ELSEVIER

European Journal of Pharmacology 315 (1996) 363–367

ejp

Molecular characterization of pharmacological properties of T-0509 for β-adrenoceptors

Yoji Sato, Hitoshi Kurose, Masafumi Isogaya, Taku Nagao *

Department of Toxicology and Pharmacology, Faculty of Pharmaceutical Sciences, University of Tokyo, Hongo, Bunkyo-ku, Tokyo 113, Japan

Received 29 April 1996; revised 24 July 1996; accepted 6 August 1996

Abstract

The pharmacological properties of T-0509, (−)-(*R*)-1-(3,4-dihydroxyphenyl)-2-[(3,4-dimethoxyphenethyl)amino]ethanol, were compared with those of isoproterenol. In the radioligand binding studies of [^{125}I]iodocyanopindolol with COS-7 cell membranes that transiently expressed β-aderenoceptor subtypes, T-0509 exhibited 11- and 97-fold greater K_i values for β_2- and β_3-adrenoceptors, respectively, compared with β_1-adrenoceptors. Affinities of β_2- and β_3-adrenoceptors to isoproterenol were 1.4- and 28-fold lower than that of β_1-adrenoceptors, respectively. The maximal stimulatory effects of T-0509 on adenylyl cyclase of CHO-K1 (chinese hamster ovary K1) cell membranes expressing β_1- or β_2-adrenoceptors were 85% or 96% of those produced by isoproterenol, respectively. These results indicate that T-0509 is a relatively specific β_1-adrenoceptor agonist with a high intrinsic activity as compared with isoproterenol.

Keywords: T-0509; β_1-Adrenoceptor agonist; β_3-Adrenoceptor; Intrinsic activity; Adenylyl cyclase

1. Introduction

Selective drugs for a certain receptor subtype have been useful tools in the studies of drug-receptor interaction and receptor-mediated physiological responses in tissues expressing a variety of receptors. β-Adrenergic stimulation activates plasma membrane adenylyl cyclase, resulting in an elevation of intracellular cyclic AMP which mediates a number of hormone-induced responses. β-Adrenoceptors have been divided into three subtypes, i.e., β_1-, β_2- and β_3-adrenoceptors. β_1-Adrenoceptor stimulation is known to have positive inotropic and chronotropic effects on cardiac tissues and lipolytic effects on adipocytes. Several selective β_1-adrenoceptor agonists have been developed in the last two decades. However, they have lower intrinsic activity for cardiac adenylyl cyclase (less than 70%) than norepinephrine and isoproterenol (Lemoine et al., 1989). Therefore, their effects can be due to their low efficacy as β_1-adrenoceptor agonist as well as their β_1-adrenoceptor subtype selectivity. The advent of a selective β_1-adrenoceptor agonist with high intrinsic activity should help to understand the functional role of β_1-adrenoceptors in various tissues. To date, norepinephrine and dobutamine are frequently used to evaluate β_1-adrenoceptor function. Although they are thought to be selective β_1-adrenoceptor full agonists, they have potent α-adrenoceptor agonist activity (Kenakin, 1981). Moreover, dobutamine is known to have low intrinsic activity in the stimulation of adenylyl cyclase (Kaumann, 1981; Lemoine et al., 1989). T-0509 [(−)-(*R*)-1-(3,4-dihydroxyphenyl)-2-[(3,4-dimethoxyphenethyl)amino]ethanol] is a catechol derivative of a β_1-adrenoceptor partial agonist, denopamine (Nagao and Nakajima, 1989; Lemoine et al., 1989), and has been reported to have selective β_1-adrenoceptor full agonist activity in cardiac contraction with less potent α_1-adrenoceptor activity than isoproterenol in aortic contraction (Yabana et al., 1992). In addition, it has been shown that T-0509 binds to cardiac membranes with higher affinity than to lung membranes, indicating its β_1-adrenoceptor selectivity (Kusayama et al., 1994). However, the intrinsic activity of T-0509 on adenylyl cyclase has not been examined and its binding properties for β_3-adrenoceptors have not been reported. In the present study, we examined the affinities and intrinsic activities of T-0509 for the β-adrenoceptor subtypes, and compared its pharmacological characteristics with those of isoproterenol.

* Corresponding author. Tel./Fax: (81-3) 5802-2943.

PII S0014-2999(96)00648-6

2. Materials and methods

2.1. Transient expression of β-adrenoceptors in COS-7 cells

The human β_1- and β_2-adrenoceptors in pBC12BI or β_3-adrenoceptor in pCMV5 were transfected into COS-7 cells by the DEAE-dextran method (Cullen, 1987). The DNA sequence encoding the human β_3-adrenoceptor was amplified from the genomic DNA prepared from HeLa cells with two primers (GCGCGAATTCACCATGGCTC-CGTGGCCTCACGAGAA and GCGCGGATCCCTACC-CGTCGAGCCGGTTGCAAA, *Eco*RI or *Bam*HI sites were underlined) and Pfu DNA polymerase. After sequencing by dideoxynucleotide sequencing method (Sanger et al., 1977), we found a mutation at position 205 from ATG (published sequence is C and amplified sequence is A) and the error was corrected by the polymerase chain reaction (PCR). The resulting sequence lacked the last 6 amino acids and it was ligated into the mammalian expression vector pCMV5. The transfected cells were grown as monolayer in 100 mm dishes containing Dulbecco's Modified Eagle Medium supplemented with 5% fetal bovine serum and gentamicin (10 μg/ml) in an atmosphere of 95% air and 5% CO_2 at 37°C.

2.2. CHO-K1 cells stably expressing the epitope-tagged β-adrenoceptors

In the preliminary study, we found that β_1- and β_2-adrenoceptors with an epitope recognized by monoclonal antibody 12CA5 at the N-terminus were expressed more stably than the wild types (the reason is not clear). Then the 9-amino acid epitope recognized by monoclonal antibody 12CA5 (sequence YPYDVPDYA) (Wilson et al., 1984; Von Zastrow and Kobilka, 1992) was inserted at the amino terminus of the receptors. The cDNAs encoding human β_1- or β_2-adrenoceptors were exercised from β_1- or β_2-pBC12BI by PCR with primer containing an epitope and primer which has internal sequence of β-adrenoceptors. The sequences of the amplified regions were confirmed by the dideoxynucleotide sequencing method. The epitope-tagged β_1- and β_2-adrenoceptors were subsequently inserted into pCMV5. The pCMV5 vectors containing the epitope-tagged β_1- and β_2-adrenoceptor cDNAs were cotransfected with pRc/CMV to confer the Geneticin (G-418 sulfate) resistance into CHO-K1 (Chinese hamster ovary K1) cells by calcium phosphate precipitation (Sambrook et al., 1989). The transfected cells were grown as monolayer in Ham's F-12 supplemented with 10% fetal bovine serum and gentamicin (10 μg/ml) in an atmosphere of 95% air and 5% CO_2 at 37°C. Stable transformants were selected in 1 mg/ml Geneticin. Expression of β_1- and β_2-adrenoceptors was determined using an [^{125}I]iodocyanopindolol binding assay, as described below.

2.3. Membrane preparation

2.3.1. COS-7 cells

Forty-eight hours after the transfection, the COS-7 cells were rinsed with 10 ml of ice-cold phosphate-buffered saline (PBS) and mechanically detached in 5 ml of lysis buffer containing 10 mM Tris-HCl (pH 7.4), 5 mM EDTA, 5 mM EGTA, 10 μg/ml benzamidine, 10 μg/ml soybean trypsin inhibitor (Type II-S) and 5 μg/ml leupeptin. The cells were homogenized and centrifuged at 45 000 × *g* for 10 min at 4°C. The resultant pellets were resuspended in the lysis buffer and frozen at −80°C until use.

2.3.2. CHO-K1 cells

The CHO-K1 cells expressing the β-adrenoceptors were washed 4 times with 10 ml of ice-cold PBS, scraped in 3 ml of the lysis buffer, homogenized and centrifuged at 45 000 × g for 10 min at 4°C. The sedimented membranes were resuspended in the lysis buffer for the radioligand binding assay or 10 mM Hepes buffer for the adenylyl cyclase assay.

2.4. Radioligand binding assay

Radioligand binding studies were carried out in a buffer containing 75 mM Tris-HCl (pH 7.4), 12.5 mM $MgCl_2$ and 2 mM EDTA at 37°C for 60 min using ~5 μg of membrane protein. The total reaction volume was 250 μl. For saturation isotherms, membranes (~5 μg) were incubated with varying concentrations of [^{125}I]iodocyanopindolol (2.5–250 pM [β_1, β_2], 150–3000 pM [β_3]) in the absence (total binding) or presence (nonspecific binding) of 1 μM (±)-propranolol for β_1- and β_2-adrenoceptors or 1 mM isoproterenol for β_3-adrenoceptor. Competition binding studies were carried out using 100 pM (β_1, β_2) or 1700 pM (β_3) [^{125}I]iodocyanopindolol and various concentrations (0–10 mM) of agonists in the presence of 100 μM (COS-7) or 1 mM (CHO-K1) of GTP. The reactions were stopped by dilution with 8 ml of cold buffer containing 25 mM Tris (pH 7.5) and 1 mM $MgCl_2$ and rapid filtration over Whatman GF/C filters. The filters were washed with an additional ice cold buffer (8 ml). The radioactivity remained on the filter was counted by a gamma counter.

2.5. Adenylyl cyclase assay

Adenylyl cyclase activities were measured by the method of Salomon et al. (1974) using CHO-K1 cell membranes. Briefly, the membranes (~15 μg of protein) were incubated with increasing concentrations (0–100 μM) of agonists in a buffer containing 40 mM Hepes (pH 7.4), 0.12 mM ATP, 0.05 mM GTP, 2.8 mM phosphoenolpyruvate, 0.1 mM cyclic AMP, 1 U of myokinase, 0.2 U of pyruvate kinase, 0.8 mM EDTA, 12 mM $MgCl_2$ and 1.0 μCi [α-^{32}P]ATP in a final volume of 50 μl, for 30 min at 37°C. The incubation was terminated by the addition of 1

ml of an ice-cold solution containing 0.5 mM ATP, 0.5 mM cyclic AMP and [^{3}H]cyclic AMP (~ 16000 cpm). The cyclic AMP was separated using sequential chromatography over Dowex resin and alumina columns.

2.6. Protein assay

Protein contents were measured by the method of Lowry et al. (1951) using bovine serum albumin as the standard.

2.7. Data analysis

Data from radioligand saturation assays were fitted to Michaelian rectangular hyperbolic curves by nonlinear least-squares analysis using a computer program, SP123 (Ikeda et al., 1991). Competition curves for agonists were analyzed with a computer program, LBS (Ikeda et al., 1991). Concentration-response curves from adenylyl cyclase assays were fitted to a logistic equation using nonlinear least-squares analysis. All results were expressed as means ± S.E.M. from n experiments. Paired t-test was performed to assess the significance of the difference. The significance level was $P < 0.05$.

2.8. Materials

T-0509 hydrochloride was generously donated by Tanabe Seiyaku, Osaka, Japan. β_1- and β_2-adrenoceptor-pBC12BI were provided by Dr R.J. Lefkowitz (Duke University). [^{125}I]Iodocyanopindolol (~ 2000 Ci/mmol, New England Nuclear), [α-^{32}P] ATP (10–25 Ci/mmol, American Radiolabeled Chemicals), (−)-ispoproterenol hydrochloride (Sigma) and the other chemicals were purchased from commercial sources.

3. Results

3.1. Affinities of T-0509 for β-adrenoceptor subtypes

We examined the affinities of T-0509 for human β_1-, β_2- and β_3-adrenoceptors transiently expressed in COS-7 cells. Table 1 shows the B_{max} and K_d values of [^{125}I]iodocyanopindolol for membranes from COS-7 cells transfected with one of the β_1-, β_2- and β_3-adrenoceptor cDNAs/genes. Specific binding of [^{125}I]iodocyanopindolol to each of the β-adrenoceptors was monophasic and saturable, indicating the single binding site. Expression of β_3-adrenoceptors in COS-7 cells was low as compared with those of β_1- or β_2-adrenoceptors and [^{125}I]iodocyanopindolol binding to the β_3-adrenoceptor was of low affinity, as reported previously (Tate et al., 1991). K_i values of the agonists for [^{125}I]iodocyanopindolol binding to the COS-7 cell membranes are shown in Table 1. In the membranes expressing one of the β-adrenoceptor subtypes, both isoproterenol and T-0509 caused monophasic displacement of [^{125}I]iodocyanopindolol, suggesting a single binding site of the agonists. The affinity of isoproterenol for β_1-adrenoceptor was 1.4-fold higher than that for β_2-adrenoceptor. Although the affinity of T-0509 for β_1-adrenoceptor was similar to that of isoproterenol, the K_i value of T-0509 for the β_2-adrenoceptor was 7-fold higher than that for isoproterenol, and the affinity of T-0509 for the β_2-adrenoceptor was 11 times lower than that for β_1-adrenoceptor, suggesting the selectivity of T-0509 for β_1-adrenoceptor. Isoproterenol and T-0509 showed 28 and 97 times lower affinity for β_3-adrenoceptor than those for β_1-adrenoceptor, respectively.

3.2. Affinity of T-0509 for CHO-K1 cell membranes

Table 1 also shows the binding properties of [^{125}I]iodocyanopindolol and β-adrenoceptor agonists for membranes prepared from CHO-K1 cells stably expressed with the epitope-tagged β_1- or β_2-adrenoceptor cDNAs. K_d values of [^{125}I]iodocyanopindolol for β_1- and β_2-adrenoceptors were similar to those obtained with the COS-7 cell membranes. Displacement curves of the β-adrenoceptor agonists were best fitted to the single-affinity binding site. The K_i values of the agonists for β_1- and β_2-adrenoceptors were comparable to those obtained with COS-7 cell membranes.

3.3. Agonist-stimulated adenylyl cyclase activity

In order to determine functional properties of T-0509, agonist-stimulated adenylyl cyclase activities were measured in membranes from CHO-K1 cells expressing either

Table 1
Binding properties of [^{125}I]iodocyanopindolol and the β-adrenoceptor agonists to membranes from COS-7 and CHO cells that express the human β-adrenoceptor subtype

Subtype		β_1 ($n = 3$)		β_2 ($n = 3$)		β_3 ($n = 2$)
Membrane		COS-7	(CHO)	COS-7	(CHO)	COS-7
K_d (pM)		32.8 ± 3.4	(24.7 ± 2.2)	20.7 ± 1.0	(20.3 ± 1.7)	178.2 ± 0.3
B_{max} (fmol/mg protein)		1851 ± 202	(247 ± 39)	844 ± 43	(339 ± 43)	331 ± 7
K_i (nM)	Isoproterenol	268 ± 99	(255 ± 23)	378 ± 54	(387 ± 27)	7475 ± 155
	T-0509	237 ± 28	(176 ± 13)	2680 ± 272	(2324 ± 51)	23000 ± 800

K_d, B_{max}: equilibrium dissociation constant and maximal binding capacity of [^{125}I]iodocyanopindolol, respectively. K_i: equilibrium dissociation constant of the agonists. Values are the means ± S.E.M. of n experiments.

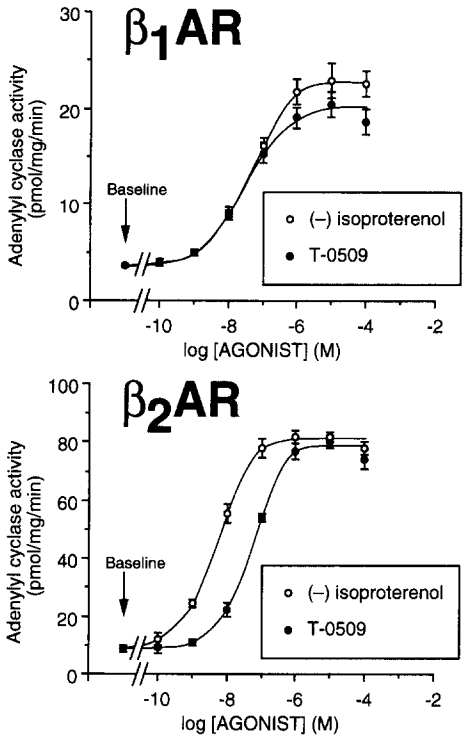

Fig. 1. Agonist-stimulated adenylyl cyclase activity measured in membranes from CHO-K1 cells expressing either the β_1- (upper) or β_2-adrenoceptors (lower). The enzyme activity was measured in the presence of increasing concentrations of isoproterenol (open circles) or T-0509 (closed circles). Values are the means ± S.E.M. of 3 experiments.

β_1- or β_2-adrenoceptors. As shown in Fig. 1, both isoproterenol and T-0509 induced a dose-dependent activation of adenylyl cyclase. The intrinsic activity of T-0509 for β_1-adrenoceptor was 85 ± 1% ($n = 3$, $P < 0.05$ vs. isoproterenol) with a EC_{50} value 1.6-fold lower than that of isoproterenol (T-0509: 24.8 ± 2.0 nM, isoproterenol: 40.9 ± 1.0 nM; $n = 3$). For β_2-adrenoceptors, T-0509 behaved as a full agonist with the EC_{50} value being 9.7-times higher than that of isoproterenol (T-0509: 46.7 ± 0.8 nM, isoproterenol: 4.8 ± 0.4 nM; $n = 3$).

4. Discussion

In the previous study, T-0509 showed a considerably higher affinity for cardiac membranes than lung membranes, suggesting its selectivity for the β_1-adrenoceptor (Kusayama et al., 1994). However, most tissues that predominantly express a certain β-adrenoceptor subtype usually contain some amount of the other subtype(s). Thus, characterization of a drug with these tissues may be affected by the minor subtype(s). The heterologous expression system using recombinant receptors allows us to evaluate properties of the drug without concerning the populations of the expressed subtypes. In particular, this approach is useful for characterizing a receptor subtype like β_3-adrenoceptor where selective and high affinity radioligand is not available.

K_d values of [^{125}I]iodocyanopindolol and K_i values of isoproterenol for COS-7 cell membranes that were transfected with the cDNAs were similar to those for β-adrenoceptor subtypes expressed in CHO-K1 cells (Tate et al., 1991; Green et al., 1992; Liggett, 1992). These results suggest that the β-adrenoceptor subtypes expressed in COS-7 cells retain the pharmacological properties. K_i values of T-0509 for β_2- and β_3-adrenoceptors were 11- and 97-fold greater than those for β_1-adrenoceptor, respectively, indicating that T-0509 has high affinity only for the β_1-adrenoceptor and extremely low affinity for the β_3-adrenoceptor.

T-0509 has been reported to have full agonist activity for the positive inotropic effect on cardiac muscle (Yabana et al., 1992). However, it has been found that partial agonists for activating adenylyl cyclase can be full inotropic agonists (Kaumann, 1981; Lemoine et al., 1989). To evaluate the ability of T-0509 to activate adenylyl cyclase, we compared a concentration-response curve of T-0509 with the β-adrenoceptor full agonist isoproterenol using the membranes from CHO-K1 cells that stably expressed epitope-tagged β_1- or β_2-adrenoceptors. The N-terminus of the β-adrenoceptors are thought to have no responsibility for both function and ligand binding of the β-adrenoceptors (Kobilka, 1992). Thus, the epitope should not affect the pharmacological characteristics of β-adrenoceptors and the signal transduction process from binding of β-adrenoceptor agonists to activation of adenylyl cyclase.

K_d values of [^{125}I]iodocyanopindolol and K_i values of the isoproterenol with the CHO-K1 cell membranes were similar to those obtained from the COS-7 cell binding experiments and the previous studies (Tate et al., 1991; Green et al., 1992). These results indicate that the epitope-tagged β-adrenoceptors were folded correctly in the CHO-K1 cells and maintained the binding properties of the wild type receptors.

With the adenylyl cyclase assays, isoproterenol had a higher potency for the β_2-adrenoceptor than for the β_1-adrenoceptor, and T-0509 had similar potencies for the β_1- and β_2-adrenoceptor. Even though isoproterenol bound to the β_1- and β_2-adrenoceptors with similar affinities, the potencies (EC_{50}) of isoproterenol to stimulate adenylyl cyclase can be different between the β_1- and β_2-adrenoceptor-mediated pathways. The potency of agonist is determined by the affinity of agonist for the receptor and the coupling between the receptor and the G_s protein. It is assumed that the latter depends on the structure of the receptor, especially intracellular domains, but not ligands (DeLean et al., 1982). The dicrepancies between the potencies of isoproterenol-stimulated adenylyl cyclase activity and the affinity of isoproterenol for β-adrenoceptor subtypes presumably owe to the intrinsic differences in the strength of coupling between the receptor subtypes (i.e., β_1- and β_2-adrenoceptors) and the G_s protein. In this context, it should be noted that the β_2-adrenoceptor showed

high efficiency of the agonist-promoted coupling between the receptor and the G_s protein than the β_1-adrenoceptors did (Green et al., 1992; Levy et al., 1993).

In the present study using CHO-K1 cell membranes stably expressed one of the β-adrenoceptors, T-0509 maximally activated adenylyl cyclase by the stimulation of β_2-adrenoceptors, to an extent comparable to isoproterenol. Although the degree of the maximum adenylyl cyclase activation by T-0509 through the β_1-adrenoceptor was considerably high, it was significantly lower than that by isoproterenol. These findings indicate that T-0509 has high but submaximal intrinsic activity for adenylyl cyclase through the β_1-adrenoceptors and full intrinsic activity through the β_2-adrenoceptors.

In summary, T-0509 appeared to have high, low and extremely low affinities for β_1-, β_2- and β_3-adrenoceptor subtypes, respectively, and to be a β_1-adrenoceptor agonist with high intrinsic activity as well as a β_2-adrenoceptor full agonist. T-0509 is a unique agonist in its selectivity and intrinsic activity. It can be a useful tool for the study of β-adrenoceptor subtypes.

Acknowledgements

We would like to thank Tanabe Seiyaku for generously providing T-0509. We are also grateful to Dr R.J. Lefkowitz for human β_1- and β_2-adrenoceptor cDNA constructs, and to Drs H. Ono (University of Tokyo) and A. Seo (Hiroshioma University) for kindly providing the data analysis program.

References

Cullen, B.R., 1987, Use of eukaryotic expression technology in the functional analysis of a cloned genes, in: Methods in Enzymology, eds. S.L. Berger and A.R. Kimmel (Academic Press, San Diego, CA), 152, 684.

DeLean, A., J.M. Stadel and R.J. Lefkowitz, 1982, A ternary complex model explains the agonist-specific binding properties of the adenylate cyclase-coupled β-adrenergic receptor, J. Biol. Chem. 255, 7108.

Green, S.A., B.D. Holt and S.B. Liggett, 1992, β_1- and β_2-adrenergic receptors display subtype-selective coupling to G_s, Mol. Pharmacol. 41, 889.

Ikeda, S., J.-I. Oka and T. Nagao, 1991, Effects of four diltiazem stereoisomers on binding of *d-cis*-[^{3}H]diltiazem and (+)-[^{3}H]PN200-110 to rabbit T-tuble calcium channels, Eur. J. Pharmacol. Mol. Pharmacol. Sect. 208, 199.

Kaumann, A.J., 1981, In Kitten ventricular myocardium, the inotropic potency of an agonist is determined by both its intrinsic activity for the adenylyl cyclase and its affinity for the β-adrenoceptors, Naunyn-Schmiedeberg's Arch. Pharmacol. 317, 13.

Kenakin, T.P., 1981, An in vitro quantitative analysis of alpha adrenoceptor partial agonist activity of dobutamine and its relevance to inotropic selectivity, J. Pharmacol. Exp. Ther. 216, 210.

Kobilka, B., 1992, Adrenergic receptors as models for G protein-coupled receptors, Annu. Rev. Neurosci. 15, 87.

Kusayama, T., J.-I. Oka, H. Yabana, S. Adachi-Akahane and T. Nagao, 1994, Binding of a catechol derivative of denopamine (T-0509) and *N-tert*-butylnoradrenaline (Colterol) to β_1- and β_2-adrenoceptors, Biol. Pharm. Bull. 17, 1023.

Lemoine, H., K.J. Teng, S.J. Slee and A.J. Kaumann, 1989, On minimum cyclic AMP formation rates associated with positive inotropic effects mediated through β_1-adrenoceptors in kitten myocardium: β_1-specific and non-adrenergic stimulant effects of denopamine, Naunyn-Schmiedeberg's Arch. Pharmacol. 339, 113.

Levy, F.O., X.Z. Zhu, A.J. Kaumann and L. Birnbaumer, 1993, Efficacy of β_1-adrenergic receptors is lower than that of β_2-adrenergic receptors, Proc. Natl. Acad. Sci. USA 90, 10798.

Liggett, S.B., 1992, Functional properties of the rat and human β_3-adrenergic receptors: differential agonist activation of recombinant receptors in Chinese hamster ovary cells, Mol. Pharmacol. 42, 634.

Lowry, O.H., N.J. Rosebrough, A.L. Farr and R.J. Randall, 1951, Protein measurement with the Folin phenol reagent. J. Biol. Chem. 193, 265.

Nagao, T. and H. Nakajima, 1989, Denopamine, Cardiovas. Drug Rev. 7,310.

Salomon, Y., C. Londos and M. Rodbell, 1974, A high sensitive adenylate cyclase assay, Anal. Biochem. 58, 541.

Sambrook, J., E.F. Fritsch and T. Maniatis, 1989, Introduction of recombinant vectors into mammalian cells: expression of cloned genes in cultured mammalian cells, in: Molecular Cloning: A Laboratory Manual (Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY) p. 16.30.

Sanger, F., S. Nicklen and A.R. Coulson, 1977, DNA sequencing with chain-terminating inhibitors, Proc. Natl. Acad. Sci. USA 74, 5463.

Tate, K.M., M.-M. Briend-Sutren, L.J. Emorine, C. Delavier-Klutchko, S. Marullo and A.D. Strosberg, 1991, Expression of three human β-adrenergic-receptor subtypes in transfected Chinese hamster ovary cells, Eur. J. Biochem. 196, 357.

Von Zastrow, M. and B.K. Kobilka, 1992, Ligand-regulated internalization and recycling of human β_2-adrenergic receptors between the plasma membrane and endosomes containing transferrin receptors, J. Biol. Chem. 267, 3530.

Wilson, I.A., H.L. Niman, R.A. Houghten, A.R. Cherenson, M.L. Connolly and R.A. Lerner, 1984, The structure of an antigenic determinant in a protein, Cell 37, 767.

Yabana, H., H. Watanabe, H. Narita and T. Nagao, 1992, Selective and full β_1-adrenoceptor agonist action of a catechol derivative of denopamine (T-0509) in the guinea-pig cardiac muscle and trachea: comparison with denopamine, xamoterol and isoprenaline, Br. J. Pharmacol. 106, 335.