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Expression and functional analysis of β-adrenoceptor subtypes in rabbit submandibular gland

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Abstract

β-Adrenoceptors (β-ARs) mediate important physiological functions in salivary glands. Here we investigated the expression and function of β-AR subtypes in rabbit submandibular gland (SMG). Both β_1- and β_2-ARs, but not β_3-AR, were strongly expressed in rabbit SMG. β_1-AR proteins were widely expressed in acinar and ductal cells whereas β_2-AR proteins were mainly detected in ductal cells. A [^{3}H]-dihydroalprenolol binding assay revealed that β-AR B_{max} was 186±11.9 fmol/mg protein and K_d was 2.71±0.23 nM. A competitive binding assay with CGP 20712A, a β_1-AR antagonist, indicated that the proportion of β_1-AR and β_2-AR was 71.9% and 28.1%, respectively. Gland perfusion with the β-AR agonist isoproterenol induced a significant increase in saliva secretion which was abolished by pretreatment with the non-selective β-AR antagonist propranolol. Pretreatment with β_1- or β_2-AR selective antagonists, CGP 20712A or ICI 118551, diminished isoproterenol-induced increase in saliva secretion by 71.2% and 28.8%, respectively. The expression of α-amylase mRNA was significantly stimulated by isoproterenol, which was eliminated by propranolol and CGP 20712A. Perfusion with isoproterenol decreased α-amylase protein storage in SMG and increased α-amylase activity in saliva. These alterations became less significant after pretreatment with propranolol and CGP 20712A. Our results suggest that both β_1- and β_2-ARs are expressed in rabbit SMG. β_1-AR is the predominant subtype and may play an important role in regulating saliva and α-amylase secretion.

Keywords: β-Adrenoceptor subtypes; Saliva secretion; Rabbit; Submandibular gland

Introduction

Adrenergic receptors (ARs) are a family of G protein-linked signaling proteins and can be functionally and structurally divided into two main classes, designated as α- and β-AR. To date, three distinct β-AR subtypes (β_1-, β_2-, and β_3-AR) have been identified through molecular cloning techniques and pharmacological assays (Hall, 2004; Machida et al., 1990; Muzzin et al., 1991; Emorine et al., 1991) and the cDNAs encoding rat and human β_1-, β_2-, and β_3-AR have also been cloned (Hall, 2004; Tate et al., 1991). It has been reported that β-AR subtypes are localized in several organs such as heart, brain, and mammary gland and mediate important physiological functions (Chu et al., 2005; Reznikoff et al., 1986; Inderwies et al., 2003).

Mammalian submandibular glands (SMG) are innervated by both sympathetic and parasympathetic autonomic nerve fibers. The secretory function of the salivary gland is primarily regulated through the complicated action of different receptors such as α-and β-ARs, muscarinic–cholinergic receptors and peptidergic receptors (Baum, 1993; Baum and Wellner, 1999). β-ARs have been found to regulate the secretion of saliva as well as protein secretion while α-ARs may have an effect on the secretion of fluid and electrolytes by the salivary glands (Baum, 1993; Baum and Wellner, 1999; Melvin et al., 2005). β_1- and

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β_2-AR subtypes have been detected in the SMGs of guinea pigs and rats (Sawiris and Enwonwu, 2000; Bahouth, 1992). However, their specific functions remain to be investigated.

Both calcium-mobilizing and cAMP-generating signaling pathways have been linked to salivation. In salivary acinar cells, the increase in the intracellular Ca^{2+} concentration is the primary signal for fluid secretion while cAMP signals mostly regulate the discharge of secretory granules (Melvin et al., 2005). β-ARs interact with catecholamines, activate adenylyl cyclase, and thereby regulate salivary secretion and other physiological functions (Baum, 1993; Baum and Wellner, 1999; Molenaar and Parsonage, 2005). The accumulation of cAMP activates cAMP-dependent protein kinase, resulting in the release of a large amount of amylase (Hu and Humphreys-Beher, 1995). Isoproterenol may regulate both the electrolyte content in primary saliva and the protein secretion in the ducts, through changing the ductal cytosolic cAMP content and Ca^{2+} concentration (Evans et al., 1996).

Rabbit is one of the useful models to study the function of salivary glands. But until now, little is known regarding the expression and function of β-AR subtypes in rabbit SMG. In this study, we examined the expression and distribution of β-AR subtypes in rabbit SMG. Using biochemical and gland perfusion approaches, we identified β_1-AR as the predominant subtype expressed in rabbit SMG, where it may play an important role in regulating saliva and α-amylase secretion.

Materials and methods

Animals and SMG isolation

Healthy male New Zealand white rabbits weighing 2.0–2.5 kg were used in the experiments. All experimental procedures were approved by the Ethics Committee of Animal Research, Peking University Health Science Center and animals were cared for in accordance with the Guidelines of the Ministry of Public Health for the Care and Use of Laboratory Animals. Rabbits were anesthetized with pentobarbital sodium (50 mg/kg weight) and the SMG was rapidly removed and frozen in liquid nitrogen until use.

Reverse-transcriptase polymerase chain reaction (RT-PCR)

Total RNA from SMG ($n=3$) was extracted using TRIzol reagent (Invitrogen, Carlsbad, CA, USA). RNA preparations were treated with DNase I to remove contamination of genomic DNA before RT-PCR. One microgram of total RNA was reverse-transcribed with M-MLV reverse transcriptase (Promega, Madison, WI, USA). All primers in Table 1 were designed according to the published cDNA sequences of rat and synthesized by AoKe Co. (Beijing, China). The amplifications were performed as follows: 94 °C for 5 min, followed by 30–35 cycles of denaturation at 94 °C for 60 s, annealing at 55–60 °C for 60 s, and polymerization at 72 °C for 60 s. Annealing temperature and the number of PCR cycles for each primer are shown in Table 1. The amplification products were visualized on 1.5% agarose gel with ethidium bromide and were sequenced to confirm their identities. The intensities of the bands were measured with a LEICA550IW (LEICA, Germany) image analysis system. Levels of mRNA were normalized to those of β-actin.

Western blot

Western blotting analysis was performed as previously described (Liu et al., 2005). Briefly, membrane fraction-rich crude protein extracts of rabbit SMG ($n=3$) were prepared. Equal amounts of protein extracts (60 μg) were separated by 9% SDS-PAGE and transferred onto polyvinylidene difluoride membrane. The membrane blots were blocked with 5% non-fat milk in PBS plus 0.05% Tween 20 and exposed to corresponding anti-β_1- and β_2-AR primary antibodies (Santa Cruz, CA, USA) overnight at 4 °C followed by 2 h incubation with secondary antibodies (Santa Cruz, CA, USA) conjugated to horseradish peroxidase at room temperature. The blots were then visualized with enhanced chemiluminescence (ECL, Amersham, Piscataway, NJ, USA) and exposed to X-ray films (Kodak, Rochester, NY, USA). The density of bands was scanned and quantified by LEICA550IW image analysis system.

Immunofluorescence

Anti-β_1- and β_2-AR antibodies (Santa Cruz, CA) were labeled with Cy3 using Cy^{tm}3 Ab Labelling Kit (Amersham Biosciences, Little Chalfont, UK). The immunofluorescence for β_1- and β_2-AR in rabbit SMG was performed as previously described (Iwamoto et al., 2000). Negative controls were incubated with normal goat IgG instead of the primary antibodies. Sections were examined with LEICA DC 300F florescent

Table 1
Primers used in PCRs (f = forward; r = reverse)

Name	Primer sequence	Product size (bp)	Annealing temperature (°C)	Cycle number
β_1-AR f	5′-ACTTCCGCAAGGCTTTCCAG-3′	237	60	30
β_1-AR r	5′-AATCGCTGTCCACAGTGGTCG-3′			
β_2-AR f	5′-TCAACTCTGCCTTCAATCCTC-3′	255	55	30
β_2-AR r	5′-GGCTAGGCACAGTACCTTGAC-3′			
β_3-AR f	5′-TTTCATCGCCCTGAACTGGT-3′	217	56	35
β_3-AR r	5′-ACGCTCACCTTCATAGCCATC-3′			
α-Amylase f	5′-GACAGCACTTGTGGCAATGAC-3′	258	55	30
α-Amylase r	5′-GCCATCGACTTTGTCTCCAGA-3′			
β-Actin f	5′-ATCTGGCACCACACCTTCTACAATGAGCTGGCG-3′	843	58	30
β-Actin r	5′-CGCCATACTCCTGCTTGCTGATCCACATCTGC-3′			

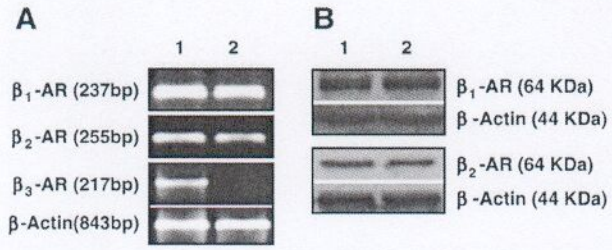

Fig. 1. The expressions of β-AR subtype mRNA and protein in rabbit SMG. A, The expression of β-AR subtype mRNA: RT-PCR products from rabbit SMGs were electrophoresed on 1.5% agarose gels and visualized with ethidium bromide. RT-PCR images were representative of three independent experiments. Lane 1 represents the RT-PCR products amplified from plasmids containing rat β_1-, β_2-AR full-length cDNA and from rabbit brown adipose tissue in correspondence with β_1-AR, β_2-AR or β_3-AR respectively as the positive control; lane 2 represents RT-PCR products amplified from rabbit SMG. B, The expression of β-AR subtype protein: equal amounts of membrane protein extracts (60 μg) were separated and immunoblotted with antibodies specific for β_1- and β_2-AR. The Western blot images are representative of three independent experiments. Lane 1, rabbit heart served as the positive control; lane 2, rabbit SMG.

microscope coupled with the computer imaging device (LEICA, Germany).

Radioligand binding assay

The radioligand binding assay was carried out as described previously (Chiarenza et al., 1998; Yeh et al., 2003). Briefly, the membrane fractions of rabbit SMG ($n=4$) were prepared and diluted to give a final protein concentration of 1.0 μg/μl with 50 mM Tris–HCl incubation buffer (pH 7.4, containing 0.25 M sucrose, 6 mM $MgCl_2$ and 1 mM EDTA) and stored at −80 °C for further use.

For saturation experiments, an aliquot of membrane suspension (100 μg) was incubated with various concentrations (0.1–6.4 nM) of [^{3}H]-dihydroalprenolol ([^{3}H]-DHA, specific activity of 100.4 Ci/mmol, PerkinElmer, Boston, MA, USA) in a final volume of 200 μl. Competitive binding assays were performed by incubation of 1.5 nM [^{3}H]-DHA with increasing concentrations of CGP 20712A, a β_1-AR antagonist (25–2500 nM, Sigma, St. Louis, MO, USA). Incubations were carried out for 20 min at 37 °C and terminated by adding 5 ml of ice-cold incubation buffer, followed by a rapid filtration over glass fiber filters (GF/C, Whatman). The filters were washed three times with 5 ml of incubation buffer. Radioactivity retained on the filters was determined by a liquid scintillation counter (BECKMAN LS 6500, Beckman, USA). All values in binding experiments were the average of duplicates. The specific binding was calculated as the difference between the binding in the absence (total binding) and in the presence (non-specific binding) of 1 μM propranolol. The equilibrium dissociation constant (K_d) and the maximum binding capacity (B_{max}) were determined by Scatchard analysis. The curve for CGP 20712A-induced displacement of [^{3}H]-DHA was analyzed by non-linear curve-fitting program of SigmaPlot (Jandel Scientific, CA).

Perfusion of the SMG

Rabbit SMG was perfused as described previously (Turner et al., 1996). Briefly, SMG was isolated, the external carotid artery and the main excretory duct were cannulated for perfusion and saliva collection, respectively. Krebs-Ringer-HEPES (KRH, containing 116.0 mM NaCl, 5.4 mM KCl, 1.25 mM $CaCl_2$, 0.4 mM $MgSO_4$, 20.0 mM HEPES, 0.9 mM Na_2HPO_4, and 5.6 mM glucose, pH 7.4) buffer was warmed to 37 °C,

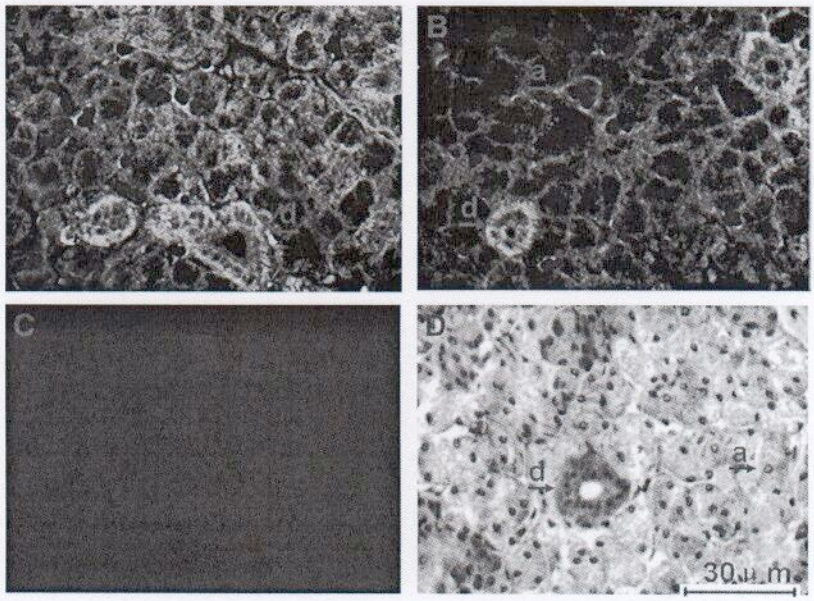

Fig. 2. Distribution of β_1- and β_2-AR in rabbit SMG. Anti-β_1- and β_2-AR antibodies were labeled with Cy3 and immunofluorescence analysis was performed as described in Materials and methods. The green fluorescence labeling specific for β_1-AR (A) and β_2-AR (B) was observed in membranes of acinar and ductal cells. Arrow a indicates acinar cells and arrow d indicates ductal cells. C, negative control; D, Hematoxylin and eosin staining from frozen sections. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

bubbled with 95% O_2 and 5% CO_2, and delivered to the SMG with a Gilson Minipuls rotary pump which provided an external carotid artery flow rate of 3 ml/min. After equilibration for at least 30 min, different antagonist and agonist substances ($n=4$ for each group) were introduced into the perfusion buffer and perfused into the gland for 20 min. The secretion of the gland was measured as the length of moisture on the filter paper (35 mm × 5 mm) after 5 min perfusion of agonist or antagonist. Saliva was then collected for 10 min and stored at −80 °C for determination of α-amylase activity. The perfused SMG specimens were stored in liquid nitrogen until further use.

Immunohistochemistry

The perfused frozen SMG tissue was sectioned 5 μm thick. The cryostat sections were fixed in acetone:chloroform (1:1) and immunostained using α-amylase antibodies (Santa Cruz, CA, USA) diluted 1:50 in 2% BSA/PBS following the routine immunohistochemistry protocols (Cohen et al., 1995). The quantitative analysis of α-amylase immunostaining was performed with an image analyzer (LEICA550IW, Germany). The immunopositive values were expressed in terms of integrate optical density (Vázquez et al., 1996).

α-Amylase activity determination

The activity of α-amylase (U/l) in saliva collected from the perfused SMG was detected by a full-automatic clinical biochemistry analyzer (Synchron LX20, Beckman, USA) and α-amylase assay kit (Zhongsheng Biotech, Beijing, China) according to the manufacturer's instructions. Values were expressed in percent of the α-amylase activity in the saliva from KRH perfused SMG.

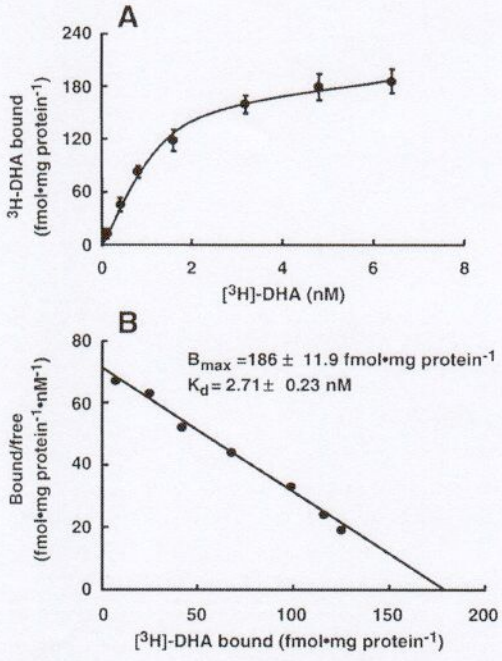

Fig. 3. Saturation curve (A) and Scatchard Plot analysis (B) of specific binding of [^{3}H]-DHA to β-ARs in rabbit SMG membrane fraction. Data are presented as mean±S.E.M. of four independent experiments. The binding assays were performed in duplicate as described in Materials and methods.

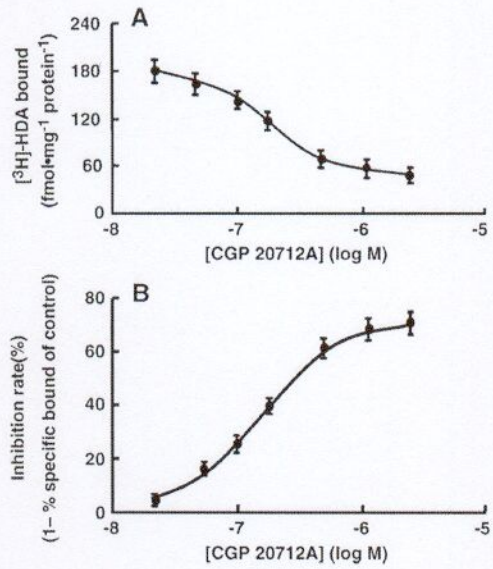

Fig. 4. Inhibition of specific [^{3}H]-DHA binding to β-ARs in rabbit SMG membrane fraction by CGP 20712A. A: Saturation curve of specific binding of [^{3}H]-DHA to β-ARs in the presence of increasing concentrations of CGP 20712A; B: The inhibition rate (1—% specific bound of control) of CGP 20712A to the specific binding of [^{3}H]-DHA to β-ARs. Data are presented as mean±S.E.M. of four independent experiments.

Statistical analysis

Data are presented as means±S.E.M. Differences were analyzed with unpaired Student's t test between two groups and with One-way analysis of variation (ANOVA) among multiple groups. P values of <0.05 were considered to be significant.

Results

Expression and distribution of β-AR subtypes in rabbit SMG

The PCR products were shown at the expected bands of 237 bp, 255 bp, and 217 bp, and their identities were further confirmed by sequencing. Both β_1- and β_2-AR mRNAs were expressed in rabbit SMG, whereas β_3-AR mRNA was not detectable. In contrast, as a positive control, β_3-AR mRNA was detected in rabbit brown adipose tissue (Fig. 1A). To further evaluate β_1- and β_2-AR protein expression, crude protein extract from SMG was examined using antibodies to β-AR subtypes. Immunoreactive β_1- and β_2-AR with a molecular mass of approximately 64 kDa were detected (Fig. 1B). Using immunofluorescence, β_1-AR proteins (Fig. 2A) were shown to be widespread in the membrane of both ductal and acinar cells, whereas β_2-AR proteins (Fig. 2B) were mainly located in the membrane of ductal cells.

The percentage of β-AR subtypes of total β-ARs in rabbit SMG

The saturation curve (Fig. 3A) and Scatchard plot analysis (Fig. 3B) from radioligand binding assay revealed that the B_{max} and K_d values for [^{3}H]-DHA binding were 186±11.9 fmol/mg protein and 2.71±0.23 nM respectively. Competitive radioligand binding assay revealed that in rabbit SMG, 71.9% of total β-ARs were β_1-AR while the rest, 28.1%, were β_2-AR (Fig. 4).

The role of β-AR subtypes in saliva secretion in rabbit SMG

In isolated rabbit SMG, marked increase of saliva secretion was observed when the SMG was perfused with isoproterenol, a β-AR agonist, at 10^{-7} M (Fig. 5). Pretreatment with propranolol, a β-AR antagonist, at 10^{-6} M, followed by isoproterenol perfusion completely abolished the increase in saliva secretion. However, with preperfusion of the SMG with CGP 20712A, the isoproterenol-induced saliva increase was diminished about 71.2% ($P<0.01$), whereas ICI 118551, β_2-AR antagonist, inhibited the saliva increase by 28.8% ($P<0.05$) (Fig. 5). Perfusion with propranolol, CGP 20712A, or ICI 118551 alone had no influence on basal salivary flow (Fig. 5).

The role of β-AR subtypes on α-amylase mRNA expression in rabbit SMG

Analysis of α-amylase mRNA expression by RT-PCR (Fig. 6) showed that the expression of α-amylase mRNA in perfused SMG with isoproterenol was increased by 119.5% compared to the basal expression ($P<0.01$). The isoproterenol-induced increase in α-amylase mRNA expression was abolished completely by preperfusion of the SMG with propranolol or CGP 20712A. However, ICI 118551 had no significant

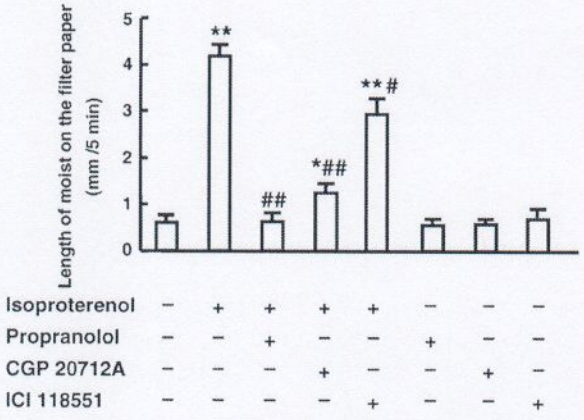

Fig. 5. Effects of different antagonist and agonist on the secretion of isolated SMG. The SMG was isolated and perfused as described in Materials and methods. After equilibration for at least 30 min, different antagonists (propranolol, 10^{-6} M; CGP 20712A and ICI 118551, 10^{-7} M) and isoproterenol (10^{-7} M) were introduced into the perfusion buffer and perfused into the gland for 20 min. The secretion of the gland was measured as the length of the moisture on the filter paper (35 mm × 5 mm) in 5 min. Data are presented as the mean±S.E.M. of four independent experiments. *$P<0.05$ and **$P<0.01$ compared with KRH perfusion; # $P<0.05$ and ## $P<0.01$ compared with isoproterenol.

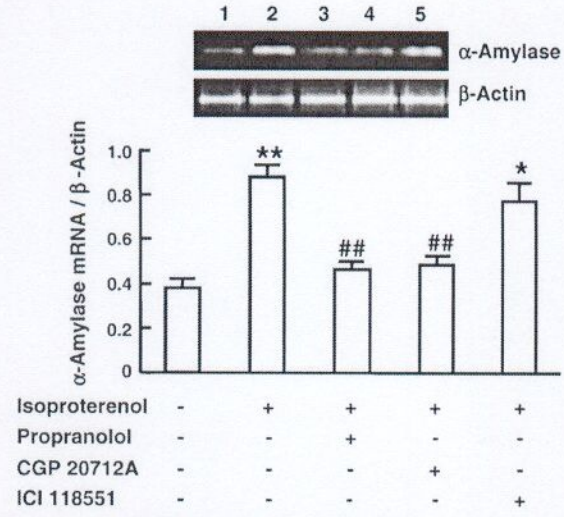

Fig. 6. Effect of β-AR subtypes activation on the α-amylase mRNA expression in the isolated rabbit SMG. Upper panel, agarose gel electrophoresis of the RT-PCR products. The α-amylase mRNA levels were normalized to β-actin level. Data are presented as mean±S.E.M. of four independent experiments. *$P<0.05$ and **$P<0.01$ compared with control; ## $P<0.01$ compared with isoproterenol.

influence on the isoproterenol-induced increase in α-amylase mRNA expression.

The role of β-AR subtypes on the α-amylase protein in perfused rabbit SMG

In the frozen sections, the α-amylase protein in perfused rabbit SMG was detected by immunohistochemistry (Fig. 7). The normal SMG (Fig. 7A) and the KRH perfused SMG (Fig. 7B) showed strong cytoplasmic reactions after staining with α-amylase antibodies. The α-amylase protein was observed in acinar cells but not in ductal cells. Quantitative analysis (Fig. 7G) revealed that the immunoreactivity of α-amylase in SMG perfused with isoproterenol (Fig. 7C) was decreased by 78.4% ($P<0.01$) compared with that of SMG perfused with KRH alone. Pretreatment with propranolol (Fig. 7D) or CGP 20712A (Fig. 7E) followed by isoproterenol perfusion, did not change the immunoreactivity of α-amylase as compared to KRH perfused SMG. However, preperfusion of the SMG with ICI 118551 followed by isoproterenol perfusion (Fig. 7F), diminished the immunoreactivity of α-amylase by about 57.9% ($P<0.05$) when compared with that of SMG perfused with KRH.

The role of β-AR subtypes on α-amylase activity in saliva from perfused rabbit SMG

Activity of α-amylase in saliva (Fig. 8) was increased by 3.77 times ($P<0.01$) in the isoproterenol group compared with that perfused in KRH alone. Pretreatment with propranolol followed by isoproterenol perfusion completely abolished the increase of α-amylase activity. However, the isoproterenol-induced increase in the activity of α-amylase in saliva was diminished by 64.9% ($P<0.01$) and 18.1% ($P>0.05$) when the

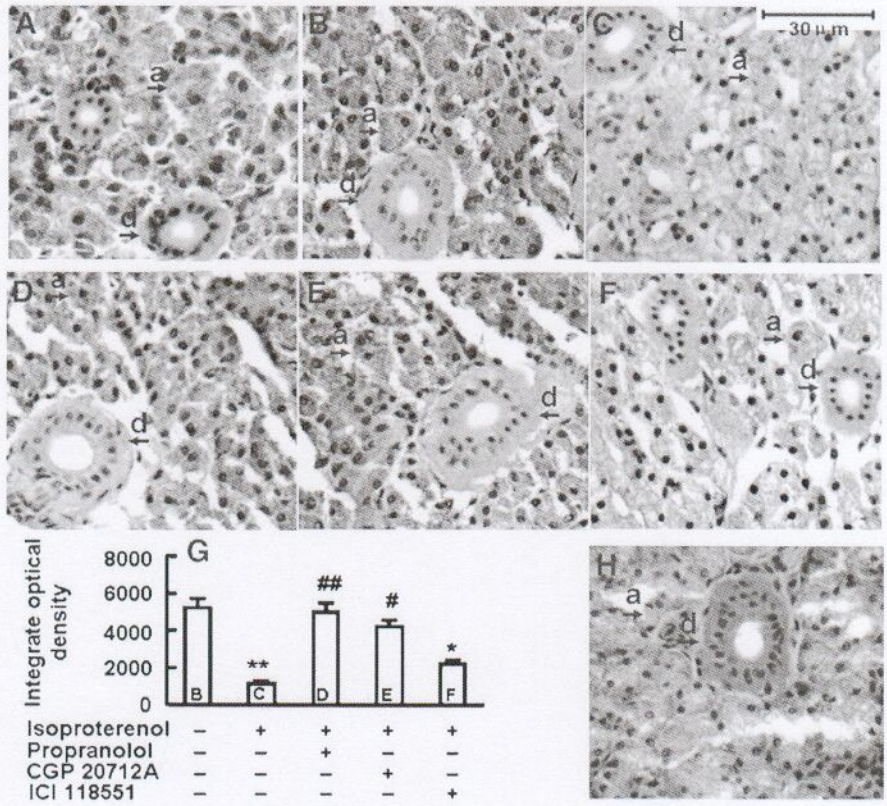

Fig. 7. The role of β-AR subtypes activation on α-amylase protein in perfused rabbit SMG. The SMG was perfused and α-amylase protein in the gland was detected and quantified as described in Materials and methods. A–F: Representative photomicrographs of immunohistochemistry showing the distribution and alterations of α-amylase protein in perfused rabbit SMG. Cytoplasmic staining was observed in acinar cells. Arrow a indicates acinar cells and arrow d indicates ductal cells. A, normal SMG; B, KRH perfusion; C, isoproterenol perfusion; D, pretreatment with propranolol followed by isoproterenol perfusion; E, pretreatment with CGP 20712A followed by isoproterenol perfusion; F, pretreatment with ICI 118551 followed by isoproterenol perfusion. G, The quantitative analysis of relative levels of α-amylase protein in perfused SMG. H, Hematoxylin and eosin staining of KRH perfused SMG from frozen sections; Data are presented as the mean±S.E.M. of four independent experiments. $^{*}P<0.05$ and $^{**}P<0.01$ compared with KRH perfusion; $^{\#}P<0.05$ and $^{\#\#}P<0.01$ compared with isoproterenol perfusion.

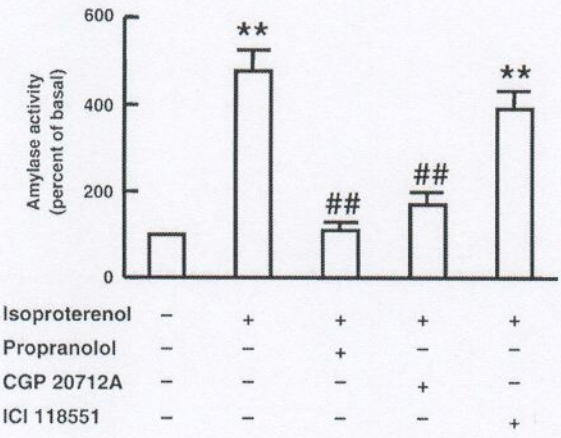

Fig. 8. Effects of activation of different β-AR subtypes on α-amylase release from rabbit SMG. The saliva was collected from the isolated perfused SMG and the α-amylase activity in saliva was measured as described in Materials and methods. Data are presented as the mean±S.E.M. of four independent experiments. $^{**}P<0.01$ compared with KRH perfusion; $^{\#\#}P<0.01$ compared with isoproterenol perfusion.

SMG was preperfused with CGP 20712A or ICI 118551 respectively. Perfusion with propranolol, CGP 20712A, or ICI 118551 alone had no influence on the basal levels of α-amylase activity (data not shown).

Discussion

The β-AR is divided into β_1-, β_2-, and β_3-subtypes (Emorine et al., 1991). Intriguing differences among β-AR subtypes have been suggested in the regulation of receptors during short- and long-term continuous exposure to agonists (Emorine et al., 1991), in G protein coupling (Hall, 2004), and in tissue distribution (Muzzin et al., 1991; Chu et al., 2005; Reznikoff et al., 1986; Inderwies et al., 2003). The subtypes of β-AR may have evolved differently to meet various physiological needs either for coupling to G proteins or for adaptive regulation in the salivary gland. Although they have been characterized in many mammalian tissues, little is known about them in rabbit SMG.

In this study, we reported for the first time the expression and function of β-AR subtypes in rabbit SMG. The detection of β_1-AR, β_2-AR but not β_3-AR mRNA in rabbit SMG was consistent with their respective presence reported in rat SMG

(Nishiura et al., 2001). Recently published data further confirmed that β_1- and β_2-AR mRNAs were present in both acinar and ductal cells of rat SMG (Nezu et al., 2005). Previously, the 64-kDa species of β_1-AR protein had been found in rat SMG membranes (Bahouth, 1992). In the present study, the existence of β_1- and β_2-AR proteins in rabbit SMG was detected using specific β-AR subtype antibodies. In addition, the distribution of β_1- and β_2-AR proteins in rabbit SMG was examined using immunofluorescence. The results showed that although both the β_1-AR and the β_2-AR proteins were expressed in acinar and ductal cells, the two subtypes were distributed differently. β_1-AR proteins were shown to be widespread in membrane of both ductal and acinar cells, whereas β_2-ARs were mainly presented in ductal cells.

Previous study by [^{3}H]-DHA binding showed that β-ARs in rat whole SMG consist of 90% β_1- and 10% β_2-ARs (Schneyer and Humphreys-Beher, 1987). Expression of β-AR subtype mRNAs is likely to vary in different cell types and species. In this study, the percentage of β-AR subtypes in total β-ARs was determined. Competitive binding assay revealed that 71.9% of total β-ARs were β_1-AR while the rest were β_2-AR in rabbit SMG. The B_{max} and K_d values from the [^{3}H]-DHA saturation assay indicated the characteristics of β-ARs concentration and their binding affinity in rabbit SMG. It has been reported that the B_{max} and K_d values for [^{3}H]-DHA binding are 174.5 fmol/mg protein and 2.62 nM respectively in rat SMG (Chiarenza et al., 1998). Our results indicated that β_1-AR was the predominant β-AR subtype in rabbit SMG and the expression pattern of β-AR subtypes was similar between rabbit and rat SMG (Schneyer and Humphreys-Beher, 1987).

Although a functional role for β-AR has been defined in the salivary glands of some species (Baum, 1993; Baum and Wellner, 1999; Melvin et al., 2005), the role of β-AR subtypes in salivary secretion of SMG remains to be fully investigated. In the present study, stimulation of the perfused rabbit SMG with isoproterenol induced a significant increase in the rate of saliva flow, and the increase was significantly inhibited by propranolol or CGP 20712A, but not markedly by ICI 118551. These results suggested that both β_1- and β_2-AR subtypes were involved in regulation of salivary secretion in rabbit SMG. Corresponding to expression pattern of β-AR subtypes, the β_1-AR plays a dominant role in the saliva secretory response of rabbit SMG.

It is known that α-ARs mostly regulate the secretion of fluid and electrolytes, while β-ARs have an effect on both the secretion of protein and the amount of saliva secretion (Baum, 1993; Baum and Wellner, 1999; Melvin et al., 2005). Stimulation of the superior cervical ganglion could increase the salivary flow rate by about five-fold in rabbit SMG and that falls about 50% with the administration of each of α- or β-AR blocking drugs (Moreno et al., 1984). In rat SMG, the secretion of fluid was increased dose-dependently by isoproterenol (Chiarenza et al., 1998). Prior exposure of acini to isoproterenol resulted in a persistent increase in basal secretion which was abolished by propranolol (Bradbury and McPherson, 1988). In both rats and hamsters, the secretory responses induced by isoproterenol were inhibited by pretreatment with metoprolol, a β_1-AR antagonist, suggesting that β_1-AR was the dominant β-AR subtype in saliva regulation of rat and hamsters SMG (Iwabuchi and Masuhara, 1992).

As a major component of saliva, amylase has multiple functions in the oral cavity. Generally the main purpose of amylase in saliva is to break down starch for initiation of digestion (Pedersen et al., 2002). The secretion of most exocrine proteins including salivary amylase was mainly initiated by β-AR activation (Hata et al., 1983; Proctor et al., 2003) and in rat salivary glands, the postsynaptic β-ARs controlled protein synthesis (Bellavia and Gallara, 2000). However, the functional roles of individual β-AR subtypes on protein synthesis and secretion in the salivary glands remain unknown. In this study, the results revealed that α-amylase mRNA in the perfused rabbit SMG was increased significantly in response to isoproterenol stimulation. The increase was abolished completely by propranolol and CGP 20712A, but not by ICI 118551. Previous studies revealed that α-amylase mRNAs in rat parotid gland were increased by short isoproterenol stimulation (Prasad et al., 1993; Woon et al., 1993). Our results suggested that the β_1-ARs rather than the β_2-ARs play a more important role in the secretory protein synthesis in rabbit SMG.

In order to clarify the role of each β-AR subtype on protein secretion in rabbit SMG, the alterations of α-amylase protein in the perfused SMG and the activity of amylase in saliva were examined. The results revealed that α-amylase protein was mainly in the cytoplasm of acinar cells. Perfusion with isoproterenol significantly stimulated the secretion of α-amylase (implied by the decreased amount of α-amylase protein in SMG and the increased α-amylase activity in saliva). The stimulation was suppressed by propranolol and β_1-AR antagonist. However, preperfusion of the SMG with β_2-AR antagonist had minimal effects on the changes of α-amylase induced by isoproterenol. These results suggested that it is β_1-AR rather than β_2-AR activation played the main role in regulating amylase secretion in rabbit SMG.

Conclusion

We have presented here the first evidence that both β_1- and β_2-AR are expressed in rabbit SMG, whereas β_1-AR was the primary subtype of β-AR and played a more important role in regulating saliva secretion, especially in the secretory protein synthesis and secretion. Our results have important significance for understanding the regulating mechanism of β-AR subtypes in saliva secretion, and may provide a potential option for artificially regulating the salivary secretion with certain agonists and antagonists of β-AR subtypes under pathological conditions.

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