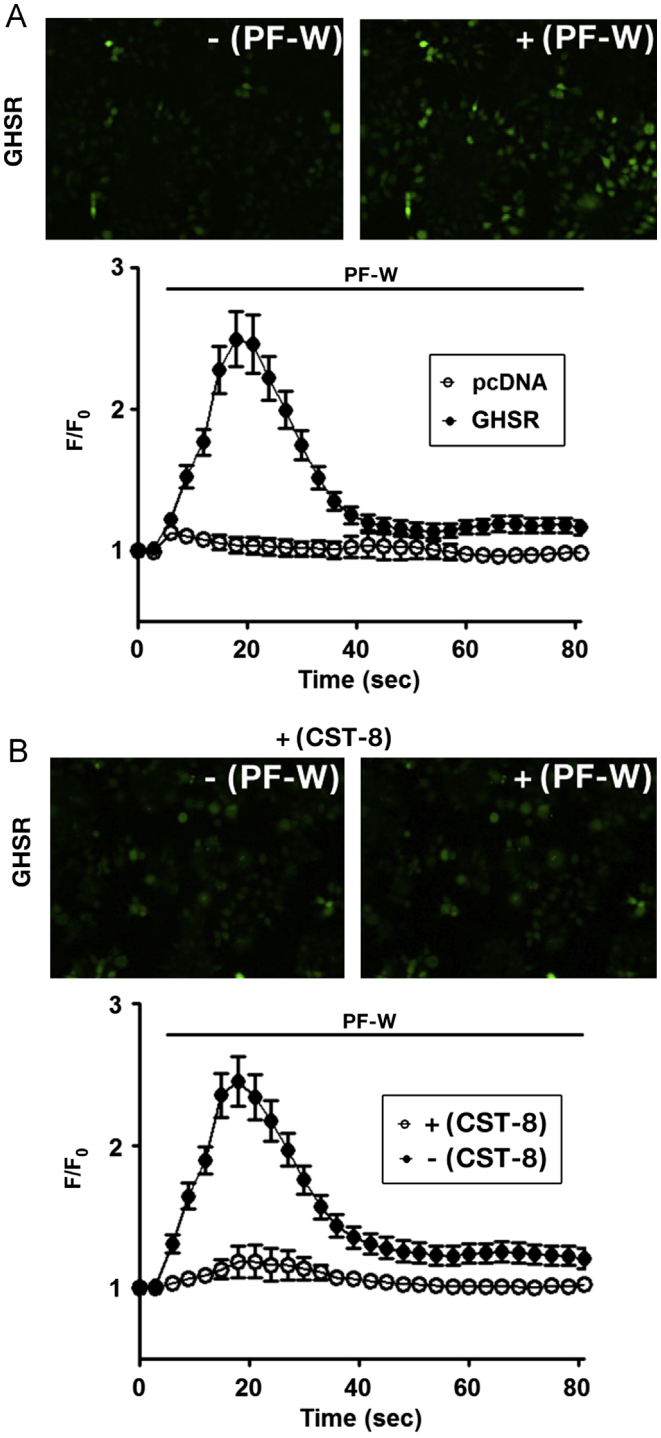


Fig. 2. PF-W specifically activates GHSR. (A) Representative Ca^{2+}-imaging data from GHSR-expressing cells before (top-left, "−(PF-W)") and after PF-W treatment (top-right, "+(PF-W)", 1 mg/ml). The treatment of PF-W (1 mg/ml) rapidly evoked transient Ca^{2+} influx in GHSR-expressing cells (bottom, filled circle, $n=36$) but not in mock-transfected (pcDNA) cells (bottom, empty circle, $n=20$). (B) The pretreatment of a GHSR antagonist CST-8 ("+(CST-8)", 2 μM), however, dramatically inhibited PF-W-induced Ca^{2+} influx (bottom, empty circle $n=20$).

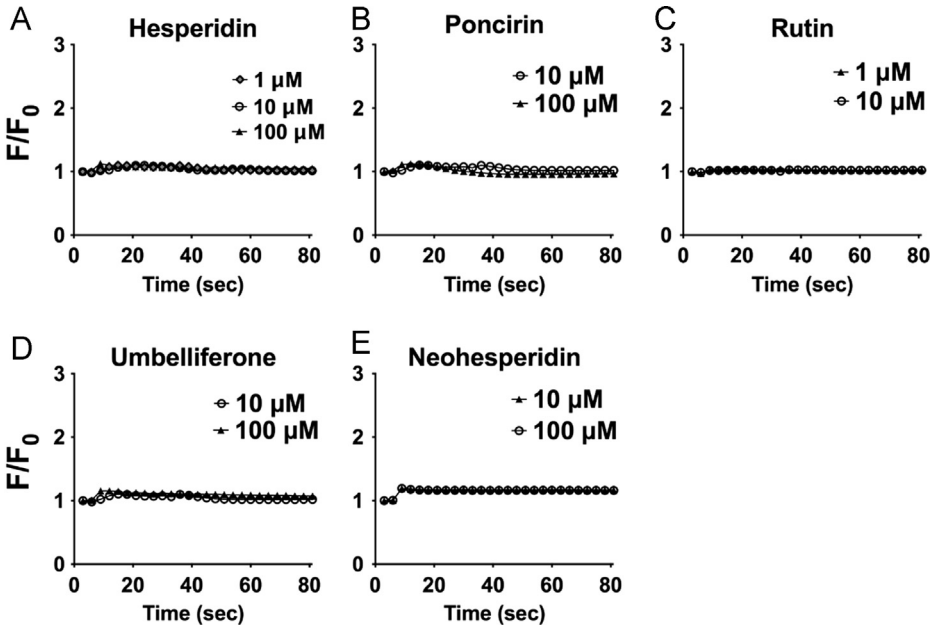


Fig. 3. The effect of various PF-W components: The effect of hesperidin (A), poncirin (B), rutin (C), umbelliferone (D), and neohesperidin (E) on GHSR-expressing cells. Various concentration ranges (1–100 μM) of these constituents failed to induce intracellular Ca^{2+} influx. $n=8$–30.

(data not shown). Taken together, it was found that PF-W contains components that specifically activate GHSR but not MLNR.

3.2. Naringin and naringenin are PF components that activate GHSR

We next asked which component of PF-W affects the activation of GHSR. To discover the active ingredient, 11 representative compounds that are known to constitute *Poncirus fructus* – namely hesperidin, neohesperidin, poncirin, rutin, umbelliferone, limonene, synephrine, linalool, citral, naringin, and naringenin (Kim et al., 2007; Lee et al., 2005b, 2009; Nizamutdinova et al., 2008; Park et al., 2005; Rajkumar and Jebanesan, 2008) – were treated on the GHSR-expressing HEK293T cells. As a result, it turned out that the treatment of hesperidin, poncirin, rutin, umbelliferone, and neohesperidin failed to evoke any $[Ca^{2+}]_i$ responses, even in higher concentrations (1–100 μM, Fig. 3). Conversely, 100 μM of limonene, synephrine, linalool, and citral moderately augmented $[Ca^{2+}]_i$ (Fig. 4). However, similar increases were also observed for mock-transfected HEK293T cells, suggesting that the observed increases of $[Ca^{2+}]_i$ were not GHSR-specific responses. We speculate that these compounds triggered yet unknown Ca^{2+} signals such as TRP channels that are endogenously present in the HEK293T cells (Jang et al., 2012b).

Among 11 ingredients, however, it was found that only naringin (Fig. 5) and naringenin (Fig. 5C) induced a strong $[Ca^{2+}]_i$ increase in GHSR-expressing cells (Fig. 5B and D). Moreover, naringin- or naringenin-induced Ca^{2+} mobilization was abolished by pretreatment of CST-8 (2 μM), suggesting that the detected responses were specifically mediated through GHSR (Fig. 5B and D). Taken together, it was concluded that only naringin and naringenin among the tested 11 constituents specifically can activate GHSR.

3.3. Naringin and naringenin generated inward currents in GHSR-expressing cells

To further investigate the putative activity of naringin/naringenin on GHSR, a whole-cell patch-clamp technique was applied and the electrical flow was measured. L-692,585, a GHSR agonist, was applied under whole-cell configuration and changes were recorded. As shown in Fig. 6, 10 μM of L-692,585 slowly evoked an

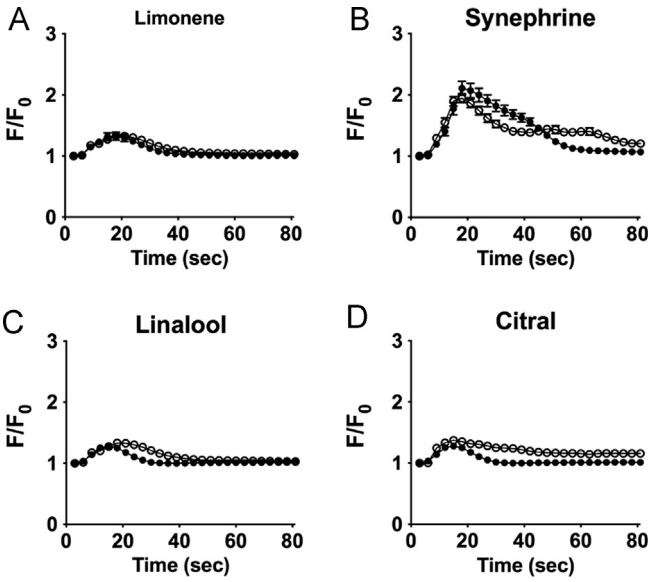


Fig. 4. The effect of other PF-W components: The effect of limonene (A), synephrine (B), linalool (C), and citral (D) on GHSR-expressing cells (filled circles, 100 μM). Although these constituents evoked similar Ca^{2+} influx in GHSR-expressing cells, the responses were also observed in mock-transfected cells (pcDNA, empty circles), indicating that the responses were not specific to GHSR. $n=12$–30.

inward current in the GHSR-expressing cells (264.0 ± 76.27 pA, $n=6$) but not in mock-transfected cells (13.50 ± 6.443 pA, $n=6$). Although the underlying mechanism of the inward current is not clear at the moment, it could be the result of $G\alpha_{q/11}$ activation, probably through the IP_3-dependent increase of intracellular calcium. Once the inward current induced by GHSR activation was confirmed, it was further verified whether naringin and/or naringenin could activate GHSR as well. As a result, both naringin (10 μM, 148.7 ± 30.90 pA, $n=6$) and naringenin (10 μM, 125.8 ± 39.51 nA, $n=6$) successfully induced inward currents in GHSR-expressing cells, but not in mock-transfected cells (Fig. 6B C, $n=6$). Therefore, it was re-verified that both naringin and naringenin can specifically activate GHSR.

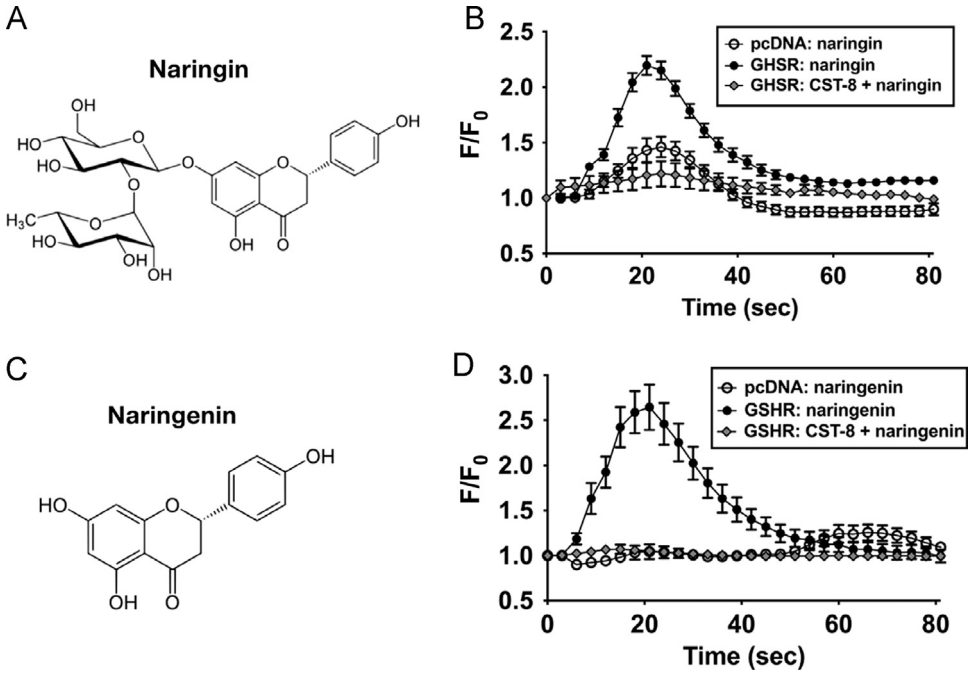


Fig. 5. Naringin and naringenin specifically activate GHSR. The application of naringin (A, 10 μM) on GHSR-expressing cells markedly increased intracellular Ca^{2+} (B, empty circle, $n=50$), but not in mock-transfected cells (B, filled black circle, $n=39$). Moreover, the effect was remarkably inhibited by pretreatment of CST-8 (2 μM) (B, gray diamond, $n=20$), suggesting that naringin specifically activates GHSR. Similarly, naringenin (C, 10 μM) also specifically activated GHSR, where GHSR-expressing cells showed profound responses (D, empty circle, $n=20$), but neither in mock-transfected cells (D, filled black circle, $n=20$) nor CST-8 pre-treated cells (D, gray diamond, $n=20$).

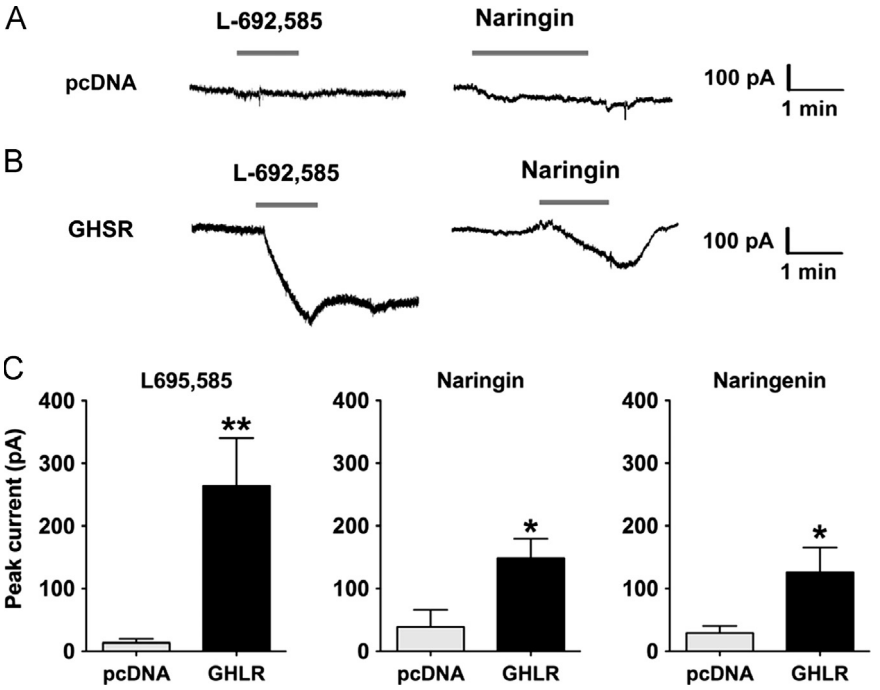


Fig. 6. Naringin and naringenin induced inward currents in GHSR-expressing cells in whole-cell configuration. The holding potential was 60 mV. In mock-transfected cells, neither L-692,585 (a GHSR agonist, 10 μM, $n=6$) nor naringin (10 μM, $n=6$) induced any noticeable changes (A). On the other hand, in GHSR-expressing cells, both L-692,585 ($n=6$) and naringin ($n=6$) robustly induced inward currents (B). Peak currents induced by L-692,585 (C, left), naringin (C, middle), and naringenin (C, right, $n=6$) were plotted.

3.4. Naringin and naringenin do not increase cAMP levels in cells expressing 5-HT4R

Meanwhile, there was a previous report that PF-W induces prokinetic activity by stimulating the 5-HT4R pathway (Shim et al., 2010). However, there was no mention which component is responsible for the 5-HT4R-mediated responses (Shim et al., 2010). Thus, it was investigated whether naringin and naringenin could be the ingredients that can also stimulate 5-HT4R. Because 5-HT4R is a GPCR coupled with $G\alpha_s$, its activation leads to an increase of cyclic-AMP (cAMP) (Blondel et al., 1997). Hence, the ELISA assay was used to measure cAMP levels. As shown in Fig. 7, 1 μM of cisapride (an agonist of 5-HT4R) treatment remarkably increased the intracellular level of cAMP (27.42 ± 2.80 pmol/mg

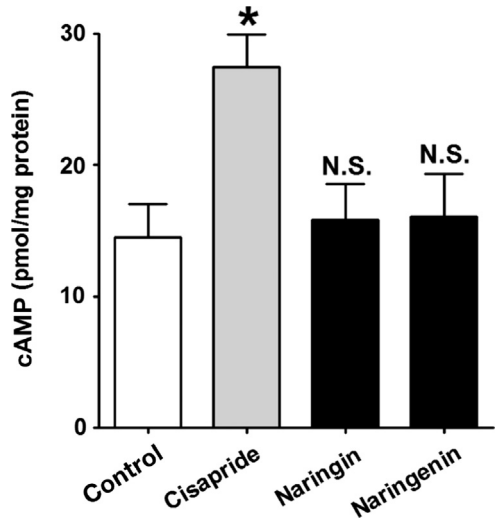


Fig. 7. Naringin and naringenin did not increase cAMP levels in cells expressing 5-HT4R. Although a 5-HT4R agonist, cisapride (1 μM, $n=5$) remarkably increased cAMP, both naringin (10 μM, $n=5$) and naringenin (20 μM, $n=5$) failed to increase cAMP levels, which were similar to control groups ($n=5$). $^*p<0.05$, N.S. not significantly different.

protein, $n=5$) on cells expressing 5-HT4R, whereas mock-transfected cells did not produce the cAMP increase (14.46 ± 2.89 pmol/mg protein, $n=5$). Likewise, it turned out that treatment of both naringin (10 μM, 15.84 ± 3.03 pmol/mg protein, $n=5$) and naringenin (20 μM, 16.10 ± 3.56 pmo/mg protein, $n=5$) failed to increase cAMP levels on cells expressing 5-HT4R. Therefore, it was found that both naringin and naringenin are compounds that cannot activate 5-HT4R.

4. Discussion

The present study revealed that PF-W contains components that can specifically activate ghrelin receptor (GHSR). Moreover, it was also found that naringin and naringenin, both of which are chemical components in *Poncirus fructus*, are agonists for GHSR, resulting in an increase of intracellular calcium levels and inward currents under whole-cell patch clamp configuration. We believe that this is the first report in which naringin and naringenin are agonists for GHSR.

It is sometimes difficult to conclude that a certain material is an agonist of a particular receptor. For instance, it was once believed that adenosine is an agonist of GHSR as well, based on findings from a calcium mobilization technique similar to the present study (Johansson et al., 2005). However, this finding was reversed in a year later that adenosine is not a GHSR agonist (Carreira et al., 2006). The misinterpretation came from the fact that GHSR antagonists such as CST-8 were not used in the experiments for verification (Carreira et al., 2006). Since all of our GHSR-activated responses were successfully inhibited by CST-8 (Figs. 2 and 5), it is highly likely that PF-W/naringin/naringenin do indeed act as agonists for GHSR.

Naringin is a natural flavonone glycoside that is abundantly present in the crude extract of PF (Kim et al., 2007). Naringenin, as its name implies, is an aglycone of naringin. Naringin and naringenin are compounds that are widely present in diverse citrus fruits, with various beneficial effects such as neuroprotective, antitussive, or antibacterial activities (Benavente-Garcia and Castillo, 2008; Gao et al., 2011; Raasmaja et al., 2013; Ross and Kasum, 2002). Since the present study revealed that naringin and naringenin activate GHSR, a receptor that enhances intestinal motility when activated, it is further expected that these compounds also have a prokinetic activity. Considering that both naringin and naringenin already have a strong anti-ulcer effect (Martin et al., 1994; Parmar, 1983), naringin and naringenin could be used as agents for improving various gastrointestinal disorders.

Gastrointestinal motility is a complex process, which is controlled by the intricate coordination of various molecular partners around neuromuscular segments in the alimentary tract. It is not surprising, therefore, that there are numerous prokinetic mechanisms that can enhance the intestinal motility. Among these mechanisms; one popular example would be the 5-HT4R, where its activation leads to enhanced intestinal motility by strong contraction of the smooth muscles in the GI tract (Sikander et al., 2009). Indeed, many clinically available prokinetic agents such as mosapride take advantage of this mechanism (Seto et al., 2011). In this regard, it is natural that PF-W also contains a fraction that can activate the 5-HT4R pathway (Shim et al., 2010). However, a single key component that can activate 5-HT4R was not revealed in the report (Shim et al., 2010). Therefore, the possibility that naringin and naringenin can activate 5-HT4R was taken into consideration in the present study. However, it turned out that both naringin and naringenin failed to activate 5-HT4R (Fig. 7). Thus, the present study clarified that constituents other than naringin/naringenin are present in PF-W that activates 5-HT4R. It means that at least two distinct prokinetic mechanisms – 5-HT4R and GHSR – could be activated by PF-W. Such a dual action could be an answer for the strong prokinetic action of PF-W.

Another molecular mechanism involved in the intestinal motility is the D_2 receptor in the dopaminergic pathway (Glavin and Szabo, 1990). As opposed to the 5-HT4R mechanism, *inhibition* of the D_2 receptor leads to increased gastrointestinal motility (Reddymasu et al., 2007). Although it has been speculated that PF-W does not seem to be involved in the D_2 pathway (Lee et al., 2005a), the possibility cannot be ruled out. Indeed, we are planning to verify the action of PF-W on D_2 receptor in the future.

As mentioned in the introduction, motilin and ghrelin are endogenous hormones essentially involved in the intestinal motility (Peeters, 2006). Not only the hormones share similarity in amino acid sequence (21% identity) (De Smet et al., 2009), their respective receptors – MLNR and GHSR – also show a noticeable amino acid sequence identity (44%), rising up to 87% in the membrane-spanning regions (De Smet et al., 2009). However, there is a huge difference between motilin and ghrelin system. That is, motilin and MLNR genes that are present in humans are *absent* in mice and rats. Indeed, *in silico* study revealed that motilin and MLNR genes are present in a form of pseudogenes in rodents (He et al., 2010). Since rats are widely used as *in vivo* animal model to test prokinetic activity, it should be considered that the activity through motilin and/or MLNR could not be evaluated in rats. In this regard, the technique used in the present study – the calcium imaging with cells expressing MLNR cDNA – could be an easy and appropriate system to screen compounds that may affect motilin and/or MLNR. Although it turned out that PF-W does not have any components that can activate MLNR in the present study, we believe that the *in vitro* system used in the current study is valuable enough for other motilin and MLNR study.

5. Conclusions

The present study revealed that PF-W specifically activated GHSR, but not MLNR. Furthermore, naringin and naringenin, components present in PF, also specifically stimulated GHSR but not MLNR. We believe that this is the first report that naringin/naringenin are compounds that can activate GHSR. Since GHSR is a

receptor responsible for the gastrointestinal motility, the present finding warrants further investigation whether naringin/naringenin could enhance gastrointestinal motility *in vivo*.

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