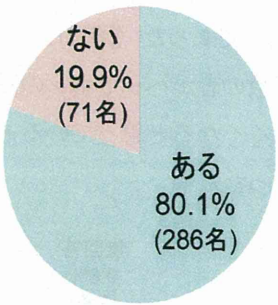

図 1. 小学生のヘッドホン使用経験の有無

「学年」と「ヘッドホン使用経験」に関連があるのではないかと仮説を立て，カイ 2 乗検定を行った．その結果，学年とヘッドホン使用経験とには関連性がみられた ($P<0.001$，図 2)．すなわち，ヘッドホンの使用経験が「ある」と回答した「3 年生」は 70.3%，「4 年生」は 78.5%，「5 年生」は 78.5%，「6 年生」は 95.2%で，学年が上がるにつれヘッドホン使用経験者の割合は多くなった．

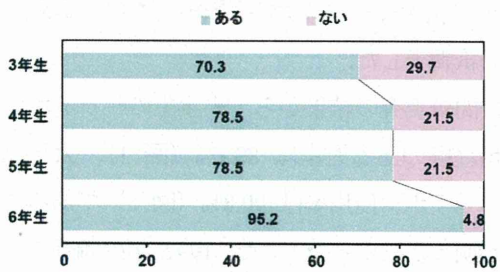

図 2. 学年とヘッドホン使用経験の有無の関係 ($P<0.001$)

質問 2 どのくらいの頻度でヘッドホンやイヤホンを使いますか．

「月に 1 ～ 2 回」と回答した者が 最も多く 36.9% (105 名)，次いで「週に 1 ～ 2 回」が 19.2% (55 名)，「週に 3 ～ 4 回」が 16.8% (48 名)，「ほぼ毎日」が 10.8% (31 名)，「その他」が 16.1% (46 名) であった (図 3)．

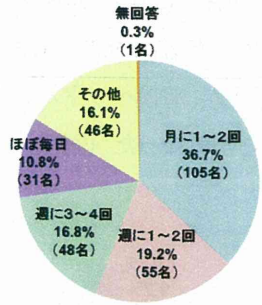

図 3. ヘッドホン使用頻度

質問 3 ヘッドホンやイヤホンを使うと，耳が聞こえにくくなると思いますか

「思う」と回答した者が 40.1% (143 名)，「思わない」と回答した者が 58.3% (208 名) であり，ヘッドホンの使用により耳が聞こえにくくなると認識している児童は 4 割程度であった (図 4)．

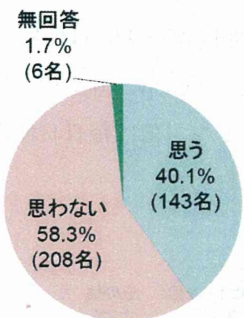

図 4. ヘッドホンの使用により耳が聞こえにくくなると思うか

C. 2. 大学生への質問紙調査

C. 2. 1. 調査対象

アンケート調査の対象者である B 大学の学生 (全 357 名) を対象とした．

C. 2. 2. 結果

質問 1 ヘッドホンやイヤホンを使っていますか．

「はい」と回答した者は 90.8%（324 名），「いいえ」と回答した者は 9.2%（33 名）であり，約 9 割の学生が，普段ヘッドホンを使用していることが明らかになった（図 5）．

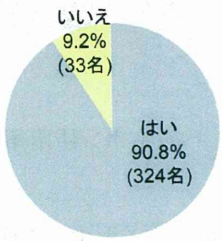

図 5.　大学生のヘッドホンの使用の有無

質問 2　どのくらいの頻度でヘッドホンやイヤホンを使いますか．

「ほぼ毎日」と回答した者が最も多く 59.3%（192 名），次いで「週に 3 ～ 4 回」が 20.7%（67 名），「週に 1 ～ 2 回」が 15.4%（50 名），「月に 1 ～ 2 回」が 3.4%（11 名），「その他」が 1.2%（4 名）であった（図 6）．従って，約 6 割がヘッドホンを「ほぼ毎日」使用していることが明らかになった．

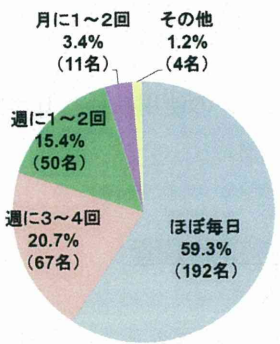

図 6.　ヘッドホン使用頻度

質問 3　ヘッドホンやイヤホンを使うと，耳が聞こえにくくなると思いますか

「思う」と回答した者が 53.8%（192 名），「思わない」が 18.5%（60 名），「わからない」が 27.2%（97 名）であった（図 7）．
従って，約半数の者がヘッドホンの使用により耳が聞こえにくくなると思っているということが明らかになった．

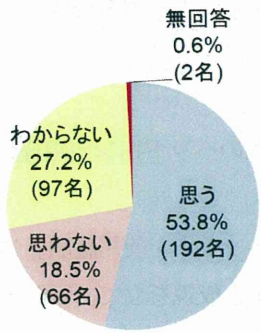

図 7.　ヘッドホンの使用により耳が聞こえにくくなると思うか

D.　考察

本分担研究は，大学生や小学生にヘッドホンの使用状況やヘッドホン難聴に対する認識を質問紙調査した．

質問紙調査の結果から，小学生のヘッドホン使用経験のある児童は 80.1%（図 1），大学生のヘッドホン使用率は 90.8%（図 5）であることが明らかになった．また，1992 年の調査では高校生のヘッドホンの所有率は 41%，1989 年の調査では中学生のヘッドホン使用率は 51%であった．これらのことから，約 20 年前と比較してヘッドホンは社会全体に広く普及し，生活に深く根付いていると考えられる．

しかし，ヘッドホン使用に関する意識は高いとは言えないことが明らかになった．本研究では，ヘッドホンの使用により耳が聞こえにくくなると思う者の割合は小学生で 40.1%，大学生で 53.8%という結果となった（図 4, 7）．また，過去に高校生を対象とした調査では，「ヘッドホ

ンを使うことによって難聴が引き起こされると思うか」という質問に対して「思う」と答えた者の割合は 55.3%，中学生を対象とした調査では，「ヘッドホン等を使うと耳が聞こえにくくなると思いますか」という質問に対して「思う」と答えた者の割合は 41.1%である．ヘッドホンの使用率は，約 20 年前と比較して約 30 ～ 40%上昇しているのにもかかわらず，ヘッドホン使用に関する意識はほとんど変わっていない．これらのことから，ヘッドホンによる聴覚への影響を意識せず，ヘッドホンを使用している者が増加しているということが言える．

ヘッドホン難聴の原因は，大音量で聴くこと，長時間聴くこと，周波数の高い音を聴くことなどである．しかし，ヘッドホン難聴の発症には，その時の体調や精神面，耳の強さ等，個人差があり，一概に，大音量で長時間の音を聴くと絶対にヘッドホン難聴になるとは言えない．本調査では，ヘッドホン使用時間と自覚症状の有無に関連性が認められ（データ未掲載），ヘッドホン使用時間が長くなるほど，自覚症状のある者の割合が増える傾向がみられた．そのため，ヘッドホン使用に関する指導の際には長時間の音を聴かないことでヘッドホン難聴のリスクを軽減できるが，普通の大きさの音を聴いていても，必ずしもヘッドホン難聴を防ぐことができるとは言えないということを伝えていく必要があると思われる．

現段階では，ヘッドホンを使っている人の聴覚の変化を数千人から数万人単位で数十年にわたって調査した例は乏しく，症例も少ない．しかし，ヘッドホンの使用率が増加し，ヘッドホンが社会全体の生活に定着しているため，今後ヘッドホン難聴になる者が増えていく可能性は十分に考えられる．従って，ヘッドホン使用による聴覚への影響が示唆されている現代においては，学校でのヘッドホン難聴についての指導や啓蒙は，児童生徒の聴覚を守るために必要なことであると考える．

ヘッドホン使用に関する指導については，児童生徒の実態を踏まえたうえで行う必要がある．影山の調査によると，ストレス感・疲労感の発散・気晴らし行動等のためにヘッドホンが多用されがちであり，ヘッドホンの過度の使用状況が存在する場合，その背景には心身の健康問題が隠れている可能性がある．従って，ヘッドホン使用に関する指導の際には，大音量・長時間の使用を控えることとあわせて，ストレス解消方法に関しての指導をすること，心身の健康問題に配慮すること等が必要である．さらに，本研究の結果から，小学生，大学生ともに，音楽聴取にのみヘッドホンを使用しているのではなく，ゲームや語学，楽器の演奏のためにヘッドホンを使用している者もいることが明らかになった（データ未掲載）．従って，指導の際には音楽聴取を目的としたヘッドホンの使用に焦点を当てるだけではなく，ゲームや楽器の演奏等，広い範囲に焦点を当てて指導をしていくことが重要であると考えられる．

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Ⅲ．研究成果の刊行に関する一覧表

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Molecular and Cellular Pharmacology

Hetero-oligomerization between adenosine A_1 and thromboxane A_2 receptors and cellular signal transduction on stimulation with high and low concentrations of agonists for both receptors

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ABSTRACT

Growing evidence indicates that G protein-coupled receptors can form homo- and hetero-oligomers to diversify signal transduction. However, the molecular mechanisms and physiological significance of G protein-coupled receptor-oligomers are not fully understood. Both ADOR1 (adenosine A_1 receptor) and TBXA2R (thromboxane A_2 receptor α; TPα receptor), members of the G protein-coupled receptor family, act on astrocytes and renal mesangial cells, suggesting certain functional correlations. In this study, we explored the possibility that adenosine A_1 and TPα receptors form hetero-oligomers with novel pharmacological profiles. We showed that these receptors hetero-oligomerize by conducting coimmunoprecipitation and bioluminescence resonance energy transfer ($BRET^2$) assays in adenosine A_1 receptor and TPα receptor-cotransfected HEK293T cells. Furthermore, coexpression of the receptors affected signal transduction including the accumulation of cyclic AMP and phosphorylation of extracellular signal-regulated kinase-1 and -2 was significantly increased by high and low concentrations of adenosine A_1 receptor agonist and TPα agonists, respectively. Our study provides evidence of hetero-oligomerization between adenosine A_1 and TPα receptors for the first time, and suggests that this oligomerization affects signal transduction responding to different concentrations of receptor agonists.

1. Introduction

The classical concept that G protein-coupled receptors function as monomers has been changing due to the increasing evidence of G protein-coupled receptor oligomerization (Dalrymple et al., 2008; Rozenfeld and Devi, 2009). Oligomerization can increase the diversity of G protein-coupled receptor phenotypes (Panetta and Greenwood, 2008). Therefore, it is important to clarify the combinations of G protein-coupled receptors which form hetero-oligomers.

Adenosine has various physiological effects, causing reductions in sympathetic and parasympathetic activities, pre-synaptic inhibition, ischemic pre-conditioning and renal mesangial cell proliferation via adenosine receptors (Fredholm et al., 2001; Martínez-Salgado et al., 2007). Adenosine receptors are subclassified into A_1, A_{2A}, A_{2B} and A_3 subtypes (Fredholm et al., 2001), all of which couple to G proteins. G proteins that are activated by G protein-coupled receptors and made up of alpha (α), beta (β), and gamma (γ) subunits. Gα subunits have many classes and behave differently in the recognition of the effectors. The adenosine A_1 receptor, the official gene name is ADOR1, which is coupled to members of the pertussis toxin (PTX)-sensitive family of G proteins, $G_{i/o}α$ (G_i) proteins, which inhibits cyclic AMP production (Ralevic and Burnstock, 1998). Hetero-oligomerization of adenosine A_1 receptors with various G protein-coupled receptors was additionally reported. For example, adenosine A_1 and $P2Y_2$ receptors formed a hetero-oligomer with novel pharmacological properties, including ligand-binding pharmacology and receptor signal responses, in human embryonic kidney 293T (HEK293T) cells cotransfected with these receptors (Suzuki et al., 2009).

The thromboxane A_2 (TXA_2) is an unstable arachidonic acid metabolite. The TXA_2 receptor (TP receptor), the official gene name is TBXA2R, also belongs to the G protein-coupled receptor family, and communicates mainly with $G_{q/11}α$ (G_q) (Johnston et al., 2001; Shenker et al., 1991), resulting in phospholipase C (PLC) activation. In addition, the TP receptor couples to other G proteins, including $G_{12}α$ (G_{12}), $G_{13}α$ (G_{13}), G_i and $G_sα$ (Cordeaux et al., 2000; Djellas et al., 1999; Hirata et al., 1996; Nakahata, 2008; Offermanns et al., 1994). TXA_2 elicits diverse physiological/pathophysiological functions including the proliferation of glial cells and proliferation and contraction of renal mesangial cells upon binding to TP receptors (Nakahata,

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2008). There are two alternatively spliced isoforms, TXA_2 receptor αTPα receptor) and TXA_2 receptor β (Hirata et al., 1991; Raychowdhury et al., 1994), which differ only at the C-terminus. Both adenosine A_1 and TP receptors are expressed in astrocytes and renal mesangial cells (Darè et al., 2007; Martínez-Salgado et al., 2007; Nakahata, 2008). In addition, both receptors are involved in decreasing the glomerular filtration rate via enhancement of renal mesangial cell proliferation and contraction (Martínez-Salgado et al., 2007; Nakahata, 2008). The contraction of mesangial cells was induced by incubation with platelet-supernatants, and abolished by a TP receptor blocker (Arribas et al., 1993). TXA_2 and adenosine are released from platelets. TXA_2 is involved in platelet activation, leading to platelet shape changes, aggregation and secretion (Nakahata, 2008). On the other hand, adenosine is a potent inhibitor of platelet activation (Cooper et al., 1995). From this evidence, it is possible that adenosine A_1 and TP receptors interact physically and functionally. In this study, we examined the hetero-oligomerization of the adenosine A_1 and TP receptors using coimmunoprecipitation and bioluminescence resonance energy transfer ($BRET^2$) techniques, and the effects on their signal transduction in HEK293T cells cotransfected with plasmids for these receptors.

2. Materials and methods

2.1. Materials

Human embryonic kidney 293T (HEK293T) were provided by Hiroyasu Nakata (Department of Molecular Cell Signalling, Tokyo, Japan). DeepBlueC was purchased from Perkin Elmer Life Sciences (Boston, MA). Forskolin was purchased from Wako Pure Chemicals (Osaka, Japan). Pertussis toxin and 4-(3-butoxy-4-methoxybenzyl) imidazolidin-2-one (Ro20-1724) were from Calbiochem (San Diego, CA). 1S-[1α,2α(Z),3α,4α]]-7-[3-[[2-[(phenylamino)carbonyl]hydrazine] methyl]-7-oxabicyclo[2.2.1]hept-2-yl]-5-heptenoic acid (SQ29548) and 9,11-dideoxy-9α,11α-epoxymethanoprostaglandin $F_2α$ (U46619) were from Cayman Chemical (Ann Arbor, MI). N^6-cyclopentyladenosine (CPA) and 8-cyclopentyl-1,3-dipropylxanthine (DPCPX) was from Sigma Aldrich (St. Louis, MO). Fura 2-AM was purchased from Dojindo (Kumamoto, Japan). Anti-HA antibody and FuGENE HD Transfection Reagent were purchased from Roche Applied Science (Manheim, Germany). HRP-conjugated anti-mouse IgG, Protein G-Sepharose™ and ECL™ Western blotting detection reagent were purchased from GE Healthcare (Piscataway, NJ). Anti-myc 9E10 antibody was purchased from Covance (Berkeley, CA). Anti-adenosine A_1 receptor antibody was from Acris Antibodies GmbH (Hiddenhausen, Germany). Anti-ERK 1/2 antibody, anti-phospho-ERK 1/2 antibody and HRP-conjugated anti-rabbit IgG were purchased from Cell Signaling Technology (Beverly, MA). HRP-conjugated anti-rat IgG was purchased from Santa Cruz Biotechnology (Santa Cruz, CA). Triple hemagglutinin-tagged adenosine A_1 receptor (3HA-adenosine A_1 receptor), hemagglutinin-tagged lysophosphatidic acid 1 receptor (HA-lysophosphatidic acid 1 receptor) and G-protein alpha q (G_q) plasmids were purchased from UMR cDNA Resource Center (Rolla, MO). Other chemicals used were of reagent grade or the highest quality available.

2.2. Construction of plasmids, cell culture and transfection

The HA-tagged TPα receptor (HA-TPα receptor), HA-tagged adenosine A_1 receptor (HA-adenosine A_1 receptor), myc-tagged adenosine A_1 receptor (myc-adenosine A_1 receptor), HA-adenosine A_1 receptor-Renilla luciferase (HA-adenosine A_1 receptor-Rluc) and HA-adenosine A_1 receptor-modified green fluorescent protein (HA-adenosine A_1 receptor-GFP^2) were constructed as described previously (Suzuki et al., 2006; Suzuki et al., 2009). HEK293T cells were grown in Dulbecco's modified Eagle's medium (DMEM; Sigma Aldrich, St. Louis, MO) containing 10% fetal calf serum, 50 units/ml penicillin, and 50 μg/ml streptomycin in a humidified incubator with a 5% CO_2 atmosphere at 37 °C. Transfections were done with FuGENE HD Transfection Reagent as described before (Suzuki et al., 2006).

2.3. Immunoprecipitation

HEK293T cells transfected with the myc-adenosine A_1 receptor, HA-TPα receptor and HA-lysophosphatidic acid 1 receptor were cultured for 48 h. Approximately 10^7 cells were collected by centrifugation at 1900×*g* and washed twice with Dulbecco's phosphate-buffered saline (137 mM NaCl, 2.7 mM KCl, 1.5 mM KH_2PO_4, and 8.1 mM Na_2HPO_4). Cells were disrupted by sonication using a Handy Sonic UR-20P (Tomy Seiko, Tokyo, Japan) in 300μl of lysis buffer (20 mM Tris–HCl pH7.4, 1 mM EDTA, 150 mM NaCl, 1% TritonX-100, and 1 mM Na_3VO_4). After incubation for 3 h at 4 °C, the solution was centrifuged at 17,400×*g* for 20 min at 4 °C, and the supernatant was pre-cleared with 30 μl/ml of 50% (w/v) Protein G-Sepharose in lysis buffer, followed by centrifugation at 17,400×*g* for 10 s to remove nonspecifically bound proteins. The supernatant was incubated with anti-myc 9E10 antibody (10 μg/ml) for 1 h, followed by Protein G-Sepharose™ (50 μl/ml) for 2 h. The mixture was centrifuged, the resulting immune complex was washed twice with 500 μl of lysis buffer, and bound proteins were eluted with 30 μl of Laemmli sample buffer (75 mM Tris–HCl, 2% SDS, 10% glycerol, 3% 2-mercaptoethanol, and 0.003% bromophenol blue).

2.4. Extracellular signal-regulated kinase1/2 (ERK1/2) assay

HEK293T cells were seeded onto 12-well plates at a density of 10^5 cells/well. At 24 h after seeding, the cells were transfected with the 3HA-adenosine A_1 receptor and HA-TPα receptor. For pertussis toxin treatment, cells were incubated with 100 ng/ml of pertussis toxin for 16 h. They were washed with a Tyrode-HEPES solution (137 mM NaCl, 2.7 mM KCl, 1.0 mM MgCl, 1.8 mM $CaCl_2$, 10 mM HEPES, and 5.6 mM glucose, pH 7.4) and preincubated for 20 min at 37 °C. The cells were incubated with DPCPX, SQ29548 or YM-254890 (Astellas, Tokyo, Japan) for 10 min prior to stimulation with CPA and/or U46619 for 10 min at 37 °C. The reaction was terminated by aspiration of the medium and cells were lysed in 150 μl of ice-cold Laemmli sample buffer.

2.5. Western blot analysis

The samples were loaded on a 10% sodium dodecyl sulfate (SDS)-polyacrylamide gel for electrophoresis (PAGE), and transferred to polyvinylidene difluoride (PVDF) membranes. The membranes were blocked with 5% non-fat milk in TBST (10 mM Tris–HCl, 100 mM NaCl, and 0.05% Tween 20, pH 7.4), incubated with anti-HA antibody (1:2000), anti-adenosine A_1 receptor antibody (1:2000), anti-ERK1/2 antibody (1:2000) or anti-phospho ERK1/2 antibody (1:2000) for 90 min followed by HRP-conjugated anti-rat IgG (1:2000) or anti-rabbit IgG (1:5000) for 90 min at room temperature, and detected with ECL™ Western blotting detection reagents.

2.6. $BRET^2$ assay

HEK293T cells (5×10^5 per 35 mm dish) were cotransfected with a fixed amount (1 μg) of HA-adenosine A_1 receptor-Rluc and HA-adenosine A_1 receptor-GFP^2 plasmids and increasing concentrations of unfused receptor plasmids (0, 0.4, 0.8, 1.2, 1.6, 2.0 μg of HA-adenosine A_1 receptor or HA-TPα receptor plasmids, and 0, 0.6, 1.2, 1.8, 2.7, 3.6 μg of HA-lysophosphatidic acid 1 receptor) using the FuGENE HD Transfection reagent. For a control, non-transfected cells were used. At 48 h after transfection, the cells were harvested and suspended in assay buffer (Dulbecco's phosphate-buffered saline containing 0.1 mg/ml $CaCl_2$, 0.1 mg/ml $MgCl_2$, and 1 mg/ml D-

glucose). Suspended cells were distributed in a white-walled 96-well Plate (OptiPlate, Perkin Elmer Life Sciences) at a density of 1×10^6 cells/well, and incubated for 20 min at 37 °C. DeepBlueC was then added at a final concentration of 5 μM. Assays were conducted immediately using a Fusion α universal microplate analyzer (Perkin Elmer Life Sciences) for the detection of Rluc at 410 nm and GFP^2 at 515 nm. The BRET ratio was calculated as the ratio between GFP^2 and Rluc emission, corrected with the background emission from non-transfected cells.

2.7. Measurement of intracellular Ca^{2+} concentrations

The measurement of intracellular free Ca^{2+} concentration ($[Ca^{2+}]_i$) was carried out as described previously (Sasaki et al., 2006). HEK293T cells were cotransfected with 3HA-adenosine A_1 receptor/G_q, HA-TPα receptor/G_q or 3HA-adenosine A_1 receptor/HA-TPα receptor/G_q and cultured for 48 h. Transfected cells were harvested and suspended in Tyrode's solution (137 mM NaCl, 2.7 mM KCl, 1.0 mM $MgCl_2$, 1.8 mM $CaCl_2$, 5.6 mM glucose, and 10 mM HEPES, pH 7.4), incubated with 5 mM fura2-AM for 15 min at 37 °C. Subsequently the cells were washed twice with Tyrode's solution and modified Tyrode's solution (137 mM NaCl, 2.7 mM KCl, 1.0 mM $MgCl_2$, 0.18 mM $CaCl_2$, 5.6 mM glucose, and 10 mM HEPES, pH 7.4), and resuspended in modified Tyrode's solution at 10^6 cells/ml. Fura2-AM fluorescence was recorded (1.5 ml aliquots) at 37 °C with gentle stirring using a FP-6500 (JASCO Corporation, Tokyo) with excitation at 340 nm and 380 nm and emission at 510 nm. Calibration of the signal was performed in each sample by adding 0.15% Triton X-100 to obtain maximal fluorescence (F_{max}) and then 2.5 mM EGTA to obtain minimal fluorescence (F_{min}). The ratio of fluorescence at 340 nm to that at 380 nm is a measure of $[Ca^{2+}]_i$, assuming a K_d of 244 nM Ca^{2+} for fura 2-AM.

2.8. Cyclic AMP assay

HEK293T cells were seeded onto 48-well plates at a density of 2×10^4/well. At 24 h after seeding, the cells were transfected with the 3HA-adenosine A_1 receptor and/or HA-TPα receptor. After 48 h, the medium was changed to Eagle's minimum essential medium—20 mM HEPES, and preincubated for 20 min at 37 °C. The cells were incubated with 100 μM of Ro 20-1724 as a phosphodiesterase inhibitor for 15 min with or without receptor antagonists. The cells were then stimulated with a receptor agonist and 100 μM forskolin, and incubated for another 10 min. Reactions were terminated by adding 2.5% perchloric acid. Acid-extracts were mixed with a 1/10 volume of 4.2 N KOH to neutralize the acid, forming potassium perchlorate as a precipitate. The cyclic AMP in the supernatant was succinylated and determined using a radioimmunoassay kit (Yamasa, Tokyo, Japan) according to the manufacturer's directions.

3. Results

3.1. Coimmunoprecipitation of myc-adenosine A_1 and HA-TPα receptors

To determine whether the adenosine A_1 and TPα receptors interact, we performed a coimmunoprecipitation analysis using HEK293T cells transiently transfected with the myc-adenosine A_1 receptor and/or HA-TPα receptor. We detected the myc-adenosine A_1 receptor in the complex precipitated with anti-myc in cells transfected with the myc-adenosine A_1 receptor and cotransfected with the myc-adenosine A_1 receptor/HA-TPα receptor (Fig. 1A, left panel, lanes 2 and 3 in 'IP', arrow), indicating the validity of this method. Importantly, we detected the band which corresponds to the HA-TPα receptor in the complex precipitated with anti-myc only in cells cotransfected with myc-adenosine A_1/HA-TPα receptors (Fig. 1B, lane 3 in 'IP', arrows). This band was not obtained from the cells transfected with

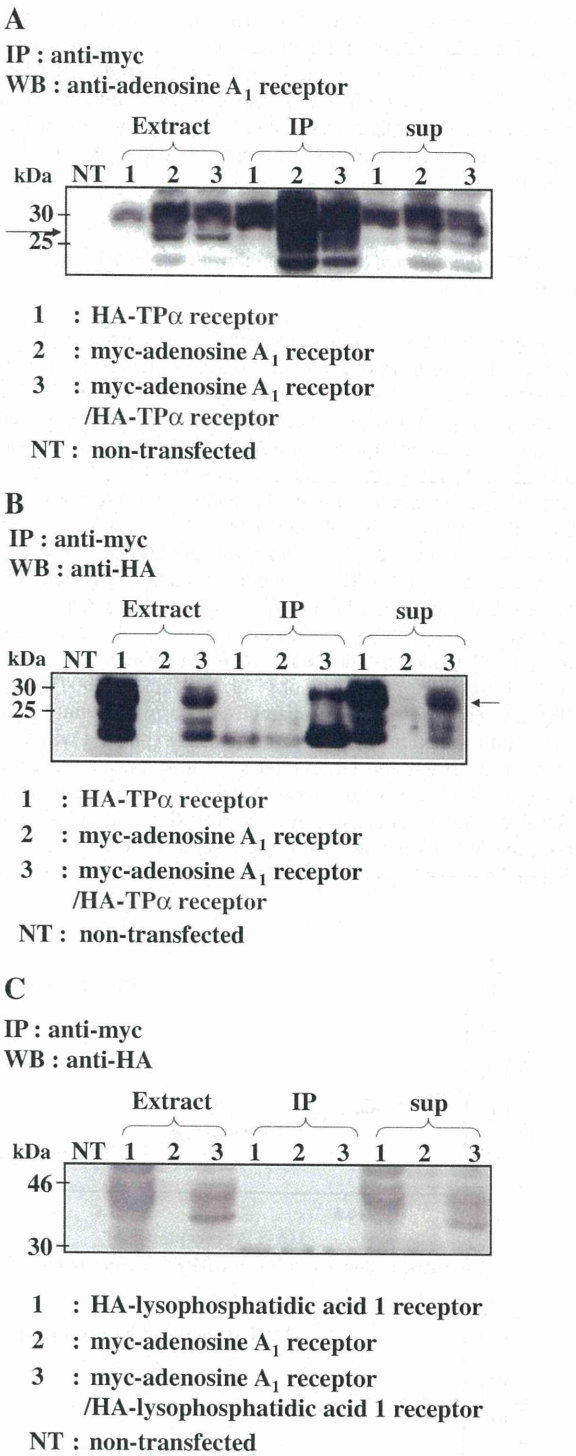

Fig. 1. Coimmunoprecipitation of the myc-adenosine A_1 receptor and HA-TPα receptor or lysophosphatidic acid 1 receptor. HEK293T cells transiently transfected with the myc-adenosine A_1 receptor and HA-prostanoid TPα receptor (A and B) or HA-lysophosphatidic acid 1 receptor (C). Cell lysates were immunoprecipitated with anti-myc antibody, and precipitates were analyzed by Western blotting with anti-HA (B and C) and anti-adenosine A_1 receptor (A) antibodies. A negative control of non-transfected cells showed no detectable bands (lane 'NT'). Arrows indicate the myc-adenosine A_1 receptor (A) and TPα receptor (B). Extract, solubilized membrane extract; IP, immunoprecipitate; sup, supernatant following immunoprecipitation; WB, Western blotting with the antibody indicated. Approximate molecular masses are shown in kDa.

the HA-TPα receptor alone (Fig. 1B, lane 1 in 'IP'). These results suggest the formation of a complex between myc-adenosine A_1 receptors and HA-TPα receptors in the cotransfected cells. We did not detect the myc-adenosine A_1 receptor in the complex precipitated with anti-myc in cells cotransfected with the myc-adenosine A_1 receptor and HA-adenosine A_1 receptor instead of HA-TPα receptor (Fig. 1C).

3.2. BRET^2 inhibition of the homo-dimerization of adenosine A_1 receptors by TPα receptors

We performed a competitive BRET^2 assay using HEK293T cells transiently transfected with Rluc- and GFP^2-fused HA-adenosine A_1 receptors and unlabeled HA-TPα receptors. With this strategy, adenosine A_1 receptor/TPα receptor interactions were assessed based on the competition of adenosine A_1 receptor homodimerization with adenosine A_1 receptor/TPα receptor heterodimerization in the living cells. The homodimerization of adenosine A_1 receptors was confirmed by BRET^2 assay using HA-adenosine A_1 receptor-Rluc and HA-adenosine A_1 receptor-GFP^2 (Suzuki et al., 2009). The specificity of adenosine A_1 receptor/TPα receptor interaction was verified by the observed decrease in the BRET^2 signals of adenosine A_1 receptor homodimerization when constant amounts of HA-adenosine A_1 receptor-Rluc and HA-adenosine A_1 receptor-GFP^2 were coexpressed with increasing concentrations of unlabeled HA-TPα receptor (Fig. 2A). For a control, a comparable increasing amount of unlabeled HA-lysophosphatidic acid 1 receptor was coexpressed with a constant amount of HA-adenosine A_1 receptor-Rluc and HA-adenosine A_1 receptor-GFP^2, which did not cause a significant decrease in the BRET^2 signal of the HA-adenosine A_1 receptor-Rluc and HA-adenosine A_1 receptor-GFP^2 pairs (Fig. 2B). These results suggested that in cotransfected living cells, the adenosine A_1 and TPα receptors formed a heterodimer whereas the adenosine A_1 receptor and lysophosphatidic acid 1 receptor did not.

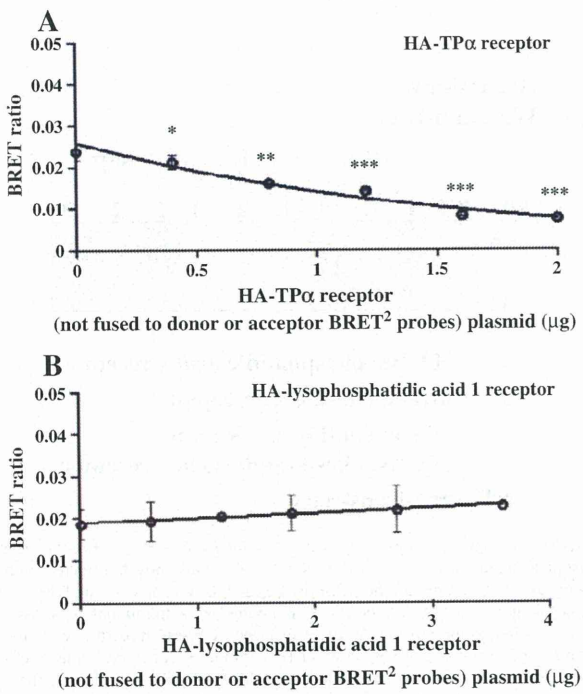

Fig. 2. Competitive BRET^2 assay. HEK293T cells were cotransfected with a fixed concentration of HA-adenosine A_1 receptor-Rluc and HA-adenosine A_1 receptor-GFP^2 plasmids and increasing amounts of (A) HA-TPα receptor, or (B) HA-lysophosphatidic acid 1 receptor plasmids. The data are the mean ± S.E.M for three experiments. *P<0.05, **P<0.005, ***P<0.001 vs. 0 (Dunnett's test).

3.3. Effect of adenosine A_1 receptor and TPα receptor agonists on forskolin-stimulated cyclic AMP accumulation

We next examined whether the adenosine A_1 receptor/TPα receptor-coexpression affects signal transduction. We studied adenosine A_1 receptor agonist-induced inhibition of adenylyl cyclase via $G_{i/o}$. As expected, CPA (1 μM), a selective adenosine A_1 receptor agonist (Ralevic and Burnstock, 1998), decreased the forskolin-evoked increase in cyclic AMP levels in the cells expressing the 3HA-adenosine A_1 receptor alone and the cells coexpressing 3HA-adenosine A_1 and HA-TPα receptors (Fig. 3A and B, 3rd column from the left). Furthermore, DPCPX (10 μM) (Ralevic and Burnstock, 1998), a selective adenosine A_1 receptor antagonist, antagonized the CPA-induced inhibition of cyclic AMP levels in both 3HA-adenosine A_1 receptor-expressing cells and 3HA-adenosine A_1 receptor/HA-TPα receptor-coexpressing cells (Fig. 3A and B, 6th column from the left). U46619, a selective TPα receptor agonist (Abramovitz et al., 2000), caused a small increase in forskolin-evoked cyclic AMP levels in cells

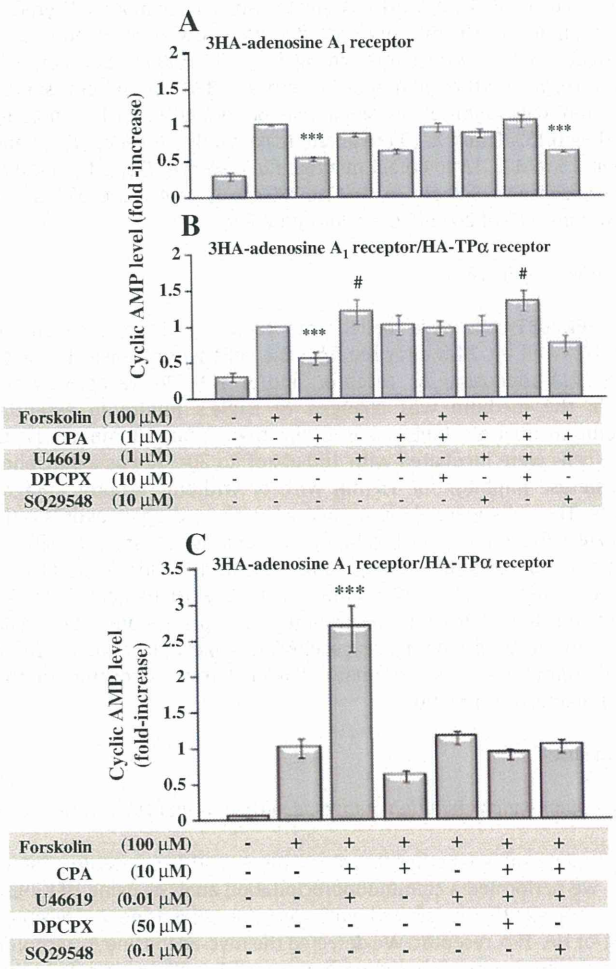

Fig. 3. Effects of adenosine A_1 receptor and TPα receptor agonists and antagonists on forskolin-stimulated cyclic AMP accumulation. Assays were performed in HEK293T cells transfected with the 3HA-adenosine A_1 receptor (A and C), HA-TPα receptor (D), and 3HA-adenosine A_1 receptor/HA-TPα receptor (B, E and F). The data were normalized to the forskolin-evoked cyclic AMP concentration in control cells (without ligands). The data are the mean ± S.E.M of triplicate determinations, and the results are representative of two similar experiments. #P<0.05, ***P<0.001, vs. forskolin-stimulated cyclic AMP accumulation (Tukey's test).

expressing the HA-TPα receptor alone (data not shown) and coexpressing 3HA-adenosine A_1 and HA-TPα receptors (Fig. 3B). Furthermore, the U46619-induced increase in cyclic AMP was inhibited by SQ29548 (10 μM), a TPα receptor antagonist (Nakahata, 2008), in 3HA-adenosine A_1 receptor/HA-TPα receptor-coexpressing cells (Fig. 3B, 7th column from the left). The simultaneous addition of CPA (1 μM) and U46619 (1 μM) decreased the forskolin-evoked increase in cyclic AMP levels slightly in cells expressing the 3HA-adenosine A_1 receptor alone (Fig. 3A, 5th column from the left), though not at all in 3HA-adenosine A_1 receptor/HA-TPα receptor-coexpressing cells (Fig. 3B, 5th column from the left). Notably, we found that the forskolin-evoked increase among 3HA-adenosine A_1 receptor/HA-TPα receptor-coexpressing cells was markedly increased by the simultaneous addition of CPA (10 μM) and U46619 (0.01 μM) (Fig. 3C, 3rd column from the left). This characteristic increase was not induced by stimulation with CPA (10 μM) or U46619 (0.01 μM) individually (Fig. 3C, 4th and 5th columns). In addition, it was not seen in cells expressing the 3HA-adenosine A_1 receptor or HA-TPα receptor alone (data not shown). DPCPX (50 μM) and SQ29548 (0.1 μM) inhibited this synergistic increase by the simultaneous addition of CPA (10 μM) and U46619 (0.01 μM) in 3HA-adenosine A_1 receptor/HA-TPα receptor-coexpressing cells (Fig. 3C, 6th and 7th columns from the left).

3.4. Effect of agonists for both adenosine A_1 and TPα receptors on ERK1/2 phosphorylation

It is reported that the individual stimulation of both the adenosine A_1 receptor and TPα receptor causes the activation of extracellular signal-regulated kinase-1/2 (ERK1/2), a major kinase of mitogen-activated protein kinase (MAPK) signaling pathways (Dickenson et al., 1998; Miggin and Kinsella, 2001). Then, we examined whether adenosine A_1 receptor/TPα receptor-coexpression affects ERK1/2 phosphorylation. Importantly, ERK1/2 phosphorylation in 3HA-adenosine A_1 receptor/HA-TPα receptor-coexpressing cells was synergistically enhanced by the simultaneous addition of CPA and U46619 in all concentrations we tested, as compared with ERK1/2 phosphorylation in cells expressing the 3HA-adenosine A_1 receptor or HA-TPα receptor alone (Fig. 4A–C). This synergistic effect was not found on stimulation with CPA or U46619 alone (Fig. 4D–F). In addition, we examined the effects of receptor antagonists and pertussis toxin and YM-254890, which block signal transduction through G_i and G_q, respectively. As shown in Fig. 5, pretreatment of 3HA-adenosine A_1 receptor/HA-TPα receptor-coexpressing cells with DPCPX significantly inhibited the synergistic effect of CPA (0.1 μM) and U46619 (0.1 μM) or CPA (1 μM) and U46619 (1 nM) (Fig. 5B and D). However, DPCPX did not block ERK1/2 phosphorylation on the addition of 1 nM CPA and 1 μM U46619 (Fig. 5C). In addition, SQ29548 did not have any inhibitory effect on ERK1/2 phosphorylation (Fig. 5A–D). Pertussis toxin and YM-254890 significantly inhibited the synergistic effect of CPA (0.1 μM) and U46619 (0.1 μM) or CPA (1 μM) and U46619 (1 nM) on ERK1/2 phosphorylation (Fig. 6A, B and D), whereas neither inhibitor had a significant effect on 1 nM CPA and 1 μM U46619-stimulated ERK1/2 phosphorylation (Fig. 6A and C).

3.5. Effect of coexpression of adenosine A_1 and TPα receptors on Ca^{2+} signaling

TPα receptor stimulation causes the activation of phospholipase C and subsequent elevation of $[Ca^{2+}]_i$ via G_q (Shenker et al., 1991). In addition, adenosine A_1 receptor stimulation also causes an elevation of $[Ca^{2+}]_i$ via Gβ and Gγ released from G_i (Dickenson and Hill, 1998; Quitterer and Lohse, 1999). We examined whether adenosine A_1 receptor/TPα receptor-coexpression affects the alteration of $[Ca^{2+}]_i$ in Fura2-AM-loaded cells. Stimulation of cells expressing the HA-TPα receptor and coexpressing the 3HA-adenosine A_1 and HA-TPα receptors with U46619 (1 μM) induced a rapid, transient elevation of $[Ca^{2+}]_i$ (Fig. 7B and C). Stimulation of 3HA-adenosine A_1 receptor-expressing cells and 3HA-adenosine A_1 receptor/HA-TPα receptor-coexpressing cells with CPA (1 μM) induced a subtle elevation of $[Ca^{2+}]_i$ (Fig. 7D and F). Simultaneous treatment with U46619 (1 μM) and CPA (1 μM) induced elevations of $[Ca^{2+}]_i$ in 3HA-adenosine A_1 receptor-expressing cells, HA-TPα receptor-expressing cells and 3HA-adenosine A_1 receptor/HA-TPα receptor-coexpressing cells (Fig. 7G, H and I), but we could not see obvious differences from stimulation with each agonist alone. Simultaneous stimulation of U46619 and CPA-induced elevation of $[Ca^{2+}]_i$ were blocked by pretreatment with SQ29548 (3 μM, Fig. 8D–F), but not DPCPX (Fig. 8A–C).

4. Discussion

The present study proves the existence of a heteromeric complex formed by adenosine A_1 and TPα receptors in solubilized and living HEK293T cells coexpressing these receptors. Fig. 1 shows that coimmunoprecipitation revealed that the adenosine A_1 and TPα receptors associate in HEK293T cells cotransfected with these receptors. The possibility of hetero-oligomerization between adenosine A_1 and TPα receptors suggested by the coimmunoprecipitation assay using solubilized cell membranes (Fig. 1) confirmed with a competitive $BRET^2$ assay (Fig. 2). $BRET^2$ assay is using living cells, so we observed the interaction in the intact cells. The specific decrease in the $BRET^2$ signal of Rluc- and GFP^2-fused adenosine A_1 receptor pairs on transfection of an increased amount of unlabeled TPα receptor was similar to the result obtained using an unlabeled adenosine A_1 receptor substituted for the TPα receptor (data not shown). Significant $BRET^2$ signal between Rluc- and GFP^2-fused adenosine A_1 receptors indicated a homodimer as reported previously (Suzuki et al., 2009). It is likely that unlabeled TPα receptors and unlabeled adenosine A_1 receptors competitively blocked the interaction between Rluc- and GFP^2-fused adenosine A_1 receptors. In the case of nonspecific interaction, a decrease in BRET signal would not be obtained (Marullo and Bouvier, 2007), as a result using the lysophosphatidic acid 1 receptor substituted for the TPα receptor (Fig. 2B). These results confirmed that adenosine A_1 and TPα receptors form hetero-oligomers in cotransfected HEK293T cells.

G protein-coupled receptor oligomerization may increase the diversity of G protein-coupled receptor phenotypes by altering the function of receptors. It is important whether oligomerization has some specific effects on the function of the receptor. In this study, in adenosine A_1 receptor/TPα receptor-coexpressing HEK293T cells, the cyclic AMP level and ERK1/2 activation were significantly altered. However, Ca^{2+} signaling was not altered by the coexpression of adenosine A_1 and TPα receptors. In the cells which were not transfected with the TPα receptor, $[Ca^{2+}]_i$ was slightly increased by stimulation with the TPα receptor agonist, possibly because of endogenous TPα receptors in HEK293T cells (D'Angelo et al., 1996). Whereas, in the adenosine A_1 receptor/TPα receptor-coexpressing cells, costimulation with a high concentration (10 μM) of adenosine A_1 receptor agonist and low concentration (0.01 μM) of TPα receptor agonist markedly increased cyclic AMP levels (Fig. 3C). However, costimulation with equal concentrations of these agonists had no significant effect on cyclic AMP levels (Fig. 3B). This is similar to the case of adenosine A_1 receptor and $P2Y_2$ receptor-hetero-oligomer, which costimulation of high concentrations of $P2Y_2$ receptor agonist and low concentrations of adenosine A_1 receptor agonist had significantly affected cyclic AMP level (Suzuki et al., 2006). This characteristic increase of cyclic AMP levels by simultaneous treatment with a high concentration of adenosine A_1 receptor agonist and low concentration of TPα receptor agonist was inhibited by the antagonists for both receptors (Fig. 3C). Furthermore, a high concentration of

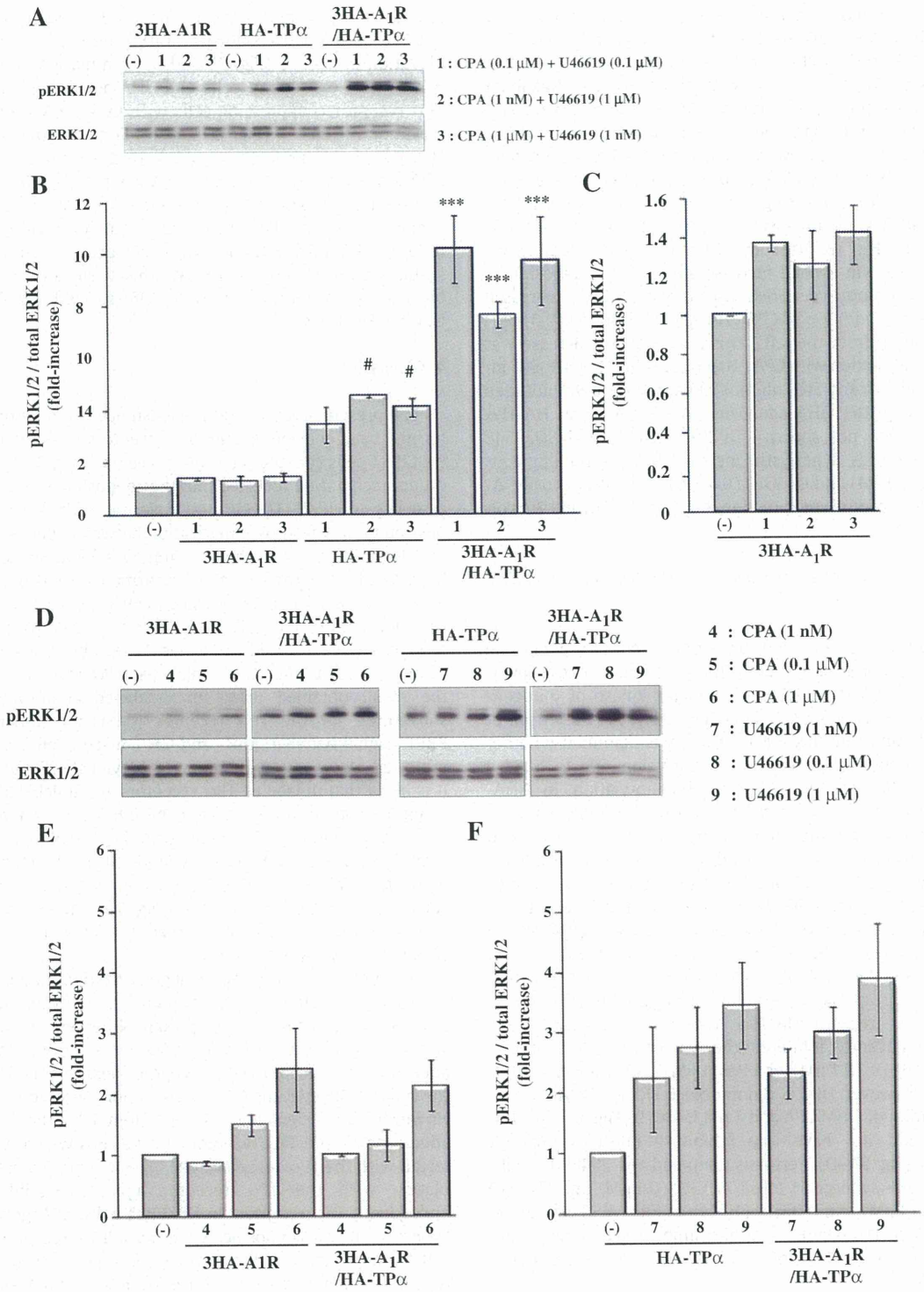

Fig. 4. Effect of CPA and U46619 on ERK1/2 phosphorylation. Assays were performed in HEK293T cells transfected with the 3HA-adenosine A_1 receptor (3HA-A_1R), and HA-TPα receptor (HA-TPα), 3HA-adenosine A_1 receptor/HA-TPα receptor (3HA-A_1R/HA-TPα) . Cells were treated with vehicle (control; (−)) or indicated concentrations of agents. The cell lysates were resolved by SDS-PAGE and analyzed by Western blotting with anti-ERK1/2 and anti-phospho ERK1/2 antibodies (A and D). Fold increases in ERK1/2 phosphorylation in A and D, are presented as mean fold increases of control phosphorylation ± S.E.M for three experiments (B, E and F). (C) Magnified image of control and adenosine A_1 receptor in B. ***$P<0.001$, #$P<0.05$ vs. (−) group (Tukey's test).

adenosine A_1 receptor agonist or low concentration of TPα receptor agonist alone did not have a significant effect on cyclic AMP levels in cells coexpressing adenosine A_1 and TPα receptors. The TPα receptor couples with not only G_q but also G_s, and a TPα receptor-mediated increase in cyclic AMP level was reported (Nakahata, 2008). In fact, stimulation with the TPα receptor agonist induced an increase in cyclic AMP levels in cells expressing the TPα receptor alone (data not shown), though this increase was rather small compared to the

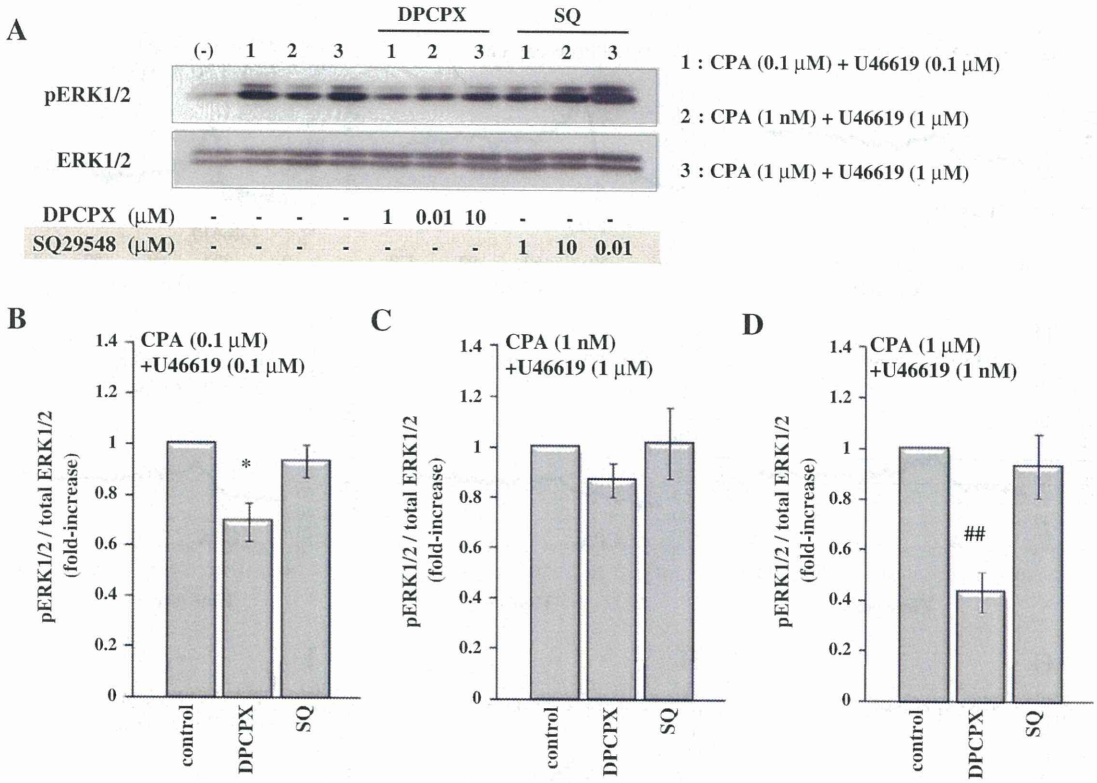

Fig. 5. Effect of DPCPX and SQ29548 on ERK1/2 phosphorylation induced by CPA and U46619. Assays were performed in HEK293T cells cotransfected with the 3HA-adenosine A_1 receptor and HA-TPα receptor. Cells were pretreated with the indicated concentration of DPCPX or SQ29548 and stimulated with vehicle (−) or indicated concentrations of agents. The cell lysates were resolved by SDS-PAGE and analyzed by Western blotting with anti-ERK1/2 and anti-phospho ERK1/2 antibodies (A). Fold increases in ERK1/2 phosphorylation in A, are presented as the mean ± S.E.M for three experiments (B, C, D). $^{*}P<0.01$, $^{\#\#}P<0.005$ vs. control (Tukey's test).

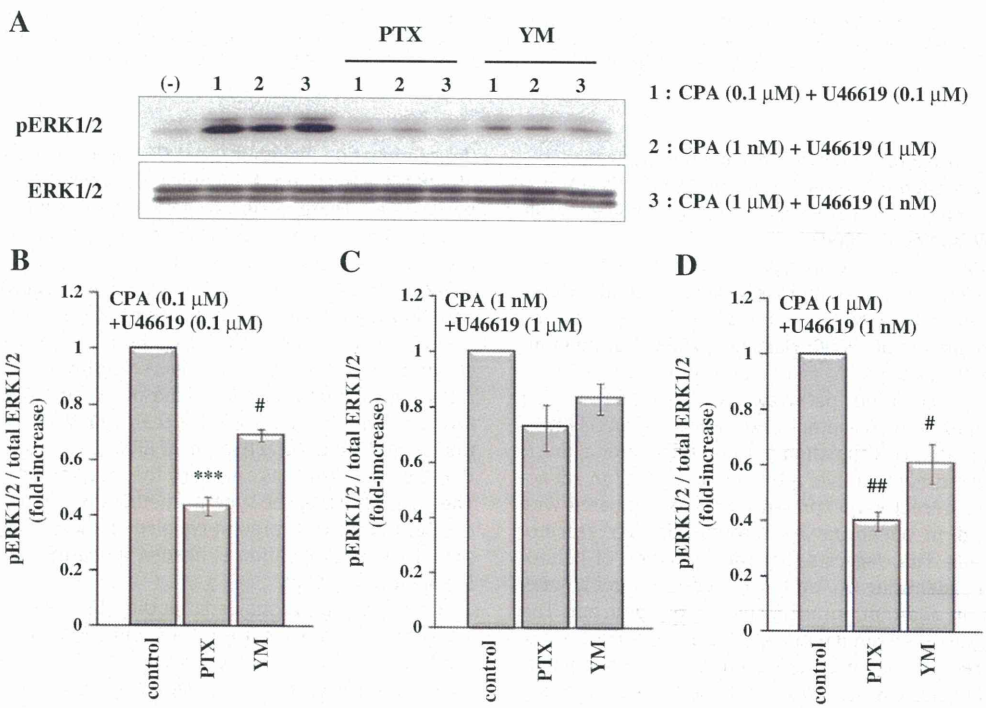

Fig. 6. Effect of pertussis toxin and YM-254890 on ERK1/2 phosphorylation induced by CPA and U46619. Assays were performed in HEK293T cells cotransfected with the 3HA-adenosine A_1 receptor and HA-TPα receptor. Cells were pretreated with 100 ng/ml pertussis toxin (PTX) for 16 h or 1 μM YM-254890 (YM) for 10 min, and stimulated with vehicle (−) or the indicated concentrations of agents. The cell lysates were resolved by SDS-PAGE and analyzed by Western blotting with anti-ERK1/2 and anti-phospho ERK1/2 antibodies (A). Fold increases in ERK1/2 phosphorylation in A, are presented as the mean ± S.E.M for three experiments (B, C, D). $^{***}P<0.001$, $^{\#\#}P<0.005$, $^{\#}P<0.05$ vs. control (Tukey's test).

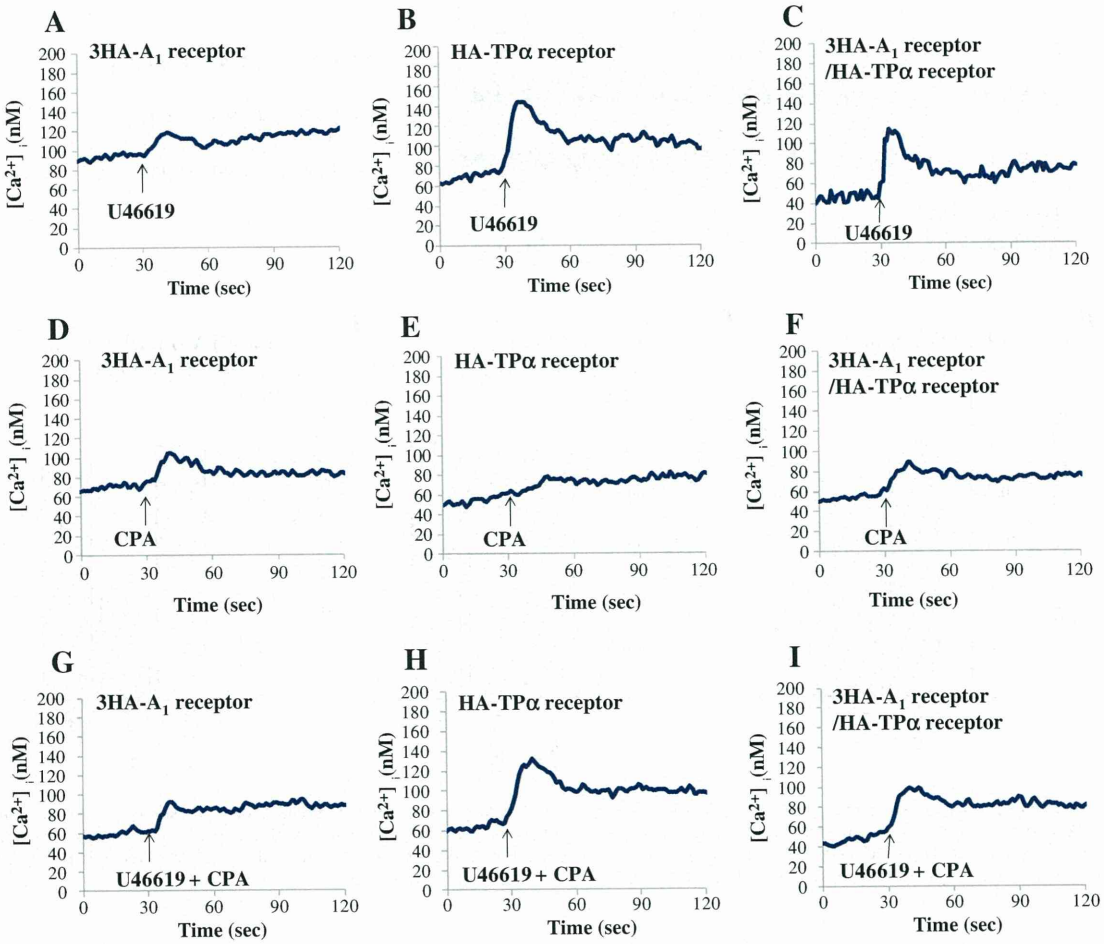

Fig. 7. Mobilization of Ca^{2+} evoked by the indicated agents in HEK293T cells transfected with the adenosine A_1 receptor and/or TPα receptor. Cells were transfected with 3HA-A_1 receptor and G_q (A, D, G), HA-TPα receptor and G_q (B, E, H), and 3HA-A_1 receptor/HA-TPα receptor and G_q (C, F, I). Cells were loaded with fura 2-AM and stimulated with the following agents at the times indicated by the arrows: (A, B, C) 1 μM U46619; (D, E, F) 1 μM CPA; (G, H, I); 1 μM U46619 and 1 μM CPA. The results are representative of two similar experiments.

notable effect of both agonists. Previously, heterodimerization between TPα and prostaglandin I_2 receptors in HEK293T cells cotransfected with both receptors facilitated TPα receptor-mediated cyclic AMP generation (Wilson et al., 2004).

Stimulation of the adenosine A_1 receptor activates ERK1/2 phosphorylation via G_i-derived βγ subunits (Dickenson et al., 1998). ERK1/2 was activated not only by βγ subunits but also by G_q, G_s and $G_{12/13}$ pathways (Honma et al., 2006; Nakahata, 2008; Norum et al., 2003). Therefore, both adenosine A_1 and TPα receptors could individually activate ERK1/2 via various pathways. We examined whether adenosine A_1 receptor/TPα receptor-coexpression affects ERK1/2 activation, and found that costimulation with both receptor agonists induced the synergistic, not additive, activation of ERK1/2 in adenosine A_1 receptor/TPα receptor-coexpressing cells as compared with the results obtained in adenosine A_1 receptor or TPα receptor alone-expressing cells. This suggests that the formation of hetero-oligomers between adenosine A_1 and TPα receptors could alter receptor functions. In addition, in adenosine A_1 receptor and TPα receptor-coexpressing cells, stimulation with the adenosine A_1 receptor agonist or TPα receptor agonist did not induce synergistic activation of ERK1/2. Namely, costimulation with both these receptor agonists on receptor-coexpressing cells was needed for the synergistic activation of ERK1/2. In adenosine A_1 receptor/TPα receptor-coexpressing cells, each concentration of U46619 in combination with 1 μM and 0.1 μM CPA induced greater ERK1/2 activation than the combination including 1 nM CPA. And the synergistic facilitation of ERK1/2 activation was significantly suppressed by DPCPX, but not by SQ29548. These results suggest that the stimulation of adenosine A_1 receptors is important in this synergistic facilitation in receptor-coexpressing cells. Also, inhibition of G_i and G_q by PTX and YM-254890 suppressed ERK1/2 activation induced by costimulation with both agonists as well as the suppression by DPCPX. The synergistic ERK1/2 activation induced by both agonists in these receptor-coexpressing cells may have diverse pathways including G_i and G_q. Although we found that the adenosine A_1 receptor and TPα receptor form a heterooligomer, and that their ligands synergized the production of cAMP and the activation of ERK1/2, we could not demonstrate that the synergism was caused by the receptor heterooligomerization. The possibility that the synergism might be induced by interaction of different types of G protein coupled to these receptors was not denied. Further exploration is needed to clarify the precise molecular mechanism in detail.

In conclusion, we showed that the adenosine A_1 receptor and TPα receptor formed a hetero-oligomer in HEK293T cells. Furthermore, the coexpression of these receptors affected receptor signal responses. Namely, the accumulation of cyclic AMP and activation of ERK1/2 were markedly enhanced by costimulation especially with different concentrations of both receptor agonists in coexpressing cells as compared with that in cells expressing either of these receptors alone. Many neurotransmitters are released from presynaptic

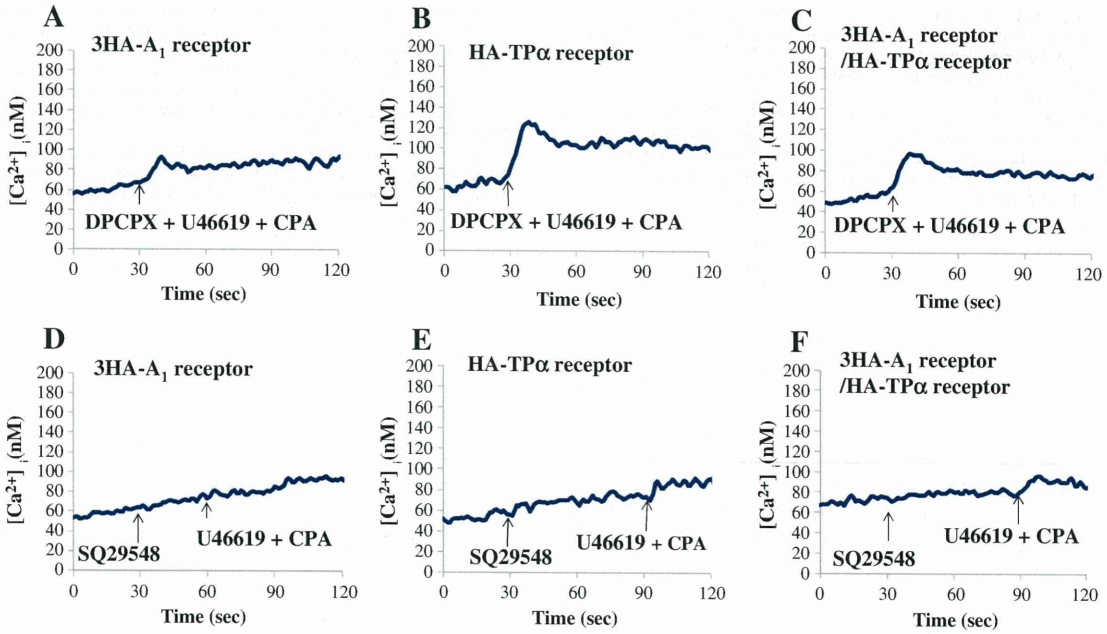

Fig. 8. Mobilization of Ca^{2+} evoked by indicated agonists and antagonists in HEK293T cells transfected with the adenosine A_1 receptor and/or TPα receptor. Cells were transfected with the 3HA-adenosine A_1 receptor and G_q (A, D), HA-TPα receptor and G_q (B, E), 3HA-adenosine A_1 receptor/HA-TPα receptor and G_q (C, F). Cells were loaded with fura 2-AM and stimulated with the following agents at the times indicated by the arrows: (A, B, C) 3 μM SQ29548 and costimulated 1 μM U46619 and 1 μM CPA; (D, E, F) 10 μM DPCPX, 1 μM U46619 and 1 μM CPA. The results were representative of two similar experiments.

regions simultaneously. Currently, many drugs are developed on the assumption that G protein-coupled receptor functions as a monomeric form. If G protein-coupled receptor forms an oligomer under certain pathological conditions, targeting of the oligomer may expand the potential for drug discovery. The present results suggest the regulation of signal transduction via G protein-coupled receptor heterodimers with different concentrations of neurotransmitters. The physiological role of G protein-coupled receptor oligomerization is still not fully understood. To identify the cells which have the receptor hetero-oligomerized of adenosine A_1 and prostanoid TPα receptors would be required to clarify the physiological roles.

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難聴患者とその家族が抱える悩みと社会現状の違いについて

中島梨香子*・石原研治**

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Deafness and the Family in Recent Social Situation

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はじめに

先天性難聴は新生児の 1000 人に 1 人の割合で誕生し、先天性疾患の中でも最も頻度の多い疾患に属する[1]。その中でも遺伝性難聴は先天性難聴の原因の約 50% を占め、実に新生児の 2000 人に 1 人の割合で誕生する。この遺伝性難聴は遺伝子の変異に起因しているため、現在、根治する治療法はなく人工内耳や補聴器等を装用して難聴者の聴力が確保されている。しかし、このような人工内耳や補聴器の装用は、遺伝性難聴に限らず感音性難聴や伝音性難聴などあらゆる種類の難聴において聴力確保のための一般的な方法といて選択されている。

近年では、新生児聴覚スクリーニングが行われるようになり難聴の早期発見が可能になった。この検査の導入に加え人工内耳や補聴器の性能も向上したことにより、難聴児がそれらを早期に装用することが可能になったため難聴児の言語習得のレベルも向上し、難聴を抱える子どもたちも通常学校に在籍するケースも珍しくない程に増えてきている。

しかし、感音性・伝音性問わず難聴者の Quality of Life (QOL) は健聴者からの認識という点で依然として十分ではない。医学や工学などの最先端研究では、難聴者の QOL を向上させるという事を目標として治療薬や医療機器の開発研究が進められている。しかしながら、難聴者も健聴者も「人」であるということを考えたとき、医学や工学などの「物」の開発発展による QOL 向上のみでは人と人とのつながりやすごしやすさである社会面・精神面までは補いきることは出来ない。

難聴者が自らの理想とする生き方および社会的にみて人間らしい生活を送ることができるためには、健聴者と難聴者の調和のとれた社会基盤を構築する教育の向上が、医学や工学に加えて必要

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不可欠である。社会基盤を構築する「人」、すなわち、医療や教育、福祉を向上させていくのも難聴者をふくめた障害者と健常者のすべて「人」なのである。

小学校、中学校あるいは高等学校といった学校は、児童生徒が学力を身につける場だけではなく、いろいろな人と出会い、考えを吸収して個々を形成・成長させていく場でもある。つまり、このような人格形成期にあたる学校での教育を児童生徒が社会に出る前の“プレ社会”と位置づけ、健聴児が難聴者の気持ちや疾病に関する理解を深めることができれば、健康な人と疾患や障害を抱える人が共に明るく楽しい生活が送れる環境および社会あるいはその基盤を作り上げる手段の一つとなり得るはずである。

そこで、本研究では、健聴者が持つ難聴や難聴者に対する知識やイメージを質問紙調査を行い明らかにし、養護教諭という立場から、難聴者やその保護者の QOL を向上させるためには学校教育でどのような働きかけができるか、学校という場を難聴者と健聴者の認識の差を埋める場としてどのように活用していけるのかを考察することを目的とした。

方法

2009 年 10 月に I 大学に所属する 252 名の学生 (教育学部・人文学部・理学部・工学部・農学部) を対象に質問紙調査を実施した。未記入事項を除いた有効回答枚数は 244 枚 (96.8%) であった。調査内容は、聴覚障害の有無、聴覚障害を持つ人と出会ったことがあるか、物が見えにくい状態と音が聞こえにくい状態のどちらが想像しにくいか、聴覚障害者に対するイメージ、聴覚障害の聞こえ方の症状に対する認識、補聴器の効果に対する認識、聴覚障害者手帳を持つ人はどのくらいの聴力レベルだと思うか、聴覚障害を持つ人と健聴者が同じ学校で学ぶことについてどう思うか(両方の立場で)、(聴覚障害を持っている人を対象に) 難聴を抱える人が生活をする上であると良いと思う工夫や配慮である。これらの間に関連性があるかどうかについては χ^2 検定を行い、統計解析した。

結果

(1) 対象の背景

質問紙調査の対象 (244 名) は以下通りである。

所属学部 ... 教育学部 141 名 (57.8%)、人文学部 17 名 (7.0%)、理学部 47 名 (19.3%)、工学部 34 名 (13.9%)、農学部 5 名 (2.0%)。

学年 ... 1 年次 89名 (36.5%)、2 年次 50名 (20.5%)、3 年次 47名 (19.3%)、4 年次 58名 (23.8%)。

性別 ... 男性 119 名 (48.8%)、女性 125 名 (51.2%)。

(2) 聴覚障害の有無

「あなたは聴覚障害をもっていますか。」という問いに対して、「聴覚障害 (難聴) をもっている

(もっていた) 」と回答した者は 5 名 (2.0%) であり、「無し」と回答した者は 239 名 (98.0%) であった。「有り」と回答した者 5 名には、難聴の程度や種類、生活する上で、あると良い配慮や工夫について自由記述で回答してもらった。これらの結果は (10) に記載した。

(3) 聴覚障害を持つ人と出会ったことがあるか

(2) で「難聴無し」と回答した者 239 名 (健聴者) のみに回答してもらった。「あなたはこれまでに聴覚障害をもつ方と出会ったことがありますか。」という問いに対して、「出会ったことがある」と回答した者は 44.8% (107 名) であり、「出会ったことがない」と回答した者は 55.2% (132 名) であった。

(4) 物が見えにくい状態と音が聞こえにくい状態のどちらが想像しにくいか

「物が見えにくい状態と音が聞こえにくい状態のどちらが想像しやすいですか。」という問いに対して、「音が聞こえにくい状態 (聴覚障害)」と回答した者は 31.4% (75 名) であったのに対し、「物が見えにくい状態 (視覚障害)」と回答した者は 68.6% (164 名) であった。

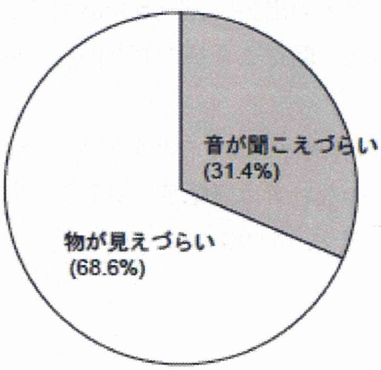

図 1. 健聴者は「物が見えにくい状態」と「音が聞こえにくい状態」のどちらが想像しやすいか (n = 239)

(5) 聴覚障害者に対するイメージ

健聴者がもつ難聴者に対するイメージを具体的に把握するために、「聴覚障害者に対してどのようなイメージを持っていますか。」という質問を行った。なお、回答は複数回答可とし、「イメージがわかない」と回答した者は他の項目を回答しないように設定した。回答は以下の表 1 の通りである。

「手話を使う」が 61.5% と一番高く、手話が難聴者の独自のコミュニケーションツールの代表格であるというイメージがある程度強いと思われる。また、「聞くことができない」および「大変そう」がともに 56.1% と半数を超え「コミュニケーションがとりづらい」が 49.0% と約半数が回答した。「話すことができない」という項目での回答は 25.9% あり、「聞くことができない」ことと「話す

ことができない」ことの関連性については人それぞれのイメージがあると考えられる。「イメージがわかない」と回答した者は4名と少なかった。

表 1. 健聴者がもつ難聴者に対するイメージ

難聴者に対するイメージ	人数 (人)	パーセント (%)
手話を使う	147	61.5
聞くことができない	134	56.1
大変そう	134	56.1
コミュニケーションがとりづらい	117	49.0
話すことができない	62	25.9
可哀想	24	10.0
イメージがわかない	4	1.7

n = 239 (複数回答可)

また、この質問においては難聴者との出会いの経験のある者と無い者の間で難聴者に対するイメージに有意に関連性があった項目は「聞くことができない」、「可哀想」、「手話を使う」の項目であった (図 2 - 4)。

図 2 は難聴者との出会い経験の有無と「聞くことができない」イメージの有無の関係を示したものである。難聴者と出会ったことのない者は難聴者に対して「聞くことができない」というイメージを抱く者が 65.2% で、ある者のそれ (44.9%) より多かった。

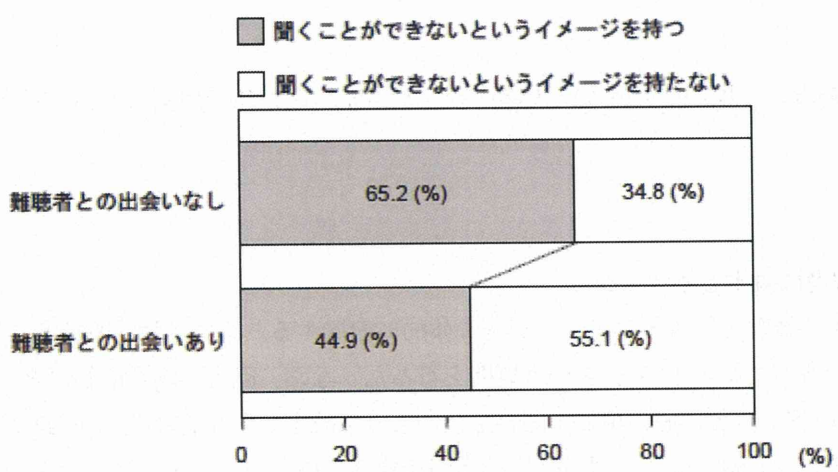

図 2. 「聞くことができない」というイメージを持つ健聴者と難聴者との出会いの経験の関連性