치의과학 석사 학위 논문

Calcium signaling of antipsychotics drug inducing xerostomia in human submandibular gland cells

타액선에서 구강건조증을 유도하는 조현병 치료제의 칼슘신호전달

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Abstract

Oral dryness is a disease that is caused by the side effects of autoimmune diseases and antibiotics, including radiation therapy and chemotherapy (Bhattarai et al., 2018). If the secretion of saliva is not smooth, it is difficult to chew or swallow the food, and it is easily exposed to various immune diseases including cavities. Human salivary glands consist of acinar cells that produce liquids and electrolytes, and duct cells that secrete the final saliva by adding various proteins (Young et al., 1987). The salivary gland cells are non-excitatory cells that do not express voltage-dependent ion channels (Moller, 2002). They are mainly regulated by the autonomic nervous system (Ambudkar, 2014; Baum et al., 1993; Melvin et al., 2005).

The neurotransmitters released from the parasympathetic nerve bind to the G-protein coupled receptors of the human submandibular gland cell membrane and cell signaling begins. Salivary glands include mainly muscarinic receptors, alpha adrenergic receptors, and neuropeptide receptors. If the function of the G-protein coupled receptor is abnormal, secretion of saliva is reduced (Lee et al., 2012; Mese and Matsuo, 2007; Proctor and Carpenter, 2007). Several drugs that cause dry mouth inhibit the function of G-protein coupled receptors (Vinayak V et al.,2013). As an example, it is already known that antidepressants regulate the signaling

of G-protein coupled receptors, but much of the effect of therapeutic drugs on the G-protein coupled receptor, an antagonist of dopamine receptors, has not been extensively studied. I found that antipsychotic drugs such as Haloperidol, a first-generation drug that is caused by xerostomia as a side effect, Hydroxyzine which is a first-generation antihistamine drug and antipsychotics drug, and Aripiprazole, Olanzapine and Risperidone, which are known to show little or no dry mouth, Binding receptor signal transduction pathway. For the first time, Haloperidol, Hydroxyzine and Olanzapine, an antipsychotics drugs, inhibit the release of calcium by muscarinic receptor stimulation (Fig2-1A,3-1A,4-1A). Secondly, Haloperidol and Hydroxyzine suppress intracellular calcium by purine receptor stimulation, but Olanzapine promotes calcium release (Fig 2-2A,3-2A,4-2A). Third, we found that Haloperidol, Hydroxyzine, and Olanzapine inhibited the action of SERCA pump in intracellular membrane of endoplasmic reticulum and decreased intracellular calcium concentration (Fig 2-3A,3-3A,4-3A). Finally, Risperidone and Aripiprazole, which are known to interact with dopamine receptors, serotonin receptors, and adrenergic and histamine receptors, do not significantly affect G-protein coupled receptors in salivary gland cells (Fig 5,6). These results suggest that Haloperidol and Hydroxyzine, which are antipsychotic drugs that cause dry mouth, not only act as antagonists of dopamine receptors in neurons but also inhibit G-protein coupled

receptor stimulation in human submandibular gland cells. As a result, we finally found that the concentration of intracellular calcium was lowered and the secretion of saliva was reduced. In addition, Risperidone and Aripiprazole, which have weak inducing effects on oral dryness, did not dramatically affect G-protein coupled receptors in human submandibular gland cells.

Key words : Dry mouth syndrome, salivary secretion, intracellular calcium, G-protein coupled receptor, salivary gland, side effects of antipsychotic

drugs

Student Number : 2011-22037

Introduction

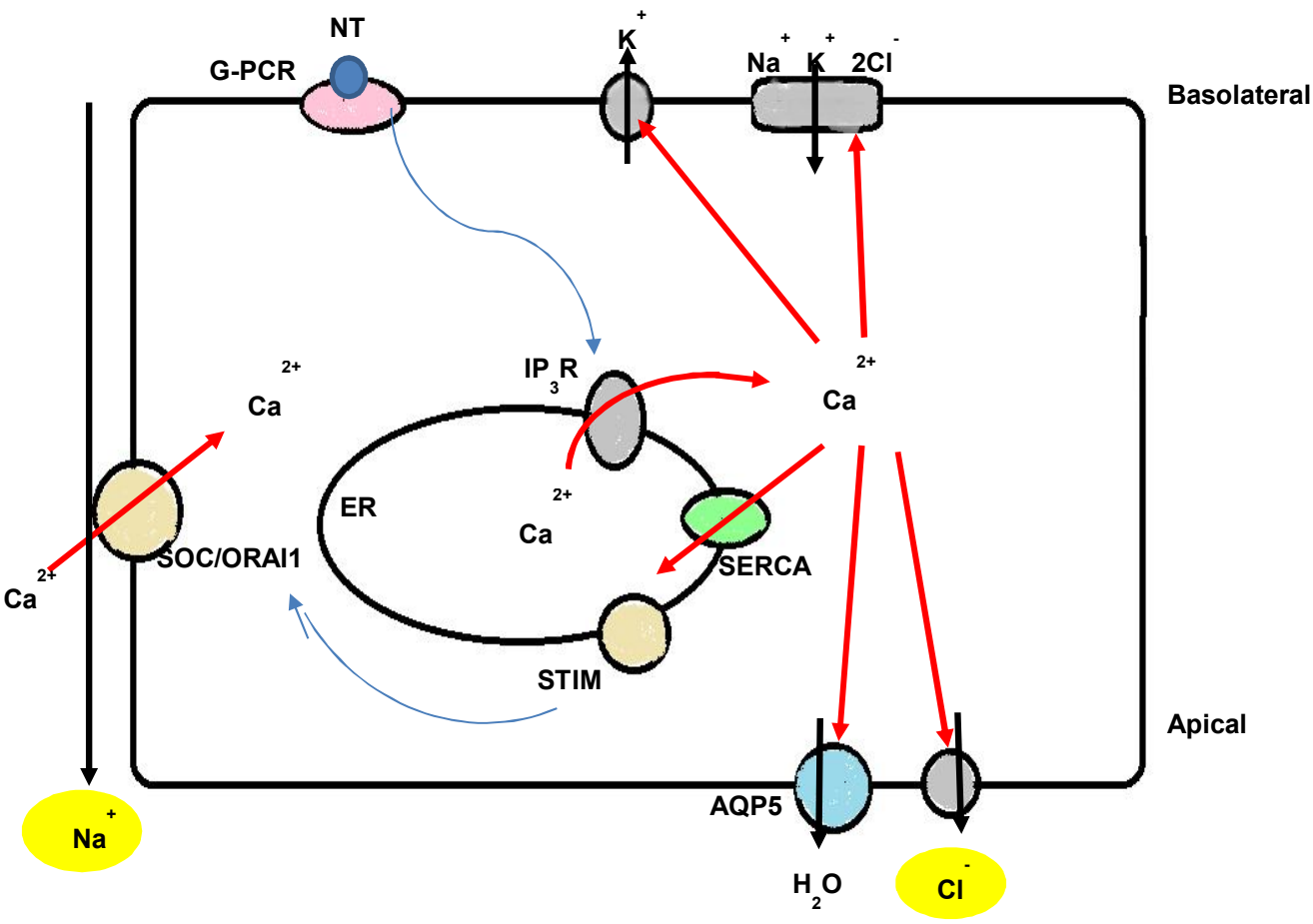


Figure 1. Dose the antipsychotic drug causing xerostomia are related in calcium signaling in HSG cells?

Introduction

Oral dryness is a disease in which the secretion of saliva is reduced. When there is no stimulation, the saliva is diagnosed when the secretion of saliva is less than 100ML for one minute. Saliva, as a mixture of protein and liquid, plays a very important role in the oral cavity (Melvin et al., 2005). If the secretion of saliva is not smooth, the mouth often dries, which makes it difficult to ingest food, talk, and other various diseases, including cavities. It consists mainly of acinar cells that produce primary saliva and ductal cells that produce secondary saliva containing multiple protein. The causes of oral dryness include radiation therapy, autoimmune diseases such as Sjogren's syndrome, metabolic diseases such as malnutrition, damage to organs associated with increased menopause or age, side effects of medications have (Bhattarai et al., 2018). There have been about 400-600 treatments for oral dryness to date, but the mechanism of its development has not been well known (Guggenheimer and Moore, 2003; Scully, 2003; Sreebny and Schwartz, 1997). In this paper, we investigated the calcium signaling mechanism of drugs that cause oral dryness among schizophrenia related therapeutic drugs. Schizophrenia has been reported to occur due to the excessive secretion of dopamine from the central nervous system, and prescribes drugs with high affinity for dopamine receptors (Konradi and Heckers, 2001; Sebel et al., 2017; Seeman and Lee, 1975; Shiozaki et al.,

2014). These hallucinations or delusions, which are mostly seen to be vain, to hear or believe, are classified as positive symptom, and hydroxyzine, which is mainly prescribed haloperidol and also has antihistamine effect, is also used. Thereafter, drugs were developed that can treat illnesses, negative symptoms such as maladies or attitudes that are unreliable or unreliable, and typically include olanzapine, aripiprazole, and risperidone. Haloperidol and hydroxyzine have been reported to cause oral dryness as a side effect, while olanzapine, aripiprazole, risperidone, etc. have been reported to have weak or rare dry mouth symptoms. In general, antipsychotic drugs use the antagonistic action of G-protein coupled receptors, and dopamine D2 receptors are known as superfamily of G-protein coupled receptors. When a neurotransmitter secreted from parasympathetic nerve binds to a G-protein-coupled receptor, it hydrolysis phosphoinositide and inositol 1,4,5-triphosphate (IP_3) stimulates the IP_3 receptor in ER, thereby releasing ER calcium into the cytoplasm (Ambudkar, 2014, 2016; Melvin et al., 2005). Increased calcium in the cytoplasm activates calcium-dependent ion channels and transpoters to allow entry of ions associated with the fluid secretion. As ions move in and out of the cell, electrical and chemical gradient of concentration occurs, causing water movement due to osmotic pressure, which induces fluid secretion. Here, calcium is a secondary messenger, which maintains a low concentration in the non-stimulated state and plays an important role in influencing the calcium-dependent modes

when the stimulation is given (Berridge et al., 2003; Putney, 1986). Therefore, the measurement of intracellular calcium concentration in cells can be used as an indicator of the mechanism of fluid secretion (Jimerson et al., 1979; Lidow, 2003).

Antipsychotic drugs are known to function as antagonists of dopamine D2 receptors and serotonin 5HTA receptors in neurons, and it has been reported that salivary gland cells with salivary secretion do not express this same receptor. Therefore, we investigated the role of antipsychotic drug in saliva secretion and calcium signaling. Human submandibular gland cells were used as a model for calcium experiments in salivary glands. To investigate the relationship between the muscarinic acetylcholine receptors and the purinergic P2X7 receptors in HSG cells, carbachol and ATP, which are their agonists, were used and thapsigargin, which releases ER calcium in their downstream, into the cytoplasm, was used. The antipsychotic drug was added to the HSG cells, the agonist of each receptor and the pump was treated, and the released calcium was stained with fura-2am to measure the absorbance.

Compared with vehicle, antipsychotic drugs affect which receptors are involved in calcium release.

Ⅱ. Material and Methods

Cell culture

The human submandibular gland ductal cells HSG cell line (Shirasuna et al., 1981) was grown in Modified Eagle's Medium supplemented with 10% (v/v) heat-inactivated fetal bovine serum, and 1% (v/v) penicillin (5000 U/mL) + streptomycin (5000 μg/mL). The human submandibular gland ductal cells A253 cell line (Marmary et al., 1989) was grown in Dulbecco's Modified Eagle's Medium supplemented with 10% (v/v) heat-inactivated fetal bovine serum, and 1% (v/v) penicillin (5000 U/mL) + streptomycin (5000 μg/mL). The cells were cultured in a humidified atmosphere of 95% air and 5% CO_2. The culture medium was changed every two days, and the cells were sub-cultured weekly.

Materials

Carbachol, ATP, and thapsigargin were purchased from Sigma (St. Louis, MO, USA). Fura-2 AM was obtained from Molecular Probes (Eugene, OR, USA). Dulbecco's Modified Eagle's Medium (DMEM), fetal bovine serum,

and penicillin/streptomycin were purchased from GIBCO (Grand Island, NY, USA).

Measurement calcium concentration

Cytosolic Ca^{2+} concentration ($[Ca^{2+}]_i$) was measured using the Ca^{2+}-sensing fluorescent indicator fura-2/AM as previously described (Kim et al., 2009). Briefly, HSG cells were harvested and cell suspension was incubated with serum-free DMEM medium containing fura-2/AM (4 μM) for 50 min at 37°C with continuous stirring. Then cells were washed with a Locke's solution (NaCl, 154 mM; KCl, 5.6 mM; $MgCl_2$, 1.2 mM; $CaCl_2$, 2.2 mM; HEPES, 5.0 mM; glucose, 10 mM, pH 7.4 with NaOH) twice and left at room temperature until experimental use. To prevent fura-2 leakage, organic-anion transporter inhibitor, sulfinpyrazone (250 μM) was added to all solutions (Di Virgilio et al., 1989). For measurement, 5 x 10^5 cells cells was placed in quartz cuvette, and changes in fluorescence ratio were monitored by Shimadzu RF-5301 spectrofluorophotometer (Nishinokyo, Kyoto, Japan) with dual excitation at 340 and 380 nm and emission at 500 nm (Grynkiewicz et al., 1985).

Statistics analysis

All quantitative data are expressed as means ± SEM. Differences were

determined by one-way ANOVA and considered significant when $P < 0.05$.

Ⅲ. Result

Haloperidol inhibit muscarinic and purinergic Ca^{2+} signaling mediated $[Ca^{2+}]i$ increase in human submandibular gland cells. It also inhibits thapsigargin-mediated $[Ca^{2+}]i$ increase.

Carbachol, which binds to and activates the muscarinic acetylcholine receptor (Liu et al., 1998; Nagy et al., 2007) inhibited the release of calcium in HSG cells treated with haloperidol. (Fig2-1A). The ATP - mediated calcium concentration, which acts on the P2X7 receptor and attracts monovalent cations and calcium ions into the cells, was reduced in haloperidol - treated cells (Fig 2-2A). Calcium release of haloperidol associated with thapsigargin, which serves to enhance calcium in the cytoplasm by blocking the ability to pump calcium into the cytoplasm and ER, was inhibited (Fig2-3A).

Hydroxyzine inhibits muscarinic receptors, purinergic receptors and thapsigargin-mediated intracellular calcium release in human salivary gland cells.

The first-generation antihistamine agent, hydroxyzine (*American Society of Health-System Pharmacists* 2018), which is reported to exhibit antagonism to several receptor systems in the brain, inhibits muscarinic-mediated calcium release. (Fig 3-1A). The ATP - mediated calcium concentration, which acts on the P2X7 receptor and attracts monovalent cations and calcium ions into the cells, was reduced in hydroxyzine - treated cells. (Fig 3-2A). When thapsigargin was added to HSG cells treated with hydroxyzine, the concentration of calcium in the cells decreased. (Fig 3-3A).

Olanzapine inhibits carbachol-mediated calcium release, promotes ATP-mediated calcium release, and slightly inhibits thapsigargin-mediated calcium release.

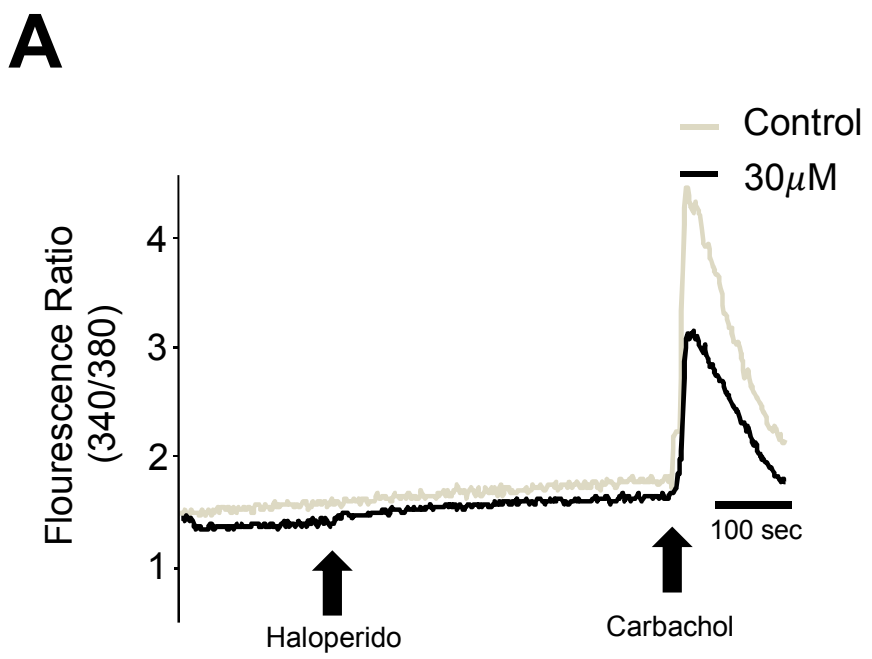
Olanzapine has been shown to be effective in the treatment of patients with recurrent schizophrenia (Harvey et al., 2016), either with or without the effects of traditional antipsychotic drugs, with positive and negative symptoms and selective antagonism to dopamine and serotonin receptors.
When carbachol was added to HSG cells treated with olanzapine, release of intracellular calcium was almost blocked.(Fig 4-A) However, when ATP was added instead of carbachol in the same way, there was no significant difference from control.(Fig 4-C). Treatment of olanzapine - treated HSG cells with thapsigargin inhibited release of intracellular calcium (Fig 4-E).

Aripiprazole does not have a significant effect on intracellular calcium concentration by muscarinic receptors, purinergic receptors and thapsigargin.

Aripiprazole is known to be a partial agonist of the dopamine D2 receptor and is known to act as an antagonist in the hypersensitivity state of dopamine and as an agonist in the underdeveloped state to enhance dopamine action (Shapiro et al., 2003). Thus, the action of dopamine and serotonin is known to help maintain proper levels. In our study, aripiprazole did not have a significant effect on muscarinic-mediated intracellular calcium release and ATP-mediated intracellular calcium release (Fig 5-A,C), and did not differ significantly in thapsigargin-mediated intracellular calcium concentration (Fig 5-E).

Risperidone has little effect on muscarinic, purinergic receptor and thapsigargin-related intracellular calcium levels.

Risperidone is an atypical antipsychotics drug which, unlike haloperidol and hydroxyzine, has been reported to bind more strongly to the 5HTA receptor than the dopamine D2 receptor (Newman-Tancredi and Kleven, 2011). In our study, risperidone did not significantly affect muscarinic- and ATP-mediated intracellular calcium concentrations (Fig 6-A,C). Similarly, there was no significant difference in the intracellular calcium concentration in the response to thapsigargin in HSG cells treated with risperidone (Fig 6-E).


A
Control
30μM
Flourescence Ratio
(340/380)
4
3
2
1
100 sec
Haloperido
Carbachol

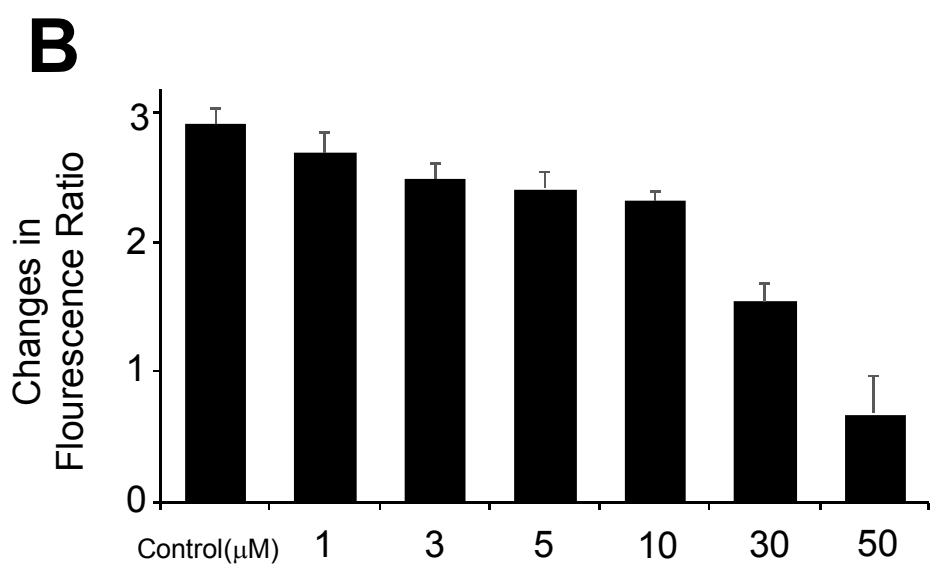
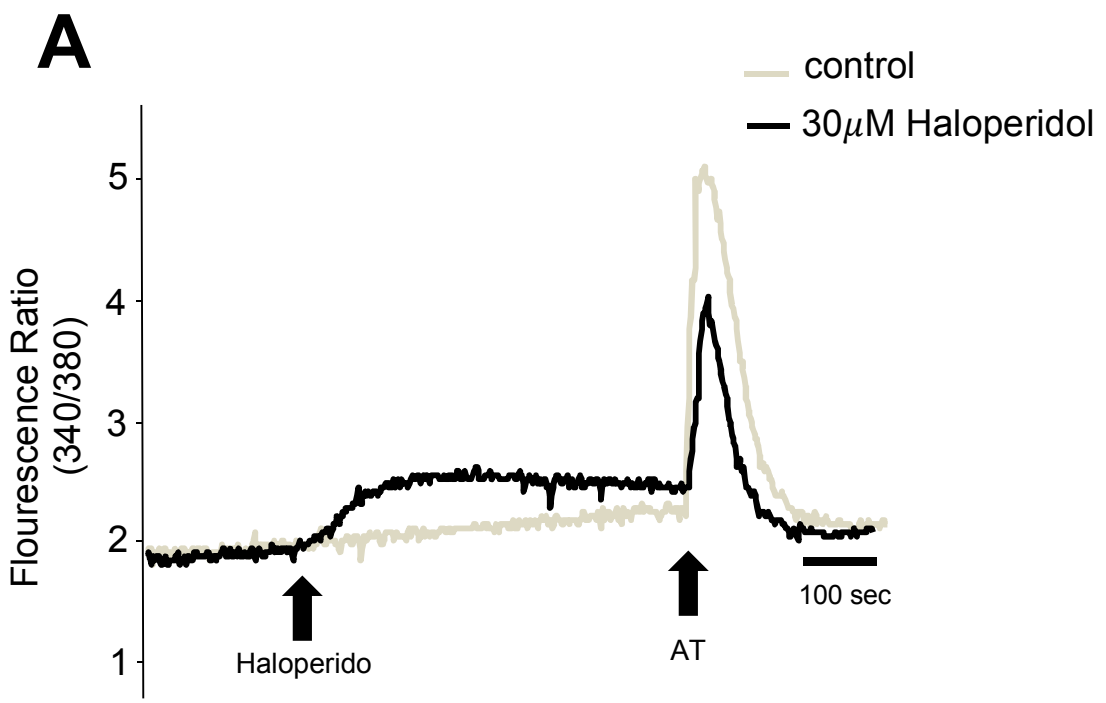

B
Changes in
Flourescence Ratio
3
2
1
0
Control(μM)
1
3
5
10
30
50

Figure 2-1. The pretreatment of Haloperidol inhibits Carbachol-induced changes in intracellular calcium concentration in Human Submandibular gland cells.

(A) Fura-2 loaded HSG cells were treated with 300 μM carbachol with (black trace) or without (gray trace) 30 μM haloperidol preincubation (5 min). The changes in F340/F380 were monitored and typical Ca^{2+} traces are presented. (B) Cells were preincubated with the indicated concentrations of haloperidol , and subsequently treated with 300 μM carbachol. The peak height of Ca^{2+} elevation was monitored. Each point represents mean ± SEM. n =8.


A
control
30μM Haloperidol
Flourescence Ratio
(340/380)
5
4
3
2
1
Haloperido
AT
100 sec

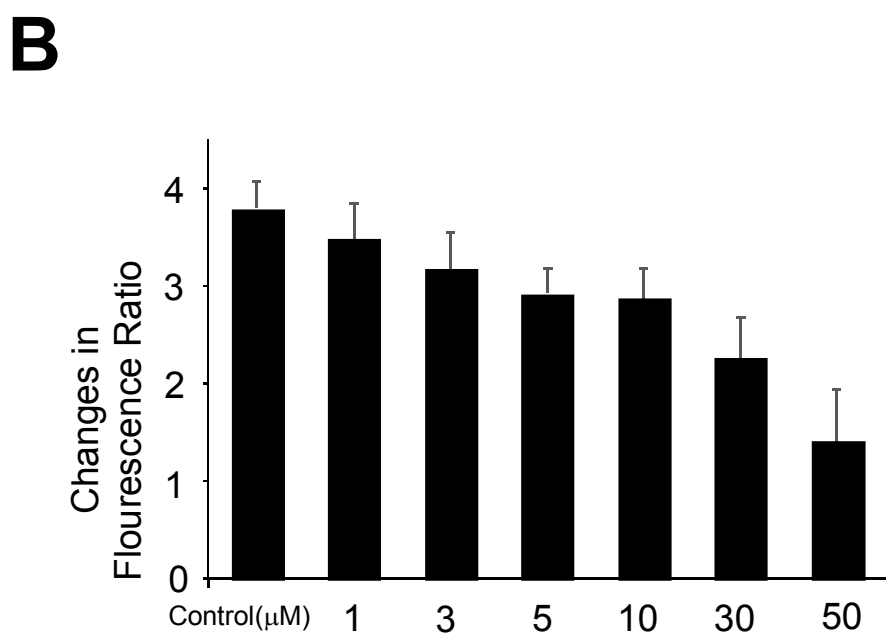
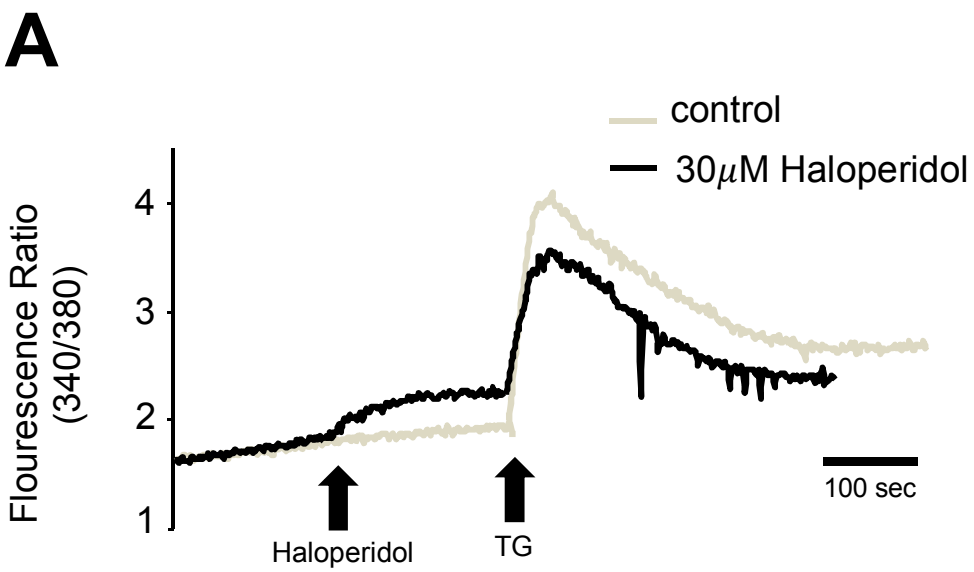

B
Changes in
Flourescence Ratio
4
3
2
1
0
Control(μM)
1
3
5
10
30
50

Figure 2-2. The pretreatment of Haloperidol inhibits ATP-induced changes in intracellular calcium concentration in Human Submandibular gland cells.

(A) Fura-2 loaded HSG cells were treated with 300 μM ATP with (black trace) or without (gray trace) 30 μM haloperidol preincubation (5 min). The changes in F340/F380 were monitored and typical Ca^{2+} traces are presented. (B) Cells were preincubated with the indicated concentrations of haloperidol, and subsequently treated with 300 μM ATP. The peak height of Ca^{2+} elevation was monitored. Each point represents mean ± SEM. n = 6.


A
control
30μM Haloperidol
Flourescence Ratio
(340/380)
4
3
2
1
100 sec
Haloperidol
TG

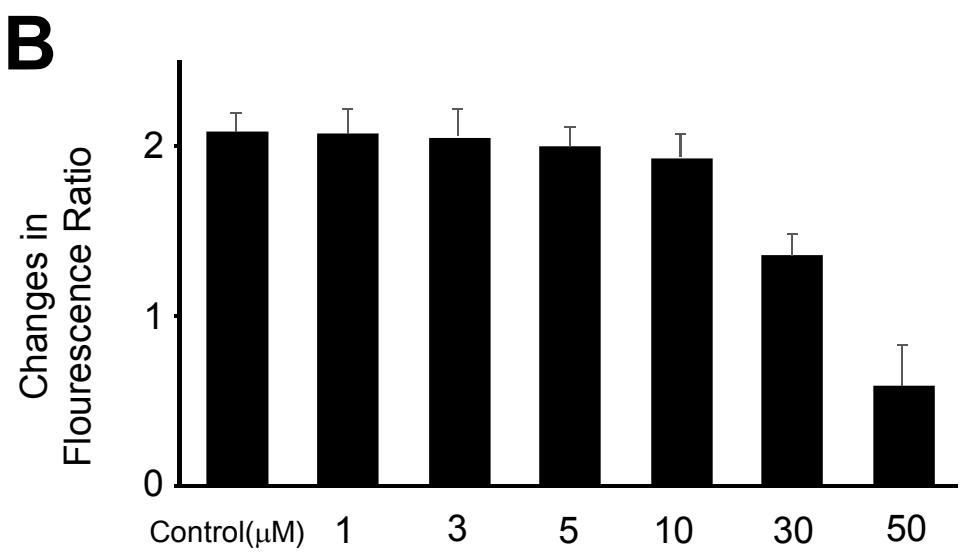
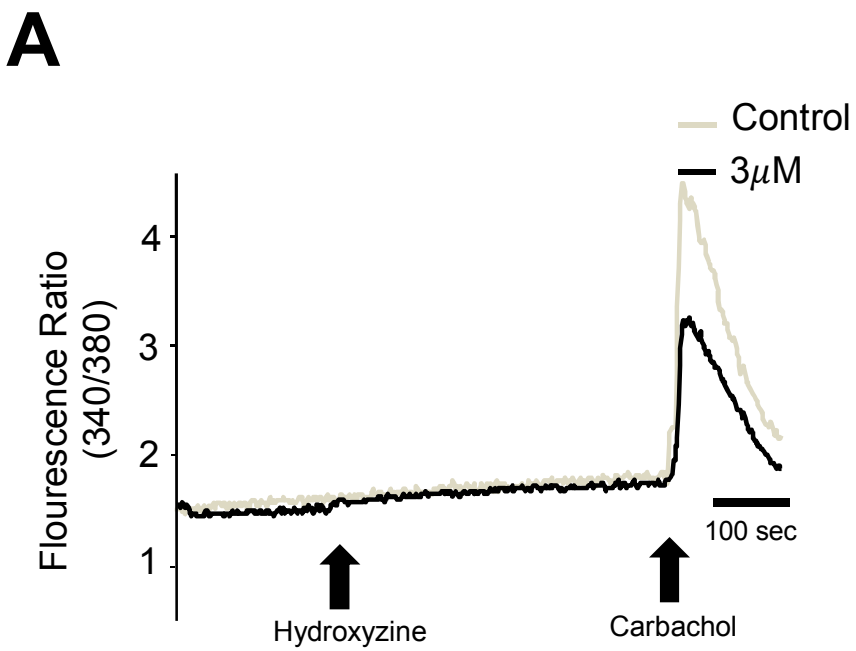

B
Changes in
Flourescence Ratio
2
1
0
Control(μM)
1
3
5
10
30
50

Figure 2-3. The pretreatment of Haloperidol inhibits Thapsigargin-induced changes in intracellular calcium concentration in Human Submandibular gland cells.

(A) Fura-2-loaded HSG cells were treated with 1μM Thapsigargin with (black trace) or without (gray trace) 30 μM Haloperidol preincubation (5min). The changes in F340/F380 were monitored and typical Ca^{2+} traces are presented. (B) Cells were preincubated with the indicated concentrations of haloperidol, and subsequently treated with 1 μM Thapsigargin. The peak height of Ca^{2+} elevation was monitored. Each point represents mean ± SEM. n = 8.


A
Control
3μM
Flourescence Ratio
(340/380)
4
3
2
1
100 sec
Hydroxyzine
Carbachol

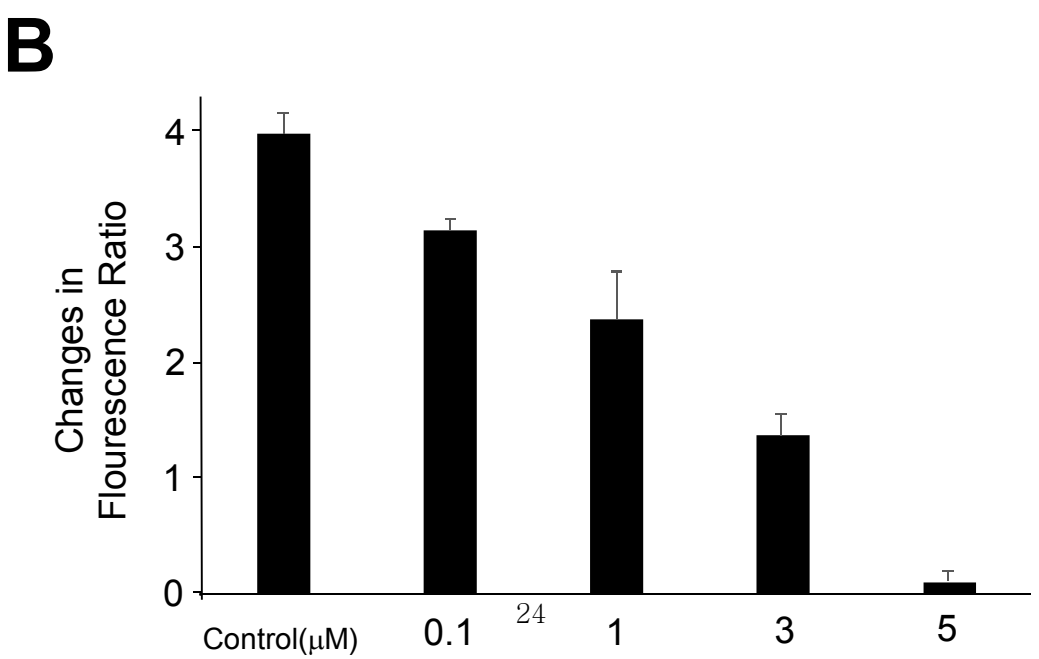
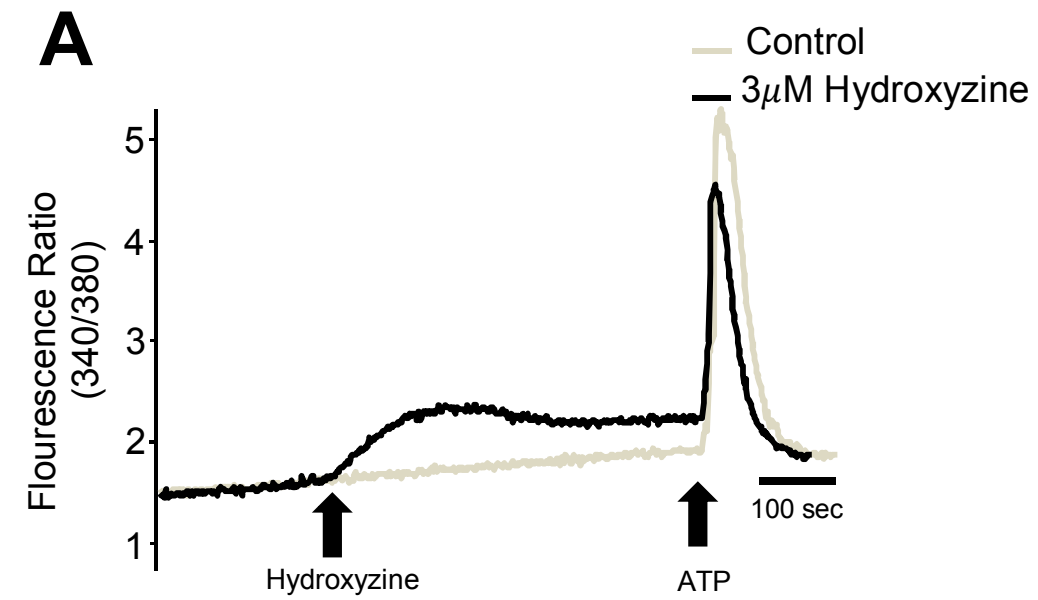

B
Changes in
Flourescence Ratio
4
3
2
1
0
Control(μM)
0.1
1
3
5

Figure 3-1. The pretreatment of Hydroxyzine inhibits muscarinic-induced changes in intracellular calcium concentration in Human Submandibular gland cells.

(A) Fura-2 loaded HSG cells were treated with 300 μM Carbachol with (black trace) or without (gray trace) 3 μM Hydroxyzine preincubation (5 min). The changes in F340/F380 were monitored and typical Ca^{2+} traces are presented. (B) Cells were preincubated with the indicated concentrations of hydroxyzine, and subsequently treated with 300 μM Carbachol. The peak height of Ca^{2+} elevation was monitored. Each point represents mean ± SEM. n = 6.


A
Control
3μM Hydroxyzine
Flourescence Ratio
(340/380)
5
4
3
2
1
100 sec
Hydroxyzine
ATP

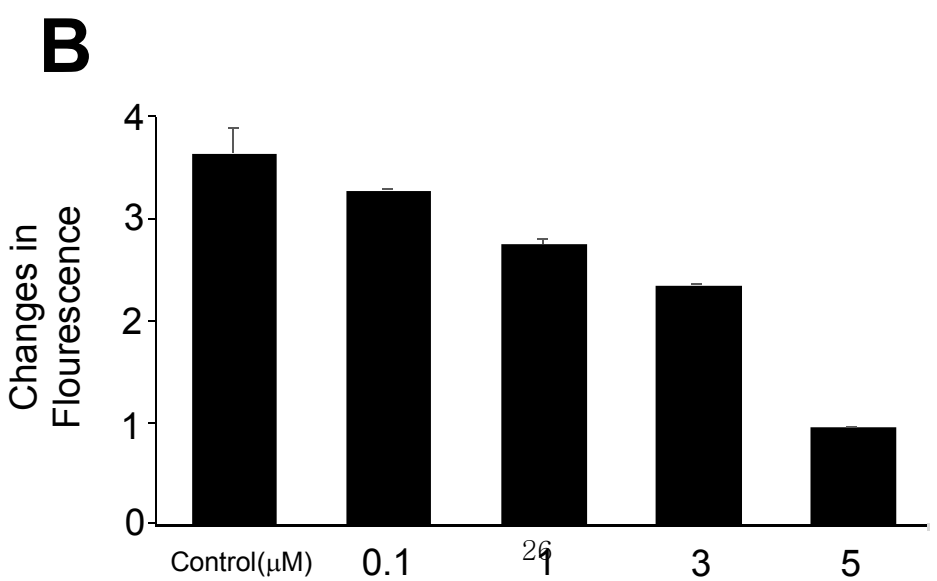

B
Changes in
Flourescence
4
3
2
1
0
Control(μM)
0.1
1
3
5

Figure 3-2. The pretreatment of Hydroxyzine inhibits ATP-induced changes in intracellular calcium concentration in Human Submandibular gland cells.

(A) Fura-2 loaded HSG cells were treated with 300 μM ATP with (black trace) or without (gray trace) 3 μM Hydroxyzine preincubation (5 min). The changes in F340/F380 were monitored and typical Ca^{2+} traces are presented. (B) Cells were preincubated with the indicated concentrations of Hydroxyzine, and subsequently treated with 300 μM ATP. The peak height of Ca^{2+} elevation was monitored. Each point represents mean ± SEM. n = 5.

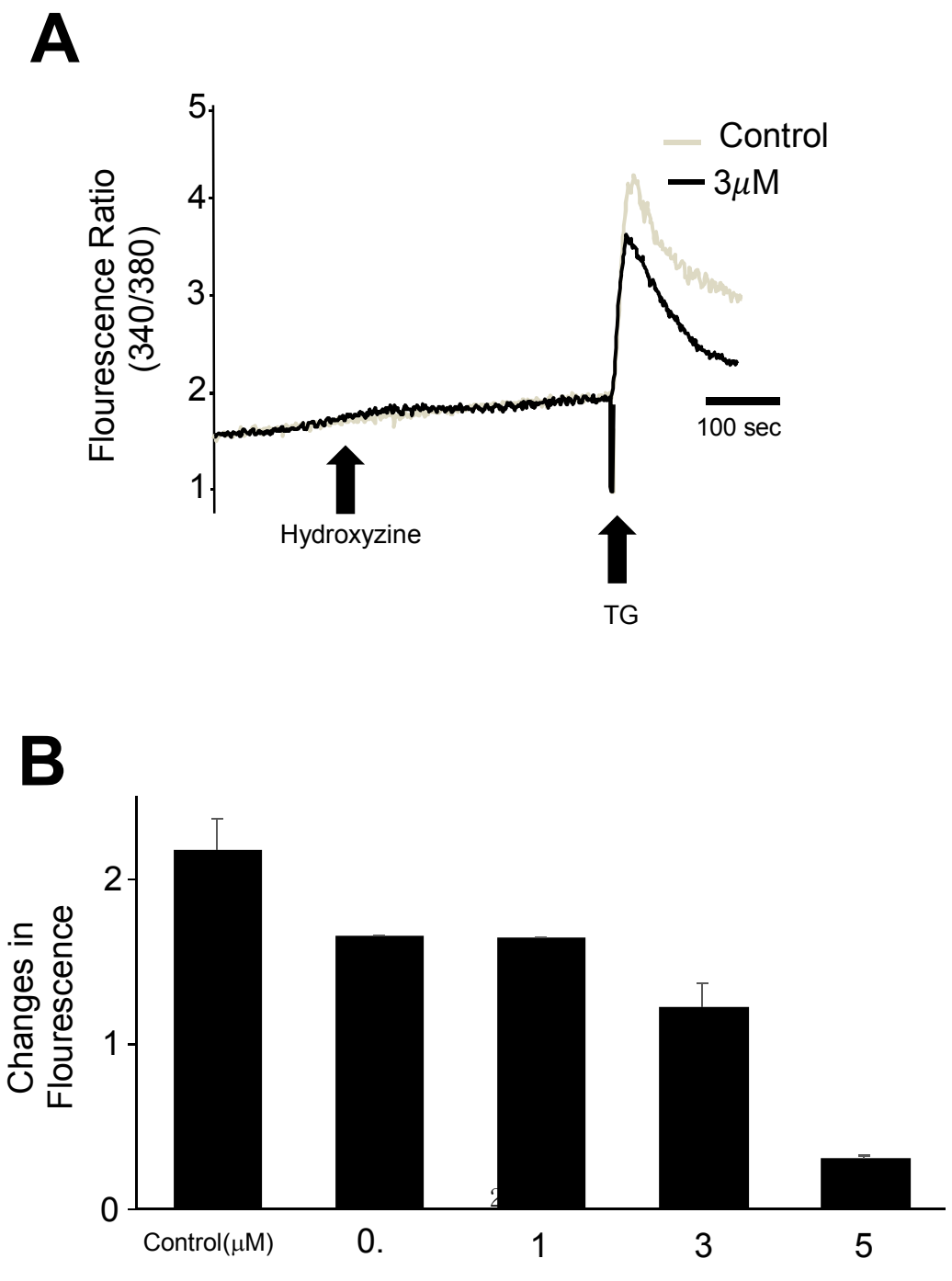

A
Flourescence Ratio
(340/380)
5
4
3
2
1
Control
3μM
100 sec
Hydroxyzine
TG
B
Changes in
Flourescence
2
1
0
Control(μM)
0.
1
3
5

Figure 3-3. The pretreatment of Hydroxyzine inhibits thapsigargin-induced changes in intracellular calcium concentration in Human submandibular gland cells.

(A) Fura-2-loaded HSG cells were treated with 1μM Thapsigargin with (black trace) or without (gray trace) 3 μM Hydroxyzine preincubation (5min). The changes in F340/F380 were monitored and typical Ca^{2+} traces are presented. (B) Cells were preincubated with the indicated concentrations of Hydroxyzine, and subsequently treated with 1 μM Thapsigargin. The peak height of Ca^{2+} elevation was monitored. Each point represents mean ± SEM. n = 7.

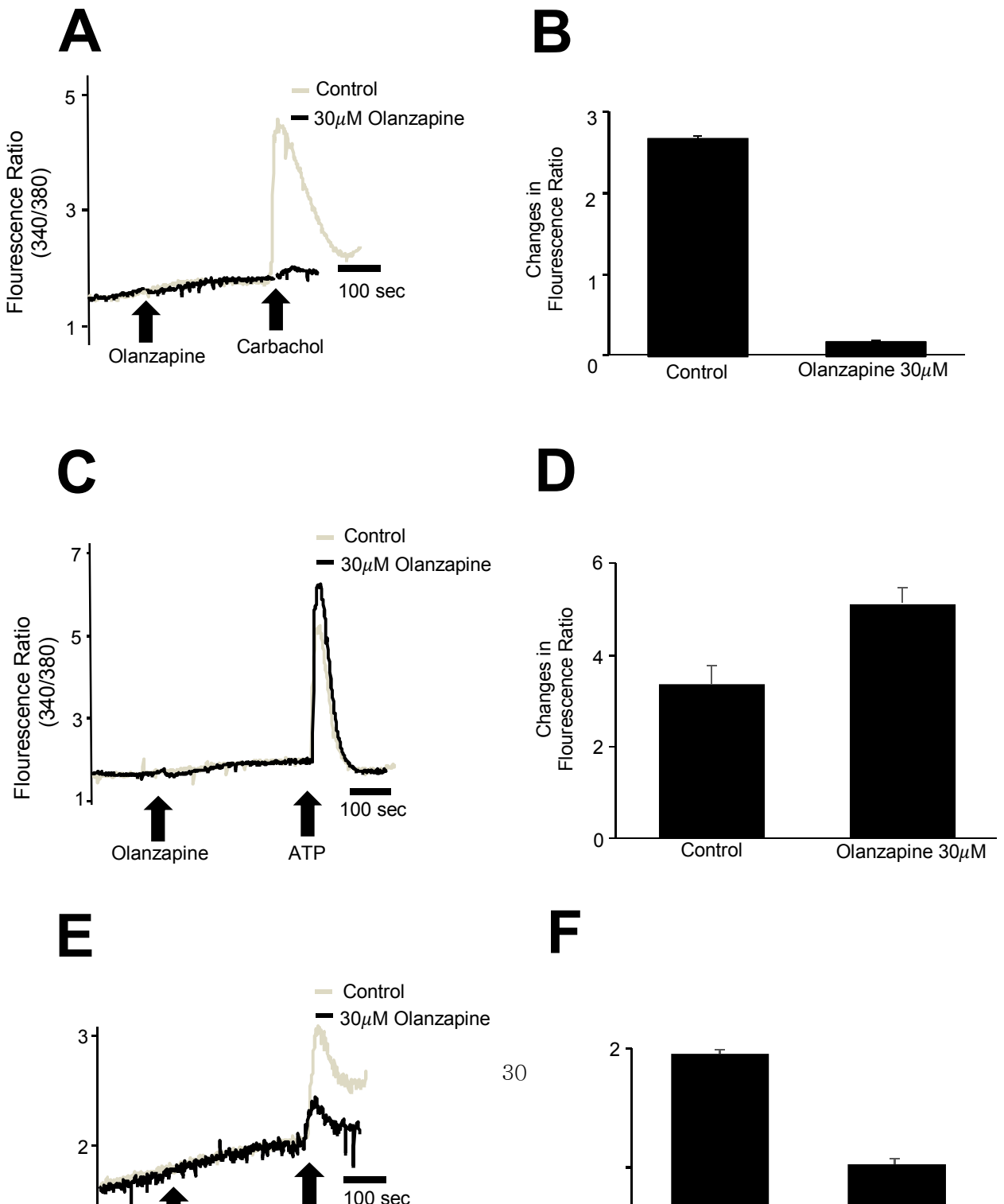

A
Control
30μM Olanzapine
Flourescence Ratio (340/380)
5
3
1
100 sec
Olanzapine
Carbachol
B
Changes in Flourescence Ratio
3
2
1
0
Control
Olanzapine 30μM
C
Control
30μM Olanzapine
Flourescence Ratio (340/380)
7
5
3
1
100 sec
Olanzapine
ATP
D
Changes in Flourescence Ratio
6
4
2
0
Control
Olanzapine 30μM
E
Control
30μM Olanzapine
3
2
100 sec
F
2

Fig 4. Olanzapine completely blocks calcium release in Carbachol-induced cells, increase calcium release in ATP-induced cells, and reduces calcium release in thapsigargin-induced cells.

(A) Fura-2-loaded HSG cells were treated with 300μM Carbachol with (black trace) or without (gray trace) 30 μM Olanzapine preincubation (5 min).

(B)Quantification of the inhibitory effect of Olanzapine on muscarinic-induced intracellular calcium increase. . The peak height of Ca^{2+} elevation was monitored. Each point represents mean ± SEM. n = 4.

(C) Fura-2-loaded HSG cells were treated with 300μM ATP with (black trace) or without (gray trace) 30 μM Olanzapine preincubation (5 min).

(D)Quantification of the effect of Olanzapine on intracellular calcium induced by purinergic receptors. . The peak height of Ca^{2+} elevation was monitored. Each point represents mean ± SEM. n = 5.

(E)Fura-2-loaded HSG cells were treated with 300 μM Carbachol with

(black trace) or without (gray trace) 30 μM Olanzapine preincubation (5min).

(F)Quantification indicating that Olanzapine does not significantly affect intracellular calcium increase induced by thapsigargin. The peak height of Ca^{2+} elevation was monitored. Each point represents mean ± SEM. n = 3.

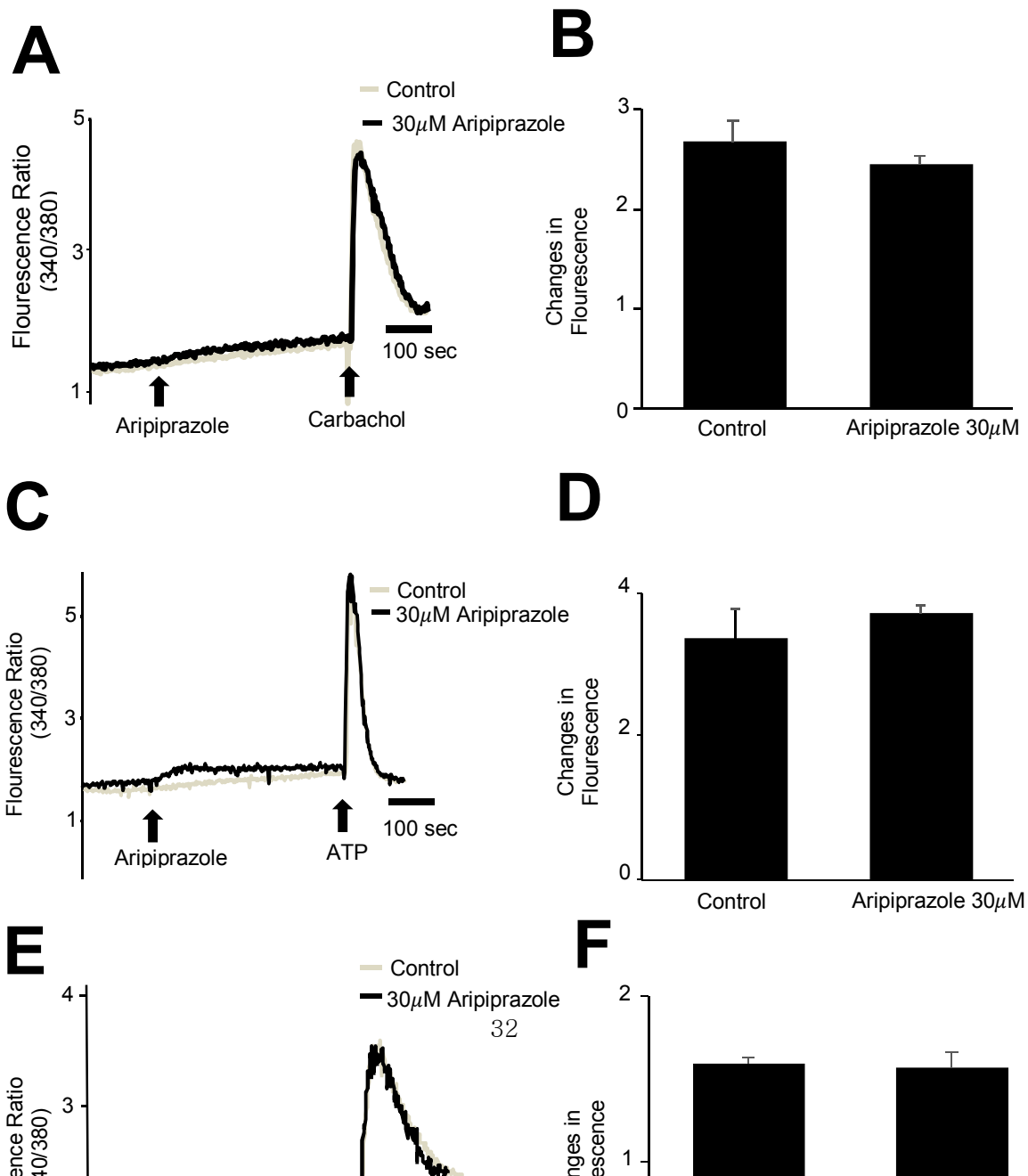


Figure 5. Aripiprazole does not significantly affect muscarinic and purinergic receptor mediated calcium release in human salivary gland cells. It is not largely involved in the calcium increase by thapsigargin.

(A) Fura-2-loaded HSG cells were treated with 300 μM Carbachol with (black trace) or without (gray trace) 30 μM Aripiprazole preincubation (5 min).

(B)Quantification of aripiprazole is not significantly altered to increase intracellular calcium induced by muscarinic. . The peak height of Ca^{2+} elevation was monitored. Each point represents mean ± SEM. n =3

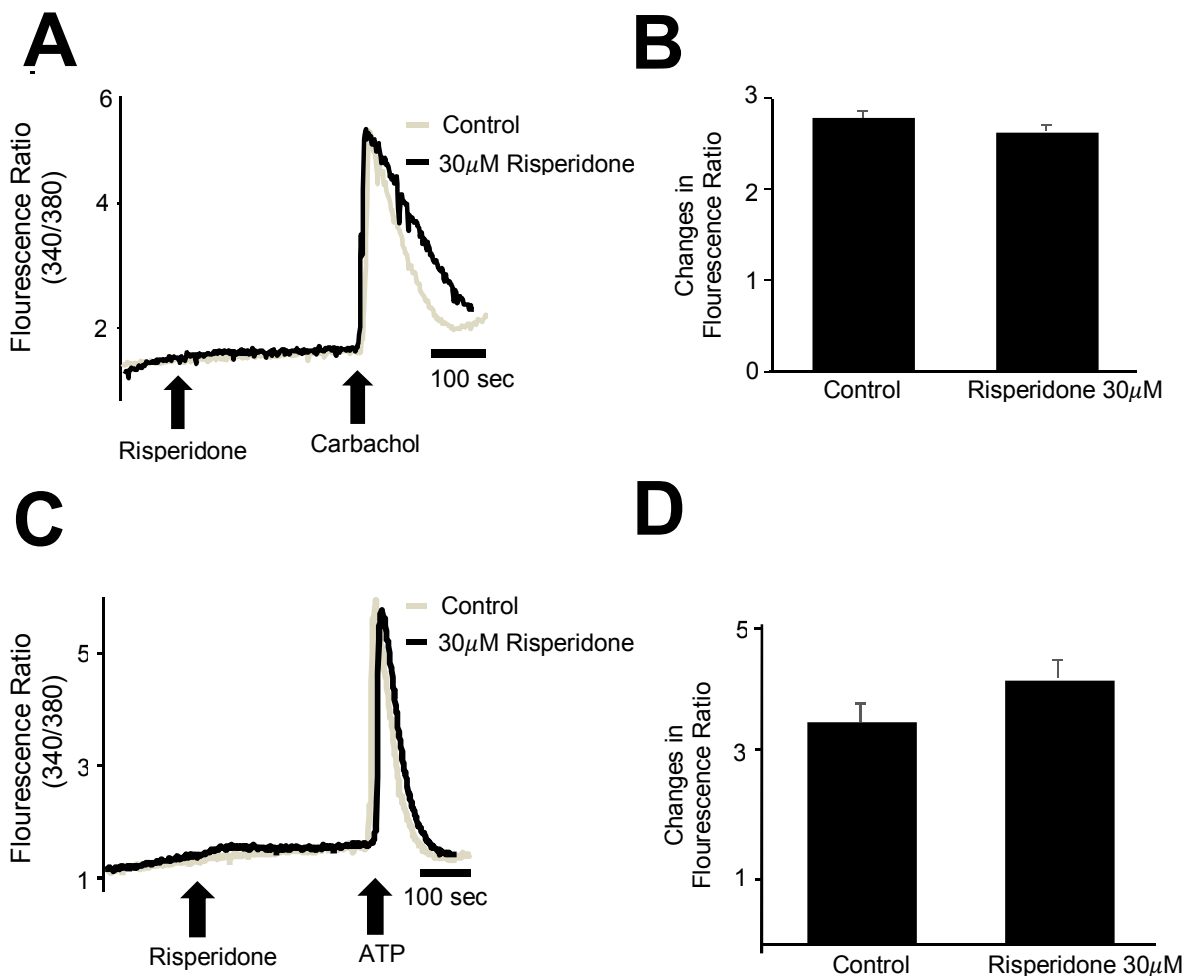
(C) Fura-2-loaded HSG cells were treated with 300 μM ATP with (black trace) or without (gray trace) 30 μM Aripiprazole preincubation (5 min).

(D) Quantification of the effect of aripiprazole on intracellular calcium induced by purinergic receptors is not large. The peak height of Ca^{2+}

elevation was monitored. Each point represents mean ± SEM. n =3

(E)Fura-2-loaded HSG cells were treated with 300 μM Carbachol with (black trace) or without (gray trace) 30 μM Aripiprazole preincubation (5min).

(F)Quantification indicating that Aripiprazole does not significantly affect intracellular calcium increase induced by thapsigargin. The peak height of Ca^{2+} elevation was monitored. Each point represents mean ± SEM. n = 5.



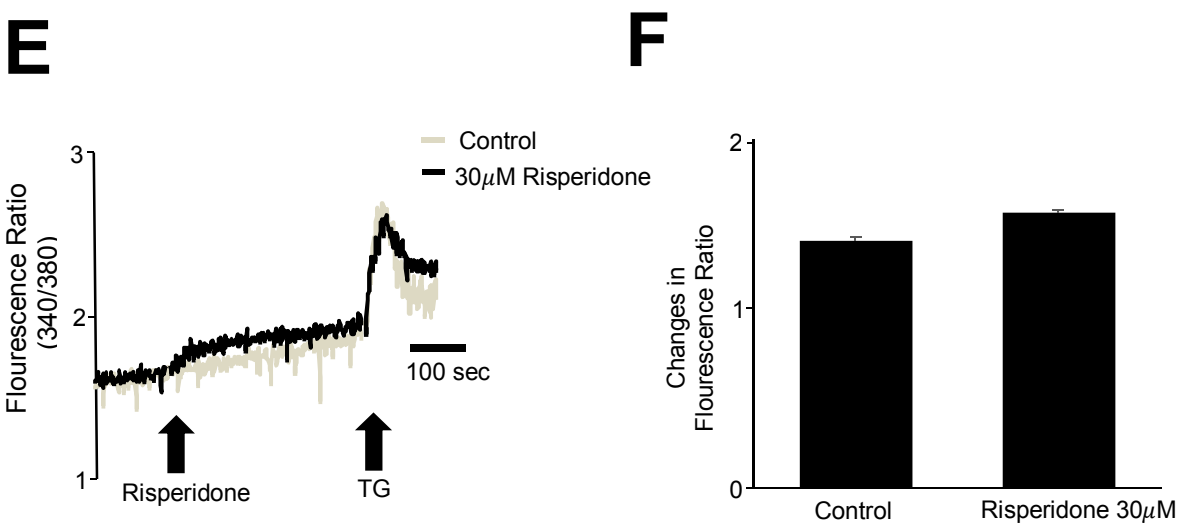


Figure 6. Risperidone does not inhibit muscarinic, ATP, Thapsigargin-induced intracellular calcium concentration increase in Human submandibular gland cells.

(A) Fura-2-loaded HSG cells were treated with 300μM Carbachol with (black trace) or without (gray trace) 30μM Risperidone preincubation (5min).

(B)Quantification of Risperidone is not significantly altered to increase intracellular calcium induced by muscarinic. The peak height of Ca^{2+} elevation was monitored. Each point represents mean ± SEM. n = 3.

(C) Fura-2-loaded HSG cells were treated with 300μM ATP with(black trace) or without (gray trace) 30μM Risperidone preincubation (5min).

(D) Quantification of the effect of Risperidone on intracellular calcium induced by purinergic receptors is not large. The peak height of Ca^{2+}

elevation was monitored. Each point represents mean ± SEM. n = 4.

(E)Fura-2-loaded HSG cells were treated with 300μM Carbachol with (black trace) or without (gray trace) 30μM Risperidone preincubation (5min).(F)Quantification indicating that Risperidone does not significantly affect intracellular calcium increase induced by thapsigargin. The peak height of Ca^{2+} elevation was monitored. Each point represents mean ± SEM. n = 4.

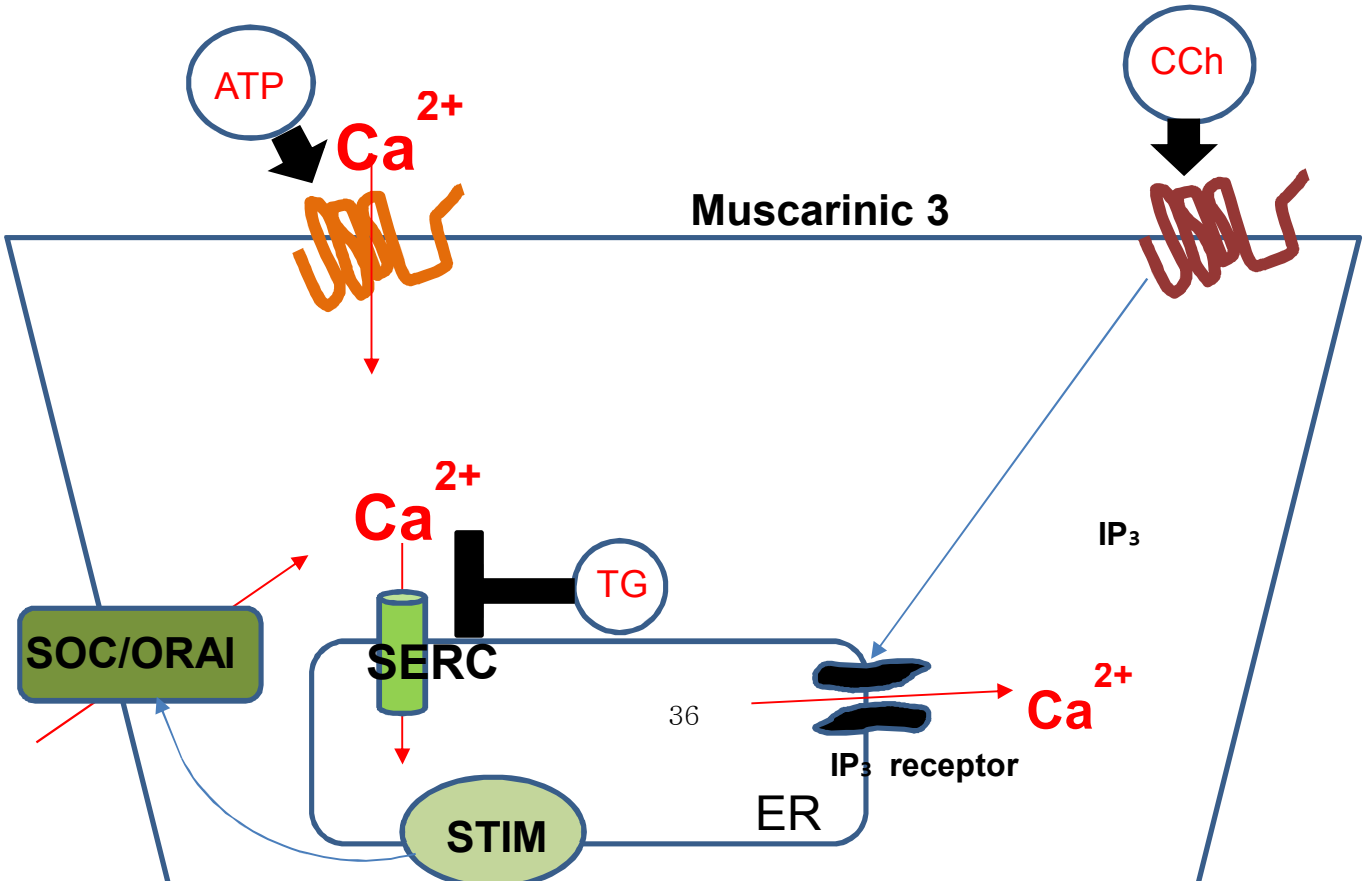


Figure 7. In human salivary gland cells, antihypertensive agents seem to be involved in calcium signaling and affect salivary secretion.

Ⅳ. Discussion

We examined the effects of first generation drugs such as haloperidol, hydroxyxyzine and second generation drugs, olanzapine, aripiprazole, and risperidone on the calcium concentration in human submandibular cells. HSG cells are composed of G-protein coupled receptors (Lee et al., 2012; Roussa, 2011), which are not voltage-dependent receptors but neurotransmitters released by the autonomic nervous system. We chose carbachol to increase intracellular calcium by stimulating muscarinic receptors, as muscarinic receptors have been reported to be involved in salivary gland intracellular calcium concentrations associated with salivary secretion (Jin et al., 2012; Nagy et al., 2007). After treatment with haloperidol and hydroxyzine, the concentration of intracellular

calcium was significantly reduced in the cells treated with carbachol. These drugs appear to be powerful antagonists of dopamine, while at the same time inhibiting muscarinic receptors and interfering with calcium increase. In addition, ATP (Hedden et al., 2011), which is known as a substance that binds to the P2X7 receptor and attracts external calcium ions into the cell, decreases the intracellular calcium concentration in cells pretreated with haloperidol and hydroxyzine. This suggests that the antagonist of the dopamine D2 receptor also inhibits the P2X7 receptor. In addition, it was confirmed that both haloperidol and hydroxyzine were inhibited by thapsigargin (Liu et al., 1998), which increases the cytosolic calcium release by blocking the action of SERCA pump which acts as downstream of these receptors (Jung et al., 2000; Thastrup et al., 1990). Haloperidol and hydroxyzine, known as powerful dopamine D2 antagonists in the brain, have been shown to affect the muscarinic receptors and purinergic receptors expressed in G-protein coupled receptors in salivary glands, thereby lowering intracellular calcium concentration. And it seems to suppress secretion of saliva. Olanzapine is known to be a strong antagonist of muscarinic 3 receptors and has been reported to have higher affinity for 5HT2A receptors than dopamine D2 receptors (Johnson et al., 2005). In our experiment, olanzapine was treated with carbachol, an agonist of the muscarinic receptor It was confirmed that calcium release was completely blocked. There was no significant difference in calcium release when ATP, an agonist of the purinergic receptor, was administered. Olanzapine seems to selectively bind strongly to muscarinic receptors, as reported previously, and it appears to influence the mechanism of salivation by lowering or removing intracellular calcium

concentration. Aripiprazole is known to exhibit a functional selective action on dopamine D2 receptors, and most atypical antipsychotics bind preferentially to external congenital receptors, while aripiprazole has low preference for this effect and receptors in the brain have a total binding capacity (Shapiro et al., 2003). In the HSG cells used in our experiments, neither the muscarinic nor the purinergic receptor showed high affinity, and therefore did not significantly affect the change of intracellular calcium concentration. Aripiprazole does not seem to selectively react with specific receptors such as muscarinic and purinergic receptors in HSG cells as well as neurons. Risperidone is known to interact even slightly with dopamine receptors, serotonin receptors, alpha 1-adrenergic receptors, alpha-2 adrenergic receptors, and histamine receptors, and it is known to have few side effects (Hecht and Landy, 2012). Therefore, it did not react significantly with any specific receptor, and the change in calcium concentration was not significant. In summary, haloperidol and hydroxyzine, which are known to be drugs that cause oral dryness, were used to lower the concentration of calcium in HSG cells and induce dry mouth, aripiprazole and risperidone, which are reported to be rarely observed, have little effect on calcium concentration. However, this has been confirmed with only three agents, and further studies on whether they affect other pathways should proceed. There are two pathways involved in water movement in a mechanism known to control saliva secretion (Ambudkar, 2011), one related to the ion transport pathway of the cell membrane as the paracellular pathway, and the other is the pathway of the AQP5 water channel (Ishikawa et al., 2005). In this regard, among the drugs we tested, haloperidol and hydroxyzine, which influenced calcium signaling, are

involved in the regulation of saliva secretion by calcium-dependent ions or the movement of a water channel called aquaporin-5, if you check that it is controlled, you will be able to explain the more accurate saliva secretion mechanism. In addition, the pathway involved in the SOCE channel that supports calcium supply in the event of calcium depletion in the ER that is the primary source of intracellular calcium should be considered (Jang et al., 2016). In HSG cells, SOCE is detected in the ER and the ER calcium binding protein STIM1 activates the plasma membrane calcium channels Orai1 and TRPC1 (Ong et al., 2014). Therefore, it is necessary to confirm whether or not these drugs affect calcium channel concentration.

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국문초록

구강 건조증을 유발하는 조현병 치료제에서의 타액선 세포내 칼슘신호에 대한 연구

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구강 건조증은 타액의 분비 저하 현상으로 방사선치료나 화학요법을 포함하여 자가 면역질환 및 조현병 치료약물의 부작용등으로 발생되는 질환이다. 타액의 분비가 원활하지 못하면 음식물을 씹거나 삼키기 어렵고, 충치를 비롯하여 각종 면역질환에 쉽게 노출이 된다.

사람 타액선은 액체와 전해질을 생성하는 선포세포와 여러 단백질들을 첨가하여 최종적인 타액을 분비하는 도관세포로 구성된다.

타액선 세포는 전압의존성 이온채널을 발현하지 않는 비흥분성 세포로서, 주로 자율신경계에 의해 조절되며, 특히 부교감 신경의 자극은 선포세포 세포막에 있는 G-단백질 결합 수용체에 신경전달물질이 결합을 함으로써 세포 외부의 신호를 내부로 전달하며 이루어진다. 타액선에는 주로 무스카린성 수용체와 알파 아드레날린성 수용체 그리고 뉴로펩타이드 수용체등이 있다. G-단백질 결합 수용체의 기능에 이상을 보이면, 타액의 분비가 저하된다. 구강 건조증을 일으키는 여러 약물이 G-단백질 결합 수용체의 기능을 억제한다. 그 예로 써, 항 우울제가 G-단백질 결합 수용체의 신호를 조절하는 것은 이미 알려져 있지만, 도파민 수용체의 길항제인 조현병의 치료약물들이 G-단백질 결합 수용체에 어떠한 영향을 미치는지는 많이 연구되지 않았다. 본 논문에서는 조현병의 주요한 치료약물들 중 구강건조증이 부작용으로 대두되는 1세대약물인 Haloperidol, 1세대 항히스타민제 이면서 조현병 치료제로도 처방되는 Hydroxyzine과 이에 대비되는 구강건조증이 적게 나타나는 2세대 약물인 Aripiprazole, Olanzapine, Risperidone 이 타액선세포에서 G-단백질 결합 수용체 신호전달 과정에 어떤 영향을 주는지 밝히고자 했다.

첫 번째로, 조현병의 정형적 치료제인 Haloperidol, Hydroxyzine과 비정형 치료제인 Olanzapine 은 무스카린성 수용체 자극에 의한 칼슘 방출을 억제한다. 두 번째로 haloperidol 과 Hydroxyzine은 퓨린성 수용체 자극에 의한 세포 내 칼슘을 억제하지만 Olanzapine에서

는 오히려 칼슘방출을 촉진하였다. 세 번째로 Haloperidol, Hydroxyzine, Olanzapine 은 세포내의 소포체 막에 있는 SERCA pump의 작용을 억제하여 세포내 칼슘의 농도를 떨어뜨린다는 것을 알게 되었다. 마지막으로 신경세포에서는 도파민 수용체, 세로토닌 수용체를 비롯 아드레날린성 수용체, 히스타민 수용체와 모두 골고루 상호작용을 한다고 알려진 Risperidone 은 타액선세포에서 G-단백질 결합 수용체에는 크게 영향을 주지 않는 것으로 나타났다. 위의 결과들로 구강 건조증을 일으키는 조현병 치료 약물인 Haloperidol, Hydroxyzine은 신경세포에서 도파민 수용체의 길항역할을 할n뿐만 아니라, 타액선세포에서는 G-단백질 결합 수용체 에서의 자극을 억제함을 알 수 있었다. 그 결과 최종적으로 세포내 칼슘의 농도를 낮추어, 타액의 분비를 감소하는 역할을 한다는 것을 알게 되었다. 한편 구강 건조증의 유발효과가 약한 Risperidone, Aripiprazole 등은 타액선 세포에서 G-단백질 결합 수용체에 많은 영향을 주지는 않음을 알게 되었다.

주요어 : 구강 건조증, 타액 분비, 세포 내 칼슘, G-단백질 결합 수용체, 타액선, 항정신병약물의 부작용

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