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Amitriptyline inhibits the G protein and K^+ channel in the cloned thyroid cell line

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Abstract

We have reported that thyroid K^+ channel is activated by extracellular application of the thyroid-stimulating hormone (TSH) using single channel recording method performed on cloned normal rat thyroid cell (FRTL-5) membrane. Treatment of dibutyryladenosine cyclic monophosphate (Bt_2 cAMP) also activated the TSH-dependent K^+ channel. These findings indicate that the thyroid K^+ channel is activated through the TSH-adenosine cyclic monophosphate (cAMP)-protein kinase A system. We examined the effects of amitriptyline on TSH-guanosine triphosphate binding protein (G protein)-adenylate cyclase-cAMP-K^+ channel system in the cloned normal rat thyroid cell line FRTL-5. Amitriptyline inhibited the cAMP production induced by TSH. Amitriptyline also inhibited the cAMP production induced by cholera toxin, indicating that amitriptyline inhibited the thyroid G protein. Amitriptyline had no effect on TSH-receptor binding and cAMP production by forskolin (adenylate cyclase stimulator). Amitriptyline inhibited the K^+ channel activation by cAMP, indicating that the suppressing mechanism is not the inhibition of TSH receptor or G protein but the direct suppression of K^+ channel. It was concluded that amitriptyline inhibited the thyroid G protein and K^+ channel.

Keywords: Thyroid gland; K^+ channel; TSH (thyroid-stimulating hormone); Thyroid-stimulating antibody; Amitriptyline; Antidepressant

1. Introduction

In the thyroid cell, Cl^-, I^- efflux and Ca^{2+} influx play a very important role in the regulation of cell function. Opening of channels for these ions is regulated mainly by voltage-dependent ion channels (Golstein et al., 1992). In several tissues, the existence and the physiological role of K^+ channels have been reported (Mokler and Van Arman, 1962; James and Nadeau, 1964; Singh and Hauswirth, 1974; Singh and Vaughan Williams, 1970; Yoshida et al., 1993). Activation of the K^+ channel increases K^+ conductance resulting in hyperpolarization of membrane and modulation of other voltage-dependent ion channels. In the pancreas, a K^+ channel blocker (sulphonylurea) can modulate intracellular Ca^{2+} through blocking pancreatic K^+ channels, resulting in secretion of insulin from pancreatic cells. We reported that the K^+ channel is activated by the thyroid-stimulating hormone (TSH)-guanosine triphosphate binding protein (G protein)-adenosine cyclic monophosphate (cAMP) system in cloned normal rat thyroid cell line FRTL-5 (Yoshida et al., 1993). FRTL-5 cells are considered to be a good model for normal thyroid cell function, because in this cell line, TSH, through its cAMP signal, regulates iodide uptake, thyroglobulin synthesis, iodination of thyroglobulin, and cell growth. So this K^+ channel is considered to be very important in the regulation of thyroid membrane potential and thyroid cell function, because activation of the K^+ channel is considered to induce the modification of the thyroid membrane potential and regulate voltage-dependent Na^+, Cl^-, I^- and Ca^{2+} channels (Golstein et al., 1992; Manley et al., 1986). The fact that TSH activates this K^+ channel suggests that some of the TSH actions are induced through this K^+ channel activation.

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It is well known that tricyclic antidepressant drugs inhibit the cardiac ion channels. These drugs inhibit cardiac Na^+ and Ca^{2+} channels and modulate the cardiac cell excitability. It is also known that thyroid hormone concentrations are decreased in antidepressant-treated animals (Atterwill et al., 1989; Brady and Anton, 1989). However physiological and electrophysiological effects of these drugs on thyroid have not been reported. In the present study, we investigated the effects of amitriptyline on thyroidal TSH receptor-G protein-adenylate cyclase-cAMP-K^+ channel system, and obtained several interesting findings.

2. Materials and methods

2.1. Chemicals

The materials used were obtained from the following suppliers: Coon's Modified Ham's F-12 Medium from Hazleton (Denver, CO, USA); calf serum from Gibco (Chagrin Falls, OH, USA); amitriptyline hydrochloride from Banyu Japan; insulin, hydrocortisone, transferrin, glycyl-histidyl-L-lysine acetate, somatostatin, dibutyryladenosine cyclic monophosphate (Bt_2 cAMP), from Sigma Chemical Co. (St. Louis, MO, USA); bovine TSH from Armour Pharmaceutical Co. (Phoenix, AZ, USA); trypsin from Difco Laboratories (Detroit, MI, USA); other chemicals from Nakarai Chemicals (Japan).

2.2. Continuous culture of FRTL-5 cells

The cloned normal rat thyroid cell line FRTL-5 was kindly provided by Dr L.D. Kohn (NIH, Bethesda, MD, USA). FRTL-5 cells were seeded in each well of 24-well Corning cell plates and grown in Coon's Modified Ham's F-12 Medium supplemented with 5% calf serum, bovine TSH (1 mU/ml), and a 5-hormone mixture consisting of insulin (10 μg/ml), hydrocortisone (10^{-8} M), transferrin (5 μg/ml), glycyl histidyl-L-lysine acetate (10 ng/ml) and somatostatin (10 μg/ml).

Cells were used for the experiments after culture at 37°C for 3 days in a humidified atmosphere of 95% air and 5% CO_2 without TSH.

2.3. Determination of the effect of amitriptyline on the induction of cAMP by TSH, 2 mg/ml Graves' immunoglobulin G (Ig G) (thyroid-stimulating antibody), cholera toxin, and forskolin

After FRTL-5 cells were cultured at 37°C for 3 days in a humidified atmosphere of 95% air and 5% CO_2 without TSH, the medium was replaced with 500 μl of Hank's balanced solution without NaCl (in the case of cholera toxin, Hank's balanced solution was used). After incubation for 15 min at 37°C with various doses of amitriptyline, 100 μU TSH, 2 mg/ml Graves' Ig G, 0.1 μg/ml cholera toxin, and 100 μM forskolin were added to the incubation mixture, and the mixture was further incubated for 2 h. After the incubation, cAMP released into the medium was measured by radioimmunoassay. In the case of cholera toxin, the reaction was stopped by the addition of 20% trichloroacetic acid, and the mixture was centrifuged at 2800 × g for 20 min. Then, cAMP content of the supernatant was determined.

2.4. TSH-receptor binding inhibition

TSH-receptor binding study in FRTL5 cells has not been well established. So TSH-receptor binding inhibition was measured using kits provided by Baxter Travenol (Tokyo, Japan). The mixture of solubilized TSH receptors and I^{125}-TSH was incubated at 37°C for 2 h with or without 500 μM of amitriptyline in the same condition as the thyroid-stimulating antibody measurement. Bound I^{125}-TSH was measured after the incubation using the polyethylene glycol method.

2.5. Preparation of Ig G

The Ig G fraction was isolated from serum samples by means of affinity chromatography on columns of protein-A-Sepharose CL-4B. The Ig G concentration of each fraction was determined by a single radial immunodiffusion method. Thyroid-stimulating level of 2 mg/ml Graves' Ig G (mean ± S.D.) measured by the method of Kasagi et al. (1987) was 980.0 ± 119.3% ($n = 5$, normal; < 133.7%).

2.6. Single channel recording

Monolayer cultures of FRTL-5 cells were perfused with a Tyrode solution consisting of 140 mM NaCl, 5.4 mM KCl 1.8 mM $CaCl_2$, 0.5 mM $MgCl_2$, 5 mM *N*-2-hydroxyethylpiperazine-*N'*-2-ethane sulfonic acid (Hepes), 5 mM glucose and with or without Bt_2 cAMP at room temperature (22°C) and at pH7.4. The pipette contained 5 mM Hepes and 145 mM KCl, with or without TSH at pH 7.4. The current data obtained were filtered with an analog two-pole active filter at 3.0 kHz, then digitized to a 14-bit resolution at 10.0 kHz and stored in a personal computer (NEC).

3. Results

3.1. Effects of amitriptyline on the induction of cAMP by 100 μU TSH

As shown in Fig. 1, amitriptyline showed dose-dependent suppression of cAMP induction by TSH, with significant suppression at concentrations of more than 1 μM.

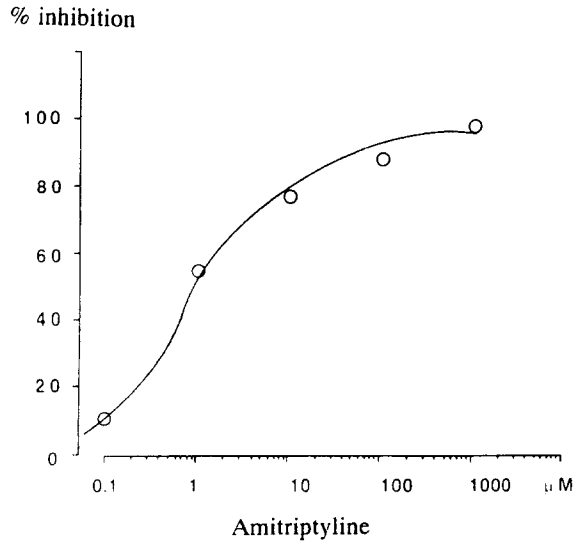

Fig. 1. Dose-dependent effect of amitriptyline on the induction of cAMP by 100 μU/ml TSH. Amitriptyline inhibited the cAMP induction by 100 μU/ml TSH, dose dependently. ID_{50} :0.8 μM; Hill coefficient 0.8 The ordinate indicates percentage inhibition of cAMP induction by amitriptyline; abscissa, concentration of the preincubated amitriptyline.

3.2. Effects of amitriptyline on the induction of cAMP by thyroid-stimulating antibody

Thyroid-stimulating antibody has been known to stimulate cAMP induction through the same receptor as TSH. So it is possible that amitriptyline inhibits the cAMP induction by thyroid-stimulating antibody. As shown in Fig. 2, 100 μM amitriptyline also showed a significant suppression of cAMP induction by thyroid-stimulating antibody in 5 patients.

The suppression of cAMP induction has been attributed

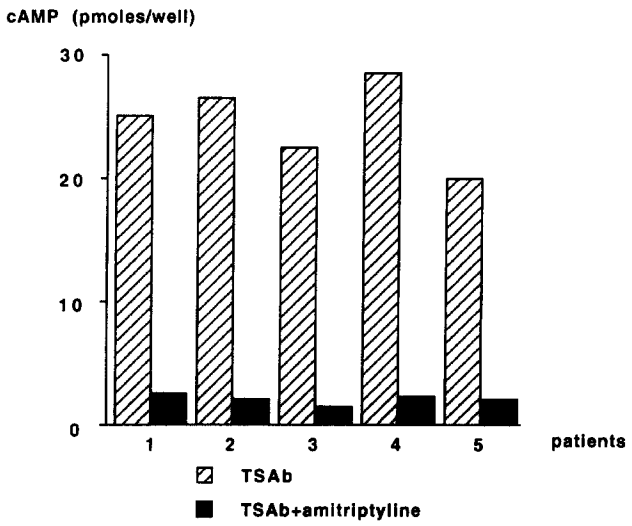

Fig. 2. Effect of amitriptyline on the induction of cAMP by thyroid-stimulating antibody. 100 μM of amitriptyline inhibited the cAMP induction by thyroid-stimulating antibody obtained from each patient. All data are the means of triplicate experiments. Paired Student's *t*-test was used for the statistical analysis.

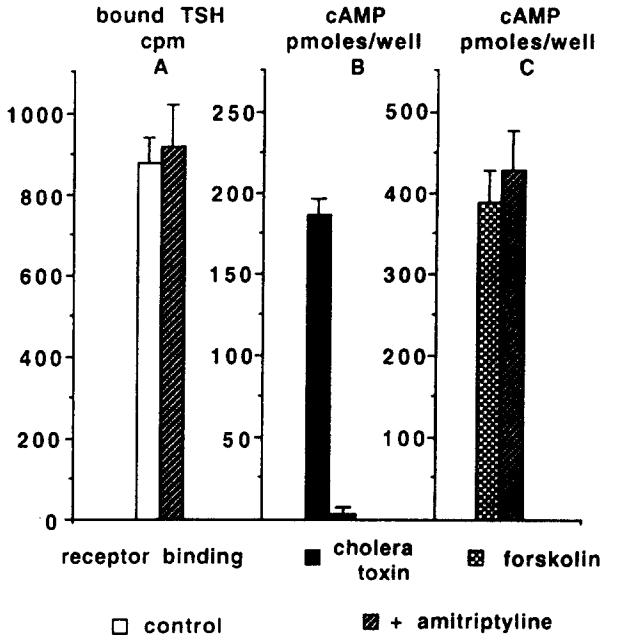

Fig. 3. Effect of amitriptyline on the TSH-receptor binding, on the induction of cAMP by 0.1 μg/ml cholera toxin and on the induction of cAMP by 100 μM forskolin. 500 μM of amitriptyline showed no influence on TSH-receptor binding and on the induction of cAMP by 100 μM forskolin. 100 μM of amitriptyline significantly inhibited the cAMP induction by 0.1 μg/ml cholera toxin. Data are expressed as means ± S.D. of 5 experiments. In forskolin and cholera toxin experiments the control value of cAMP was 1.5 ± 1.0 pmol/well and 2.5 ± 1.9 pmol/well, respectively.

to several mechanisms; TSH-receptor binding inhibition, G protein inhibition, and adenylate cyclase inhibition. To clarify the mechanism of inhibition of cAMP induction, we performed the following experiments.

3.3. Influence of amitriptyline on TSH-receptor binding

We examined whether amitriptyline inhibits the TSH binding to solubilized TSH receptors. As shown in Fig. 3A, 500 μM of amitriptyline had no effect on TSH-receptor binding.

3.4. Effect of amitriptyline on G protein activity

To examine the effect of amitriptyline on G protein, we examined the effects on cAMP induction by cholera toxin. Induction of cAMP by FRTL-5 cells is considered to be regulated mainly by G_s protein, and cholera toxin directly stimulates G_s protein. Thus inhibition of the effect of cholera toxin indicates the inhibition of G_s protein activity. As shown in Fig. 3B, amitriptyline at 100 μM significantly inhibited the cAMP induction by cholera toxin.

3.5. Effect of amitriptyline on cAMP induction by forskolin

Forskolin stimulates adenylate cyclase activity directly. We therefore studied the effect of amitriptyline on the

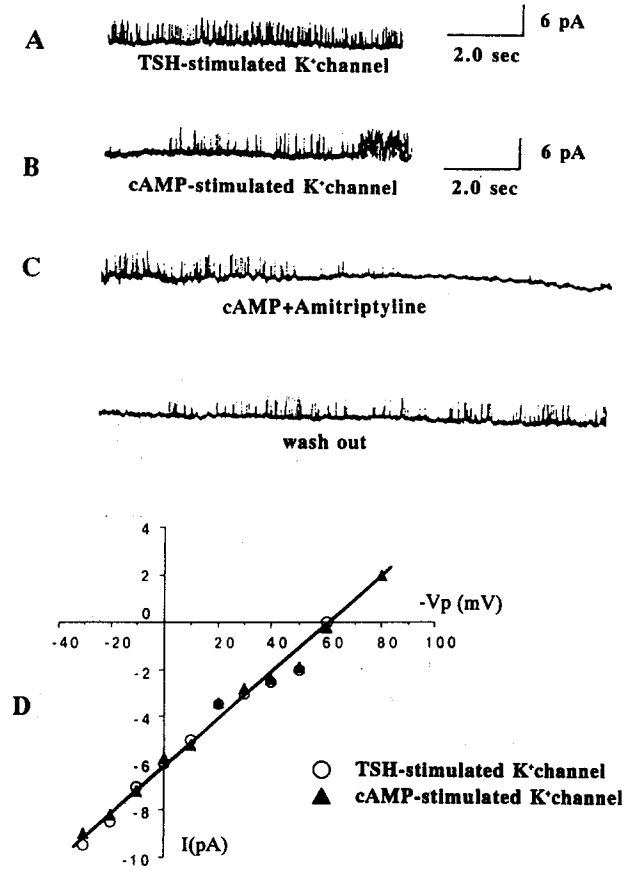

Fig. 4. Characteristics of TSH-stimulated or Bt_2 cAMP-stimulated K^+ channel under the condition of cell-attached patch and effects of amitriptyline on the activation of thyroid K^+ channels. (A) Original single channel recording of channel activity obtained with micropipettes containing 100 μU/ml TSH. (B) In Bt_2 cAMP experiments, pipette did not contain TSH and channel activity was not observed, and after exposure to 100 μM Bt_2 cAMP, the channel activities were observed. (C) 10 μM amitriptyline inhibited the K^+ channel activation by 100 μM Bt_2 cAMP. This channel is activated again after the wash out of amitriptyline. (D) Current-voltage relationships of K^+ channel activated by TSH (open circle) and by Bt_2 cAMP (closed triangle). The ordinate indicates current amplitude; abscissa, −(pipette potential); −Vp.

induction of cAMP by forskolin to investigate the effect on adenylate cyclase. As shown in Fig. 3C, 500 μM of amitriptyline had no effect on the induction of cAMP by forskolin.

3.6. Characteristics of the K^+ channel in the thyroid cells

We have reported the existence of thyroid K^+ channel activated by TSH and Bt_2 cAMP. In that report we revealed that thyroid K^+ channel is activated by the TSH-G_s protein-adenylate cyclase-cAMP system.

We re-examined the characteristics of K^+ channel in the thyroid cells.

Fig. 4 shows the original current traces, effect of amitryptyline on the activation of thyroid K^+ channel and the current-voltage relationships obtained from the cell-attached patch performed on the 100 μU/m TSH stimulated or 100 μM Bt_2 cAMP stimulated FRTL-5 cell membrane. The upward deflection means open state and downward deflection indicates closed state. A current began to fluctuate rapidly between the open state and the closed state. Under the condition of cell-attached patch, the amplitude of the unitary channel current at resting potential was −6 pA. In Fig. 4, the abscissa indicates deviation of the membrane potential from the resting potential; −(pipette potential). Current traces reversed at −(pipette potential) was around +60 mV (no rectification). The mean open time was 8.4 ± 2.0 msfor TSH, and 8.3 ± 2.5 msfor Bt_2 cAMP (means ± S.D.; $n = 4$). The mean single channel conductance calculated from the slope of current-voltage relationship was 94.6 ± 6.2 pS for TSH, and 95.3 ± 5.5 pS for Bt_2 cAMP (means ± S.D.; $n = 4$). There are no significant differences in the characteristics of TSH-stimulated and Bt_2 cAMP-stimulated K^+ channel, indicating that TSH and Bt_2 cAMP activate the same K^+ channel. Na^+ and Cl^- ions were unlikely to carry the current because the current was not observed with a K^+-free Na^+-rich solution in the pipette and a current of similar amplitude was consistently recorded when KCl in the pipette was substituted by K-aspartate (data are not shown).

3.7. Effect of amitriptyline on the activation of thyroid K^+ channel

Because amitriptyline has been revealed to inhibit the thyroid G protein, and cAMP induction by TSH, we examined the effect of amitriptyline on the activation of K^+ channel by cAMP. As shown in Fig. 4C, amitriptyline inhibited the K^+ channel activation by cAMP, indicating the direct inhibition of K^+ channel.

4. Discussion

The cardiac electrophysiological effects of tricyclic antidepressant agents, such as imipramine, amitriptyline, desipramine, chlorimipramine, and dexepin, have been attributed to an inhibition of the fast inward Na^+ current (Tamargo and Rodriguez, 1979; Tamargo et al., 1979; Brannan, 1980; Rodriguez and Tamargo, 1980; Muir et al., 1982). Imipramine also depressed the slow action potentials in K^+-depolarized guinea pig papillary muscles (Garcia et al., 1978). Furthermore, a current voltage clamp study by Isenberg and Tamargo (1985) has demonstrated that imipramine decreases the slow inward current of bovine ventricular myocytes. These observations indicate that imipramine depresses the slow Ca^{2+} channel as well as the fast Na^+ channel of the heart. These drugs possibly inhibit the cardiac K^+ channels, but only few reports have been presented.

In this study, we demonstrated the inhibitory effects of amitriptyline on the activation of K^+ channel and the induction of cAMP by TSH. Amitriptyline affects the

receptor-G protein-adenylate cyclase-cAMP-K^+ channel system, as has been reported in several antiarrhythmic drugs (Nakajima et al., 1989; Mirro et al., 1980; Mokler and Van Arman, 1962; James and Nadeau, 1964; Yoshida et al., 1995). The mechanism of the cAMP suppression is considered to be the suppression of G protein because this drug has no effect on TSH receptor binding and adenylate cyclase stimulation by forskolin. Results showing that this drug inhibited the cAMP production by cholera toxin indicate the direct evidence of G protein inhibition because cholera toxin directly stimulates G protein.

Amitriptyline significantly inhibited the cAMP production by TSH and thyroid-stimulating antibody. This decrease in cAMP production is considered to result in a decrease in thyroid cell function, such as iodine uptake, hormone synthesis, hormone secretion, and thyroid cell growth.

10 μM amitriptyline inhibited the thyroid K^+ channels. This inhibitory effect is due to the direct channel protein suppression, because these drugs suppressed the K^+ channels activated by cAMP. Amitriptyline possibly changes the membrane potential and opening of voltage-dependent I^-, Cl^-, Na^+, Ca^{2+} channels through the inhibition of K^+ channel activation induced by TSH receptor-G protein-cAMP system. Physiological effects of amitriptyline on the thyroid function are unknown. It is very interesting to investigate the effects of amitriptyline on the thyroid cell proliferation and hormone synthesis.

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