INTRODUCTION

The esophagus is a muscular tube that connects the back of the pharynx to the top of the stomach and ranges from approximately 18-25 cm in length, and 1-2 cm in diameter. The muscles in the upper portion of the esophagus, also called the upper esophageal sphincter (UES), are under voluntary control. The lower esophageal sphincter (LES) is a bundle of muscles at the low end of the esophagus, where it connects the stomach. When the LES is closed, it prevents acid and stomach content reflux. The LES consists of smooth muscle like the rest of the digestive tract. Smooth muscle in the gastrointestinal tract is controlled by the autonomic nervous system. Contraction or relaxation of smooth muscle has a key role in gastrointestinal motility. Digestive motility disorders can lead to impaired peristalsis resulting in slow contractions, rapid contractions, or combination of both slow and fast contractions (Vantrappen et al., 1986).

P2 receptors are membrane-bound receptors for extracellular nucleotides such as ATP and UTP. P2 receptors have been classified as ligand-gated ion channels or P2X receptors and G protein-coupled P2Y receptors. Recently, purinergic signaling has begun to attract attention as a potential therapeutic target for a variety of diseases especially associated with gastroenterology. This study determined the ATP and UTP-induced receptor signaling mechanism in feline esophageal contraction. Contraction of dispersed feline esophageal smooth muscle cells was measured by scanning micrometry. Phosphorylation of MLC$_{\text{iso}}$ was determined by western blot analysis. ATP and UTP elicited maximum esophageal contraction at 30 s and 10 $\mu$M concentration. Contraction of dispersed cells treated with 10 $\mu$M ATP was inhibited by nifedipine. However, contraction induced by 0.1 $\mu$M ATP, 0.1 $\mu$M UTP and 10 $\mu$M UTP was decreased by U73122, chelerythrine, ML-9, PTX and GDP$_{\beta}$S. Contraction induced by 0.1 $\mu$M ATP and UTP was inhibited by Go$_i$ or Go$_q$ antibodies and by PLC$_{\beta}$ or PLC$_{\beta}$ antibodies. Phosphorylated MLC$_{\text{iso}}$ was increased by ATP and UTP treatment. In conclusion, esophageal contraction induced by ATP and UTP was preferentially mediated by P2Y receptors coupled to Go$_i$ and Go$_q$ proteins, which activate PLC$_{\beta}$ and PLC$_{\beta}$. Subsequently, increased intracellular Ca$^{2+}$ and activated PKC triggered stimulation of MLC kinase and inhibition of MLC phosphatase. Finally, increased pMLC$_{\text{iso}}$ generated esophageal contraction.

Keywords: ATP, contraction, Esophagus, P2Y receptor, UTP
variety of diseases (Burnstock, 2006). Exploratory studies fo-
cused on purinergic receptors as the future therapeutic targets
of GI diseases (Burnstock, 2008; Yangou et al., 2001).

It was previously demonstrated that both P2X and P2Y re-
cipients exist in esophageal smooth muscle and mediate
esophageal contraction (Cho et al., 2010). However, the de-
tailed signaling mechanism of ATP- and UTP- induced contrac-
tion via P2X and P2Y receptors in feline esophageal smooth
muscle has not been studied. Understanding the signaling
mechanism of purinergic receptors on esophagus can contrib-
ute to the treatment of esophageal diseases. In addition, coex-
istence of P2X and P2Y receptors raises the question of which
receptor subtype preferentially mediates the action of the en-
dogenous ligand. The purpose of this study was to investigate
which receptor preferentially induces contraction activated by
ATP and UTP and the signaling mechanism involved in feline
esophageal smooth muscle cells. The nucleotides ATP and
UTP were utilized to activate P2 receptors and to identify pref-
errentially activated receptor and the signaling pathways. Selective
G-protein antibody was utilized to identify the coupling of
specific G-proteins to effector enzymes, and selective inhibitors
were used to characterize the pathways involved in MLC20 (20
kDa, regulatory light chain of Myosin II) phosphorylation and esophageal
smooth muscle cell contraction.

MATERIALS AND METHODS

Materials and reagents

G protein antibodies (Gα11, Gαq, Gα15, Gαq, Gαq, and Gq) and
PLC antibodies (β1, β3, γ1) from Santa Cruz Biotechnology
(USA); Chelerythrine chloride from Research Biochemicals
(USA); goat anti-rabbit IgG-HRP from Bethyl Laboratories Inc.
(USA); rainbow molecular weight marker from Amersham
(USA); enhanced chemiluminescence (ECL) agents from
PerkinElmer Life Sciences (USA); sodium dodecyl sulfate
(SDS) sample buffer from Owl scientific Inc. (USA); nitrocellu-
lose membrane, Tris/Glycine/SDS buffer and Tris/Glycine buffer
from BioRad (USA); phosphate-buffered saline (PBS) from
Roche Diagnostics Co. (USA); RestoreTM Western Blot Strip-
ing Buffer from Pierce (USA); and 4-(2-hydroxyethyl)-1-
piperazine-N'-2-ethane sulfonic acid (HEPES), collagenase
type F, ammonium persulfate, ponceau S, bovine serum albu-
mun (BSA), leupeptin, aprotinin, β-mercaptoethanol, N,N,N',
tetramethylethylenediamine (TEMED), ethylene glycol-bis-(β-
aminoethyl ether)-N,N',N'-tetraacetic acid (EGTA), ethylene-
diaminetetraacetic acid (EDTA), phenylmethyl-sulfonylfluoride
(PMSF) and other reagents from Sigma Chemical Co. (USA).

Tissue dissection and dispersion of smooth muscle cells

Adult male cats weighing between 3 and 5 kg were anesthe-
tized and esophageal smooth muscle tissue was prepared as
previously described (Biancaniet al., 1987; Murthy and Makhlof,
1998; Nam et al., 2013). The tissue was digested overnight
maintaining a temperature of 4°C with HEPES-buffered solution
that contained 1 mg/ml papain, 1 mM diithiothreitol, 1 mg/ml BSA
and 0.5 mg/ml collagenase (type F, Sigma). The HEPES-
buffered solution contained 1 mM CaCl2, 250 μM EDTA, 10 mM
glucose, 10 mM HEPES, 4 mM KCl, 131 mM NaCl, 1 mM
MgCl2 and 10 mM taurine.

Next day, the tissue was warmed in HEPES-buffered solution
at room temperature (15-20°C) for 30 min and then heated in a
water bath at 31°C for 30 min. After heating, the digested tissue
was poured out over a 400 μm nylon mesh, rinsed in colla-
genase-free HEPES buffer to remove any trace of collagenase
and then incubated in this solution at 31°C, which was gassed
with 95% O2-5% CO2. The cells were allowed to dissociate
freely for 10 to 20 min. Before beginning the experiment, the
cells were kept at 31°C for at least 10 min to relax the cells.
Throughout the procedure, care was taken not to agitate the
fluid in order to avoid cell contraction in response to mechanical
stress.

The experiments were performed in accordance with the
guidelines of the Institutional Animal Care and Use Committee
of Chung-Ang University (No.14-0045).

Preparation of permeabilized smooth muscle cells

Cells were permeabilized, when required, to diffuse agents
such as G protein antibodies and PLC isozyme antibodies,
which do not diffuse across intact cell membrane. The process
of preparation of permeabilized cells did not affect cell contrac-
tion (Cao et al., 2001; Horowitz et al., 1996; Murthy et al., 2003;
Shim et al., 2002;Sohn et al., 1997). After completion of the
enzymatic phase of the digestion process, the partly digested
muscle tissue was washed with an enzyme-free cytosolic buffer
of the following composition: 20 mM NaCl, 100 mM KCl, 5.0
mM MgSO4, 0.96 mM NaH2PO4, 1.0 mM EGTA and 0.48 mM
CaCl2 and 2% bovine serum albumin. The cytosolic buffer
was equilibrated with 95% O2-5% CO2 to maintain pH 7.2 at
31°C. The muscle cells were dispersed spontaneously in this
medium. The cytosolic buffer contained 0.48 mM CaCl2 and 1 mM
EGTA, yielding 0.18 mM free Ca2+ (Fabio and Fabio, 1979).

After dispersion, the cells were permeabilized by incubation
for 5 min in cytosolic buffer containing saponin (75 μg/ml). After
exposure to saponin, the cell suspension was spun at 350 g,
and the resulting pellet was washed with saponin-free modified
cyto
cular buffer that contained antimycin A (10 μM), ATP (1.5
mM) and an ATP-regenerating system that consisted of crea-
tine phosphate (5 mM) and creatine phosphokinase (10
units/ml) (Bitar et al., 1986). The procedure was repeated twice
to ensure complete removal of saponin. After the cells were
washed free of saponin, they were resuspended in modified
cytic buffer.

Measurement of contraction by scanning micrometry

Contraction of isolated muscle cells was measured by scanning
micrometry (Murthy and Makhlof, 1998; Sohn et al., 1993;
1995b). An aliquot of cell suspension containing 106
cells/ml was added to HEPES medium containing the test agents.
The reaction was terminated by addition of acrolein (1% final
concentration). The length of 30 to 40 muscle cells treated with a
contraction was measured at random by scanning micro-
metry, with a phase contrast microscope (ULWCDC 0.30
Olympus,Japan) and digital closed-circuit video camera (CCD
color camera, Japan) connected to a Macintosh computer (Ap-
ple, USA) with a software program, NIH Image 1.57 (National
Institutes of Health, USA). It was then compared with length of
untreated cells. Contraction was expressed as the percentage
decrease of mean cell length, as compared with control group.
All measurements were done in the presence of adenosine A1
and A2 antagonists (1 mM DPCPX and 0.1 mM CGS-15943,
respectively) (Murthy et al., 1995).

Protein assays

The protein concentration of supernatant was determined by
the Bradford reagents, according to the instruction of the
manufacturer (Bio-Rad Chemical Division, USA). The ab-
sorbance was measured spectrophotometrically at a wave-
length of 595 nm.
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Fig. 1. Time course curves and concentration-response curves of contractile response induced by ATP and UTP in dispersed feline esophageal smooth muscle cells. (A) Time course curve of contraction in response to 1 μM ATP. The response was detected at 10, 20, 30, 45, 60, 120 and 300 s. (B) Time course curve of contraction in response to 1 μM UTP. (C) ATP-induced contraction of dispersed smooth muscle cells was measured at 30 s after treatment of ATP in different concentration (10^-6, 10^-5.5, 10^-5, 10^-4.5, 10^-4, 10^-3.5, 10^-3 M). (D) Concentration-response curve induced by UTP was measured at 30 s after ATP treatment at different concentrations (10^-5 to 10^-3 M). Muscle cell contraction was measured by scanning micrometry and expressed as percent decrease in cell length from control. Data are expressed as the mean ± S.E.M (n = 5).

Western blot analysis
Dispersed muscle cells isolated from the esophagus were resuspended in DMEM, containing penicillin (100 unit/ml), streptomycin (0.1 mg/ml), amphotericin-b (0.25 μg/ml) and 10% fetal bovine serum (FBS). The muscle cells were plated at a concentration of 10^4 cells/ml and incubated at 37°C in a CO2 incubator. DMEM-10 medium was replaced every 3 days for 2-3 weeks until confluence was attained. All experiments were done on cells in the second passage (Murthy et al., 2003).

Phosphorylated MLC20 was determined by immunoblot analysis using a phospho-specific antibody (Huang et al., 2005; Murthy et al., 2003). Previously frozen samples of dispersed muscle cells were homogenized in a buffer containing 20 mM Tris-HCl (pH 7.4), 0.5 mM EDTA, 0.5 mM EGTA, 1% (w/v) Triton X-100, 0.01% (w/v) SDS, 10 μg/ml leupeptin, 10 μg/ml aprotinin, 1 mM PMSF, phosphatase inhibitor cocktail-3 10 μg/ml and β-mercaptoethanol 0.7 μg/ml. Samples of the homogenates were then centrifuged for 10 min at 4°C, and the supernatants collected. Equal amounts of proteins from each sample were resolved on an SDS-polyacrylamide gel by electrophoresis. Prestained molecular mass marker in loading buffer: 25 mM Tris (pH 8.3), 192 mM glycine and 0.1% SDS, was also run in an adjacent lane to permit molecular mass determination using a power supply (Power Pac 1000, Bio-Rad, USA). The separated proteins were transferred to 0.45 μm nitrocellulose membrane in transfer buffer: 25 mM Tris (pH 8.3), 192 mM glycine and 0.1% SDS, was also run in an adjacent lane to permit molecular mass determination using a power supply (Power Pac 1000, Bio-Rad, USA). The separated proteins were transferred to 0.45 μm nitrocellulose membrane in transfer buffer: 25 mM Tris (pH 8.3), 192 mM glycine and 20% (v/v) methanol, using a power supply (Power Pac 1000, Bio-Rad, USA). To confirm uniformity of gel loading, blots were stained with Ponceau S. After confirmation, the membrane was washed twice for 5 min using TBST and incubated with horseradish peroxidase-conjugated secondary antibody (1:5,000 dilution) for 1 h at room temperature. The immunoreactive bands, detected by enhanced chemiluminescence agents (ECL, Perkin Elmer, USA), were developed by X-ray film developer and fixer. Developed films from ECL were scanned and analyzed densitometrically using Scion Image. Phosphorylation of MLC20 was calculated as the ratio of phosphorylated MLC20 to total MLC20 (Cao et al., 2001; Ijzer et al., 2009; Nam et al., 2013). All measurements were done in the presence of adenosine A1 and A2 antagonists (1 mM DPCPX and 0.1 mM CGS-15943, respectively) (Murthy et al., 1995).

Analysis of data
The results were expressed as mean ± S.E.M. of n experiments. P values were determined by one-way ANOVA with post-hoc Tukey HSD (Honest Significant Differences) using GraphPad PRISM (GraphPad Software, USA). Each experiment was done on cells obtained from different animals. Values were considered statistically significant when P value < 0.05.

RESULTS

Contraction induced by ATP and UTP in dispersed smooth muscle cells
Dispersed feline esophageal smooth muscle cells were treated for 10, 20, 30, 45, 60, 120, and 300 s with 1 μM ATP or UTP. Exposure of dispersed smooth muscle cells to 1 μM ATP caused immediate contraction that significantly increased until 30 s. Maximal contraction was attained at 30 s followed by a decline to lower levels. The contraction almost disappeared after 5 min (Fig. 1A).
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Fig. 2. Inhibition of ATP- and UTP-induced contraction in dispersed feline esophageal smooth muscle cells in the absence of Ca^{2+} from the medium (0 Ca^{2+} / 2 mM EGTA). Dispersed smooth muscle cells were preincubated in Ca^{2+}-free medium and then treated with 10 μM ATP, 0.1 μM ATP, 10 μM UTP, and 0.1 μM UTP for 30 s. Muscle contraction was measured by scanning micrometry. Data are expressed as means ± S.E.M (n = 4). **P < 0.01 versus control.

The time course was identical to that observed with 1 μM UTP (Fig. 1B). The maximal response at 30 s was used in concentration-response curves. Contraction of dispersed feline smooth muscle cells were measured at 30 s after treatment of 10^{-6}, 10^{-5}, 10^{-4}, 10^{-3}, 10^{-2} M ATP or UTP respectively. Treatment of ATP caused concentration-dependent contraction of dispersed feline esophageal smooth muscle cells. Each concentration of ATP was treated for a 30 s duration. The maximal peak was attained by 10^{-3} M concentration of ATP (Fig. 1C). The concentration-response curve showed the same pattern to that observed with treatment of UTP (Fig. 1D).

In calcium-free medium, contraction induced by 10^{-5} M ATP was inhibited but contraction induced by ATP 10^{-7} M or UTP was not inhibited. This result supported that contraction induced by 10^{-3} M ATP or UTP was activated by intracellular IP_{3} pathway although contraction induced by 10^{-5} M ATP depends on extracellular calcium ion (Fig. 2).

The signaling of contraction induced by ATP was altered depending on its concentration
P2X receptors are ligand-gated ionotropic channel family, especially Ca^{2+} channel, and P2Y receptors are involved in pertussis toxin-sensitive and -insensitive G proteins that regulate diverse enzymes (Akbar et al., 1996; Chang et al., 1995; Cowen et al., 1990; Dubyak and el-Mostassim, 1993; Harden et al., 1995; Lazarowski and Harden, 1994).

Dispersed smooth muscle cells were pretreated with Ca^{2+} channel blocker nifedipine 1 μM for 10 min or with pertussis toxin PTX 400 ng/ml and GDPβS 10 μM for 1 h respectively, and then treated with 10 μM or 0.1 μM ATP and UTP for 30 s. Contraction induced by 10 μM ATP were abolished by only the Ca^{2+} channel blocker, nifedipine but were not affected by pretreatment of dispersed cells with PTX, and GDPβS (Fig. 3A). In contrast, contraction induced by 0.1 μM ATP was inhibited by PTX or GDPβS but not affected by nifedipine (Fig. 3B). Contractions induced by 10 μM and 0.1 μM UTP were abolished by PTX, and GDPβS but were not affected by pretreatment of dispersed cells with nifedipine (Figs. 3C and 3D). The signaling of contraction induced by ATP was concentration-dependent. Higher concentration of ATP mediated contraction via P2X receptors. In contrast, lower concentration of ATP mediated contraction via P2Y receptors in accordance with UTP-induced contraction.

Identification of the G protein subtypes related to ATP- and UTP-induced contraction
The above experiment revealed that preferential signaling of ATP- and UTP-induced contraction was mediated by P2Y receptors. A previous study showed that G_{i1}, G_{i2}, G_{i3}, G_{i4} (40 kDa), G_{o} (40 kDa), G_{q} (42 kDa), and G_{s} (46 kDa) proteins are expressed in cat smooth muscle cells (Yang et al., 2000).
The subtypes of G proteins activated by ATP and UTP in smooth muscle were identified by contractile blockade with G protein-specific antibodies. Permeabilized feline esophageal smooth muscle cells were preincubated with specific antibodies to Go1i, Go2i, Go3i, Goq, G12i, G13i, Gq, and Gi1 for 1 h respectively, and then treated with 0.1 μM ATP or 0.1 μM UTP for 30 s. Contraction induced by 0.1 μM ATP was partially abolished by Go1i and Gi1. Treatment of 0.1 μM UTP also produced the same results as treatment of 0.1 μM ATP (Fig. 4).

The signaling pathway of contraction activated by ATP and UTP P2Y receptors involve diverse enzymes including phospholipase C (Akbar et al., 1996; Dubyak and el- Moatassim, 1993; Harden et al., 1995; Lazarowski and Harden, 1994), and protein kinase C (van der Weyden et al., 2000), which can induce activation of MLC kinase and inhibition of MLC phosphatase (Ikebe et al., 1987). Dispersed smooth muscle cells were pretreated with PLC inhibitor (U73122), PKC inhibitor (chelerythrine), MLC kinase inhibitor (ML-9). Dispersed smooth muscle cells were preincubated with U73122 (1 μM) and ML-9 (10 μM) for 10 min and chelerythrine (10 μM) for 1 min respectively, and then treated with (A) 0.1 μM ATP and (B) 0.1 μM UTP for 30 s. Muscle contraction was measured by scanning micrometry. Data are expressed as the mean ± S.E.M (n = 5). *P < 0.05, **P < 0.01 versus control.

ATP- and UTP-induced phosphorylation of MLC20
The primary mechanism of smooth muscle contraction is phosphorylation of the 20 kDa myosin light chain (MLC20). (Ikebe et al., 1987; Puettz et al., 2009; Webb, 2003). To verify that phosphorylation of MLC20 is related in esophageal contraction, Western blot analysis was performed to measure phosphorylated MLC20 (pMLC20) and total MLC20 (tMLC20) using specific antibodies to pMLC20 and tMLC20, respectively. Phosphorylation of pMLC20 was significantly increased in cultured cells treated with ATP and UTP, as compared to control (Fig. 7). The results suggested that both ATP and UTP mediated contraction of feline esophageal smooth muscle via phosphorylation of MLC20.

DISCUSSION
A variety of signaling mechanisms are involved in contraction or...
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relaxation of esophageal smooth muscle. However, the detailed signaling mechanism of contraction induced by P2 receptors in esophageal smooth muscle has not been studied. It was reported that the NANC neurotransmitters play a key role in the diseased condition of GI tract (Lefebvre, 1993; Matsuda and Miller, 2010). Investigation of the NANC nucleotide ATP- and UTP-induced signaling mechanism of esophageal smooth muscle contraction contributes to understanding pathophysiology of esophageal diseases.

In the present study, we found that contraction induced by 10 μM ATP was mediated by P2X receptors that can induce influx of Ca²⁺ from extracellular space. In contrast, contraction induced by 0.1 μM ATP was triggered by P2Y receptors linked to PTX-sensitive Gαq and PTX-insensitive Gqi proteins, which activate PLCβ1 & PLCβ3. This result was in concordance with the hypothesis that P2Y receptors preferentially induce esophageal smooth muscle contraction in response to ATP.

These conclusions derive from the following findings:

ATP preferentially activated P2Y receptors to elicit smooth muscle contraction

P2 receptors have been classified into 2 classes comprising P2X and P2Y receptors (Fredholm et al., 1994; 1997). P2X receptors are ligand-gated ionotropic channel family members that can induce transfer of calcium ion from extracellular space to cytoplasm (Fredholm et al., 1994; 1997). P2Y receptors are G-protein coupled receptor (GPCR) family that activate phospholipase C (PLC) (Fredholm et al., 1994; 1997; Lee et al., 2000). Previous study demonstrated that the co-existence of ligand-gated P2X and G protein-coupled P2Y receptors in freshly dispersed gastric smooth muscle cells and that ATP preferentially activates P2Y receptors to elicit Ca²⁺ mobilization and muscle contraction (Murthy and Makhlouf, 1998). Contraction induced by 10 μM ATP was abolished by only the Ca²⁺ channel blocker, nifedipine that can inhibit P2X receptor (Murthy and Makhlouf, 1998). P2Y receptors trigger release of Ca²⁺ from endoplasmic reticulum that leads to increase of calcium concentration. From our findings intracellular calcium concentration plays a key role in smooth muscle contraction induced by P2 receptors. It seems that contraction was not inhibited by Gai antibody because increase of calcium concentration induced by P2Y receptors is negligible compared to that of P2X receptors when treated with high concentration of ATP. In contrast, contraction induced by 0.1 μM ATP, 10 μM UTP and 0.1 μM UTP was inhibited by PTX or Gqi/S.

The ATP-induced contraction signaling was concentration-dependent. Response to UTP and lower concentration of ATP was mediated by PTX-sensitive G protein and PTX-insensitive G protein but contraction induced by higher concentration of ATP occurred via ligand-gated calcium channel. Therefore, these results suggested that the purine nucleotide, ATP released from nerves as extracellular transmitters preferentially activate P2Y receptors to elicit smooth muscle contraction.

P2Y receptors were linked to Gai, and Gqα proteins activating PLCβ, and PLCβ3

G proteins, also known as guanine nucleotide-binding proteins, are a family of proteins involved in transmitting signals from a variety of intracellular signaling such as activation of PLCβ that can cleave PIP₂ into IP3 and DAG (Gilman, 1987; Lechleiter et al., 1990; Somlyo and Somlyo, 1994). Contraction mediated by P2Y receptors was decreased by Gαi3 and Gαq specific anti-
Contraction mediated by P2Y receptors was involved in G-protein- and PKC-dependent pathway

PLC catalyses the generation of inositol triphosphate (IP3) and diacylglycerol (DAG). IP3 then binds to IP3 receptors, particularly Ca2+ channels in the smooth endoplasmic reticulum (ER). This induces increased cytoplasmic concentration of Ca2+, causing a cascade of intracellular changes and the activation of calmodulin and PLC. Subsequently, increased intracellular Ca2+ by IP3 and activated PKC by DAG triggered stimulation of MLC kinase and inhibition of MLC phosphatase, respectively. Finally, increased pMLC20 generated smooth muscle contraction of the feline esophagus.

bodies and by PLCβ1 and PLCβ3 antibodies. Thus, P2Y receptors are involved in Gq11 and Gqq proteins triggering activation of PLCβ1 and PLCβ3.

Contraction mediated by P2Y receptors was involved in IP3- and PKC-dependent pathway

P2Y receptors triggered contraction by MLC20 phosphorylation

The primary mechanism of smooth muscle contraction is phosphorylation of the 20 kDa myosin light chain (MLC20) by a MLC20 kinase that is activated by Ca-calmodulin (Ikebe et al., 1987). Relaxation, then, is primarily the result of dephosphorylation of MLC20 by MLC phosphatases (Bialojan et al., 1987; Haeberle et al., 1985). Phosphorylation of MLC20 was increased by activating P2Y receptors. Our results demonstrated that P2Y receptors modulate smooth muscle contraction through myosin light chain phosphorylation, caused by the IP3- and PKC-dependent pathways.

In conclusion, ATP- and UTP-induced contraction of feline esophageal smooth muscle cells was preferentially mediated by P2Y receptors coupled to Gq11 and Gqq proteins, which activate PLCβ1 and PLCβ3. Subsequently, increased intracellular Ca2+ by IP3 and activated PKC by DAG triggered stimulation of MLC kinase and inhibition of MLC phosphatase, respectively. Finally, increased pMLC20 generated smooth muscle contraction of the feline esophagus (Fig. 8).

In time course ATP- and UTP-induced contraction curves, the contraction completely disappeared 300 s after ATP and UTP treatment. This pattern was different from other neurotransmitters that induce sustained contraction. It can be hypothesized that P2 receptor activation is associated with lower esophageal sphincter (LES) relaxation induced by GERD or other esophageal diseases. Further research is required to investigate P2 receptor induced-signaling of contraction in esophageal smooth muscle under pathophysiological disease conditions.

In summary (Fig. 8), esophageal contraction induced by ATP and UTP was preferentially mediated by P2Y receptors coupled to Gq11 and Gqq proteins, which activate PLCβ1 and PLCβ3. Subsequently, increased intracellular Ca2+ and activated PKC triggered stimulation of MLC kinase and inhibition of MLC phosphatase, and increased pMLC20 generated esophageal contraction.

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