Available online at www.sciencedirect.com
SCIENCE DIRECT®
European Journal of Pharmacology 473 (2003) 79–82
www.elsevier.com/locate/ejphar

Short communication

Function of β_1-adrenoceptors and mRNA expression of β_1- and β_2-adrenoceptors in guinea-pig esophagus

Takahiro Horinouchi, Yoshio Tanaka, Katsuo Koike*

Department of Chemical Pharmacology, Toho University School of Pharmaceutical Sciences, 2-2-1 Miyama, Funabashi, Chiba 274-8510, Japan

Received 6 March 2003; received in revised form 27 May 2003; accepted 3 June 2003

Abstract

β-Adrenoceptor subtypes mediating relaxation were examined by using pharmacological and molecular analyses in guinea-pig esophageal muscularis mucosae. (−)-Isoprenaline-induced relaxations were antagonized by (±)-propranolol ($pA_2 = 8.47 \pm 0.07$), a selective β_1-adrenoceptor antagonist, (±)-2-hydroxy-5-[2-[[2-hydroxy-3-[4-[1-methyl-4-(trifluoromethyl)-1*H*-imidazol-2-yl]phenoxy]propyl]amino]ethoxy]-benzamide methanesulfonate (CGP20712A; $pA_2 = 9.43 \pm 0.09$), and a selective β_2-adrenoceptor antagonist, (±)-1-[2,3-(dihydro-7-methyl-1*H*-inden-4-yl)oxy]-3-[(1-methylethyl)amino]-2-butanol hydrochloride (ICI-118,5511; $pA_2 = 7.11 \pm 0.04$), indicating that β_1-adrenoceptors but not β_2- or β_3-adrenoceptors were essentially involved in β-adrenoceptor-mediated relaxations. However, the expression of messenger RNA (mRNA) for β_1- and β_2-adrenoceptors, but not for β_3-adrenoceptors, was detected by reverse transcription-polymerase chain reaction (RT-PCR). These results clearly suggest that not all β-adrenoceptor mRNA expressed strictly reflect functional receptors in guinea-pig esophageal muscularis mucosae.

Keywords: β_1-Adrenoceptor; β_2-Adrenoceptor; β_3-Adrenoceptor; Smooth muscle relaxation; Messenger RNA (mRNA); Esophageal muscularis mucosae, guinea-pig

1. Introduction

β-Adrenoceptors were initially classified into β_1- and β_2-adrenoceptor subtypes based on the relative rank order of potency for 15 catecholamines and tissue localization (Lands et al., 1967a,b). The relaxations of gastrointestinal smooth muscles in response to sympathomimetic amines have been considered to be mediated via β_1-adrenoceptors (Lands et al., 1967a,b). β-Adrenoceptors in esophageal and ileal smooth muscles of guinea-pig were actually described as β_1-adrenoceptors (Grassby and Broadley, 1984; Kamikawa and Shimo, 1987). Recently, the presence of β_3-adrenoceptors, which have low sensitivity to β_1- and/or β_2-adrenoceptors antagonists such as propranolol, have been definitely established by pharmacological and molecular evidence (for review, see Arch and Kaumann, 1993) and β_3-adrenoceptors are also responsible for the relaxations of ileum isolated from guinea-pig (Horinouchi and Koike, 2000; Manara et al., 1995). In addition, it is well known that functional β_3-adrenoceptors are abundantly coexpressed with β_1-adrenoceptors in many tissues including brown adipocytes and gastrointestinal smooth muscles (Bronnikov et al., 1999; Brown and Summers, 2001).

The present study was therefore carried out to determine the function and the messenger RNA (mRNA) expression of β-adrenoceptor subtypes in esophageal muscularis mucosae of guinea-pig with a special attention on the possible contribution of β_3-adrenoceptors.

2. Methods

2.1. Animals

Male Hartley guinea-pigs weighing 300–500 g (Saitama Experimental Animals, Saitama, Japan) were used in the present study. Animals were housed under laboratory standard conditions on a 12-h light/dark cycle (lights on 8:00 a.m.; lights off 8:00 p.m.) in rooms in which temperature (20–22 °C) and relative air humidity (50 ± 5%) were strictly regulated. Food and water were available ad libitum. Guinea-pigs were used in accordance with the Guideline for

* Corresponding author. Tel./fax: +81-47-472-1419.
E-mail address: ktkoike@phar.toho-u.ac.jp (K. Koike).

0014-2999/03/$ - see front matter © 2003 Elsevier B.V. All rights reserved.
doi:10.1016/S0014-2999(03)01943-5

the Care and Use of Laboratory Animals of Toho University School of Pharmaceutical Sciences (which is accredited by the Ministry of Education, Culture, Sports, Science and Technology, Japan), and the protocol of the present study was approved by the Institutional Animal Care and Use Committee.

2.2. Tissue preparation

Guinea-pigs were stunned and killed by cervical dislocation. The esophagus was immediately removed and the outer striated muscle coat was carefully dissected away. The longitudinal muscularis mucosae tube was cut into approximately 15 mm in length for organ bath study. Atrium and ileal longitudinal smooth muscle isolated from guinea-pig were also used in order to compare mRNA expression. All three types of preparations were rapidly frozen in liquid nitrogen for molecular analysis.

2.3. Functional analysis by organ bath study

Preparations were suspended vertically under an initial tension of 0.5 g in a 20-ml organ bath filled with a Ringer–Locke solution (mM: NaCl, 154; KCl, 5.6; $CaCl_2$, 2.2; $MgCl_2$, 2.1; $NaHCO_3$, 5.9 and D-(+)-glucose, 2.8). Ringer–Locke solution was bubbled continuously with a mixture of 95% O_2/5% CO_2 and maintained at 32 °C (pH = 7.4). The following drugs were present in the bath solution throughout the experiments: imipramine (1 μM, a neuronal uptake inhibitor); normetanephrine (10 μM, an extraneuronal uptake inhibitor); phentolamine (10 μM, an α-adrenoceptor antagonist); and L-ascorbic acid (10 μM, an antioxidant for catecholamines). Preparations were contracted with histamine (10 μM) after equilibration for 30 min and were then exposed to increasing half-log cumulative concentrations of (−)-isoprenaline. To evaluate the effects of β-adrenoceptor antagonists, each drug was added to the bath and allowed to equilibrate with the tissue for 30 min before addition of histamine.

2.4. Detection of mRNA by reverse transcription-polymerase chain reaction (RT-PCR)

Total RNA from each tissue was extracted following the method of Chomczynski and Sacchi (1987). mRNA was subsequently purified from total RNA using a mRNA purification kit (Amersham Biosciences, Buckinghamshire, UK), in accordance with the manufacturer's instructions. cDNA was synthesized by using both Omniscript™ RT Kit (Qiagen, Tokyo, Japan) for reverse transcription (RT) and HotStarTaq™ Master Mix Kit (Qiagen) for polymerase chain reaction (PCR). Briefly, cDNA was synthesized by RT of 2.0 μg of each mRNA using oligo$(dT)_{15}$ (Promega, Madison, USA) as a primer, and RT reaction mixture was incubated at 37 °C for 60 min, then at 95 °C for 5 min before being placed on ice. A negative control without reverse transcriptase was made to verify that amplification did not ensue from residual genomic DNA.

PCR amplification was carried out on cDNA equivalent to 100 ng of starting mRNA, using specific oligonucleotide primers for β_1-adrenoceptors (forward, 5′-CCGCTGCTACAACGACCCCAAG-3′ and reverse, 5′-AGCCAGTTGAAGAAGACGAAGAGGCG-3′), β_2-adrenoceptors (forward, 5′-CTGGTCATCACAGCCATTGCC-3′ and reverse, 5′-TGGTTCGTGAAGAAGTCACA-3′), β_3-adrenoceptors (forward, 5′-GTGGGAGGCAACCTGCTGGT-3′ and reverse, 5′-CGCCACCACTGGCTCAT-3′), and β-actin (forward, 5′-ATCCTGCGTCTGGACCTGGCTG-3′ and reverse, 5′-CCTGCTTGCTGATCCACATCTGCTG-3′) synthesized at TaKaRa Biotechnololgy (Shiga, Japan). cDNA was heated for 15 min at 95 °C, then amplified by 35 cycles at β-adrenoceptors (95 °C for 30 s, 60 °C for 30 s, 72 °C for 30 s) or 25 cycles at β-actin (95 °C for 30 s, 64 °C for 30 s, 72 °C for 30 s) followed by 5 min of extension at 72 °C. The PCR products were electrophoresed on 2.0% ethidium bromide stained agarose gels. Because the sequence for guinea-pig β_1-adrenoceptor was unknown, the primer set for β_1-adrenoceptors (Nevzorova et al., 2002) based on rat and mouse sequences was used in the present study. Other primer sets were designed on the basis of the guinea-pig sequence.

2.5. Drugs

The following drugs were used in the present study: (−)-isoprenaline hydrochloride, (±)-propranolol hydrochloride, histamine dihydrochloride, imipramine hydrochloride, normetanephrine hydrochloride, L-ascorbic acid (Sigma-Aldrich, St. Louis, MO, USA); phentolamine mesylate (Novartis, Basel, Switzerland); (±)-2-hydroxy-5-[2-[[2-hydroxy-3-[4-[1-methyl-4-(trifluoromethyl)-1*H*-imidazol-2-yl]phenoxy]propyl]amino]ethoxy]-benzamide methanesulfonate (CGP20712A), (±)-1-[2,3-(dihydro-7-methyl-1*H*-inden-4-yl)oxy]-3-[(1-methylethyl)amino]-2-butanol hydrochloride (ICI-118,551; Research Biochemicals International, Natick, MA, USA). The other chemicals used were of analytical grade. CGP20712A was dissolved in dimethylsulfoxide at a stock solution of 20 mM, and further diluted in distilled water. Final dimethylsulfoxide concentrations in the bath solution did not affect muscle responses (data not shown). All other drugs were dissolved in distilled water.

2.6. Data analyses

The results are expressed as means ± S.E.M. of n number of experiments. Concentration–response curve fitting and calculations of the pD_2 value for (−)-isoprenaline and pA_2 values for β-adrenoceptor antagonists were performed using GraphPad Prism™ (version 2.01, GraphPad Software, San Diego, CA, USA). pA_2 values were calculated according to the method of Arunlakshana and Schild (1959). Statistical significance was evaluated by

paired *t*-test. A *P* value of less than 0.05 was considered statistically significant.

3. Results

3.1. Effects of β-adrenoceptor antagonists

(−)-Isoprenaline elicited concentration-dependent smooth muscle relaxations in guinea-pig esophagus muscularis mucosae with the pD_2 value of 8.31 ± 0.09 (n = 12).

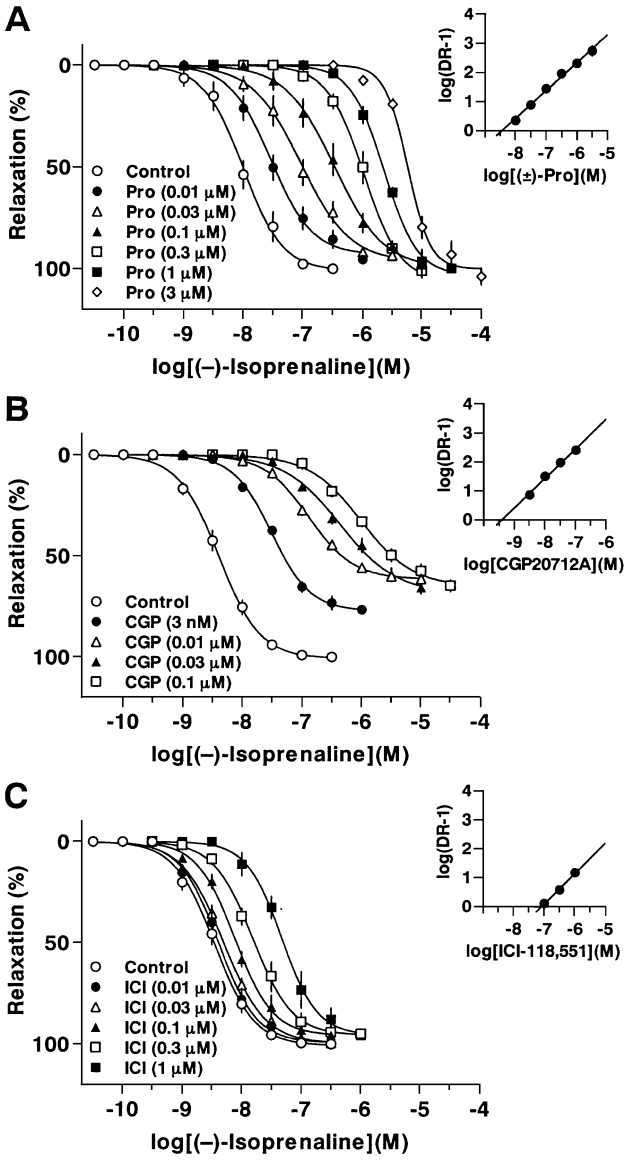

Fig. 1. Effects of (±)-propranolol (A), CGP20712A (B) and ICI-118,551 (C) on concentration–response curves for (−)-isoprenaline. Ordinate: relaxation (%), expressed as a percentage of the maximum relaxation induced by (−)-isoprenaline (0.3 μM) in the absence of β-adrenoceptor antagonists; abscissa: the logarithm concentration (M) of (−)-isoprenaline. Each point represents the mean ± S.E.M. of four experiments. The insets show the corresponding Schild plots.

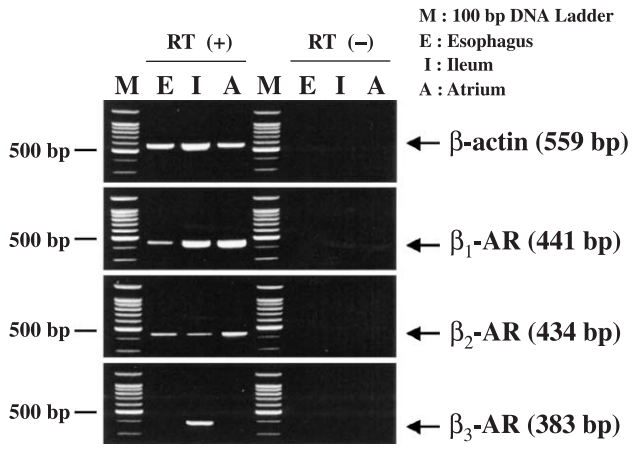

Fig. 2. Detection by RT-PCR of β_1-, β_2-, β_3-adrenoceptor and β-actin mRNA in esophageal muscularis mucosae, ileum and atrium of guinea-pig. The PCR products were separated in 2.0% ethidium bromide stained agarose gels. M: 100-bp DNA ladder (Promega), E: esophagus, I: ileum, A: atrium, RT (+): reverse transcription, RT (−); none reverse transcription.

Concentration-response curves for (−)-isoprenaline were shifted to the right by (±)-propranolol (a non-selective β_1- and β_2-adrenoceptor antagonist), CGP20712A (a selective β_1-adrenoceptor antagonist) and ICI-118,551 (a selective β_2-adrenoceptor antagonist) (Fig. 1A–C). Schild plot analyses of the effects of these antagonists against (−)-isoprenaline gave pA_2 values of 8.47 ± 0.07 ((±)-propranolol), 9.43 ± 0.09 (CGP20712A) and 7.11 ± 0.04 (ICI-118,551), respectively, and all Schild slopes were not significantly different from unity.

3.2. Detection of mRNA for β-adrenoceptors

β_1-, β_2-, β_3-Adrenoceptor and β-actin mRNA were detected using RT-PCR. Each expected size was 441 (β_1-adrenoceptors), 434 (β_2-adrenoceptors), 383 (β_3-adrenoceptors) and 559 bp (β-actin), respectively. The PCR product from esophageal muscularis mucosae showed the expression of β_1-, β_2-adrenoceptors and β-actin mRNA and the lack of mRNA expression for β_3-adrenoceptors (Fig. 2). On the other hands, β_1-, β_2-, β_3-adrenoceptor and β-actin mRNA were detected in ileal smooth muscles and β_1-, β_2-adrenoceptor and β-actin mRNA expressed in atrium (Fig. 2). The PCR products without reverse transcription provided evidence that there is no non-specific amplification and these bands derived from mRNA but not contaminating genomic DNA.

4. Discussion

In the present study, β-adrenoceptors in esophageal muscularis mucosae of guinea-pig were characterized by pharmacological and molecular approaches. (−)-Isoprenaline induced potent relaxations of histamine (10 μM)-precontracted preparations and the responses to (−)-isoprena-

line were antagonized by (±)-propranolol, CGP20712A and ICI-118,551 in a concentration-dependent manner. The pA_2 values for these antagonists are in good agreement with the affinity values (as pA_2 or pK_i values) at β_1-adrenoceptors which were reported as follows: 8.6 for (±)-propranolol, 8.5–9.3 for CGP20712A, and 7.2 for ICI-118,551 (Alexander et al., 2001; Arch and Kaumann, 1993). These results clearly suggested that the smooth muscle relaxations in response to (−)-isoprenaline were due to activation of β_1-adrenoceptors rather than β_2- and β_3-adrenoceptors.

RT-PCR technique was frequently used to demonstrate the expression of mRNA for three subtypes for β-adrenoceptors on many tissues isolated from rat (Evans et al., 1996), but there is no genetic information about the β-adrenoceptor expression on gastrointestinal smooth muscles of guinea-pig. In the present study, RT-PCR methods for detecting the mRNA expression of β-adrenoceptors in guinea-pig tissues were first established. Newly devised methods demonstrated that very low or no expression of β_3-adrenoceptor mRNA in esophageal muscularis mucosae and atrium compared to ileum and the mRNA expression of β_1- and β_2-adrenoceptors in all of three tissues described functionally as β_1-adrenoceptors. These data indicate that β_3-adrenoceptors do not necessarily coexist with β_1-adrenoceptors and there is the difference between function and expression of β-adrenoceptor subtypes. The later finding obtained in esophagus is consistent with the study showing that β_2-adrenoceptor mRNA is predominately expressed in rat ileum despite β_2-adrenoceptors are not responsible for smooth muscle relaxations (Roberts et al., 1999). Although the reason for this discrepancy between the lack of function and the expression of mRNA for β_2-adrenoceptors remains unknown, function of β_2-adrenoceptors may be lost with the continued expression of β_2-adrenoceptor mRNA as a result of switching functional β-adrenoceptors described in brown adipocyte where functional receptors switched from β_1-adrenoceptors to β_3-adrenoceptors during development, whereas β_1-adrenoceptor mRNA expression is maintained after this switching (Bronnikov et al., 1999).

In conclusion, the present studies provide functional and molecular evidence that despite the participation of only β_1-adrenoceptors in the smooth muscle relaxation, both β_1- and β_2-adrenoceptor mRNA were expressed and β_3-adrenoceptors appear to be essentially nonexistent in esophageal muscularis mucosae of guinea-pig.

References

Alexander, S.P.H., Mathie, A., Peters, J.A., 2001. Nomenclature supplement 2001. Trends Pharmacol. Sci., 12th ed., p. 17.

Arch, J.R.S., Kaumann, A.J., 1993. β_3- and atypical β-adrenoceptors. Med. Res. Rev. 13, 663–729.

Arunlakshana, O., Schild, H.O., 1959. Some quantitative uses of drug antagonists. Br. J. Pharmacol. Chemother. 14, 48–58.

Bronnikov, G., Bengtsson, T., Kramarova, L., Golozoubova, V., Cannon, B., Nedergaard, J., 1999. β_1 to β_3 switch in control of cyclic adenosine monophosphate during brown adipocyte development explains distinct β-adrenoceptor subtype mediation of proliferation and differentiation. Endocrinology 140, 185–197.

Brown, K.J., Summers, R.J., 2001. β_1- and β_3-adrenoceptor mediated smooth muscle relaxation in hypothyroid rat ileum. Eur. J. Pharmacol. 415, 257–263.

Chomczynski, P., Sacchi, N., 1987. Single-step method of RNA isolation by acid guanidinium thiocyanate–phenol–chloroform extraction. Anal. Biochem. 162, 156–159.

Evans, B.A., Papaioannou, M., Bonazzi, V.R., Summers, R.J., 1996. Expression of β_3-adrenoceptor mRNA in rat tissues. Br. J. Pharmacol. 117, 210–216.

Grassby, P.F., Broadley, K.J., 1984. Characterization of β-adrenoceptors mediating relaxation of the guinea-pig ileum. J. Pharm. Pharmacol. 36, 602–607.

Horinouchi, T., Koike, K., 2000. Functional identification of β_3-adrenoceptors in the guinea pig ileum using the non-selective β-adrenoceptor antagonist (±)-bupranolol. J. Auton. Pharmacol. 20, 253–258.

Kamikawa, Y., Shimo, Y., 1987. Different spasmolytic effects of smooth muscle relaxants on the guinea-pig esophageal muscularis mucosae contracted by carbachol or high potassium in vitro. Eur. J. Pharmacol. 136, 39–48.

Lands, A.M., Arnold, A., Mcauliff, J.P., Luduena, F.P., Brown Jun, T.G., 1967a. Differentiation of receptor systems activated by sympathomimetic amines. Nature 214, 597–598.

Lands, A.M., Luduena, F.P., Buzzo, H.J., 1967b. Differentiation of receptors responsive to isoproterenol. Life Sci. 6, 2241–2249.

Manara, L., Croci, T., Landi, M., 1995. β_3-adrenoceptors and intestinal motility. Fundam. Clin. Pharmacol. 9, 332–342.

Nevzorova, J., Bengtsson, T., Evans, B.A., Summers, R.J., 2002. Characterization of the β-adrenoceptor subtype involved in mediation of glucose transport in L6 cells. Br. J. Pharmacol. 137, 9–18.

Roberts, S.J., Papaioannou, M., Evans, B.A., Summers, R.J., 1999. Characterization of β-adrenoceptor mediated smooth muscle relaxation and the detection of mRNA for β_1-, β_2- and β_3-adrenoceptors in rat ileum. Br. J. Pharmacol. 127, 949–961.