Changes in Human Lymphocyte β-Adrenergic Receptors after Administration of Xamoterol and Atenolol

Ken-ichi WATANABE[1], Yoichi HIROKAWA[2], Kaoru SUZUKI[2], Fumiaki MASANI[1], Akira SHIBATA[2], Hiroshi TSUCHIHASHI[3] and Takafumi NAGATOMO[3]

[1]Division of Cardiology, Kuwana Hospital, 6-4 Furukawa-cho, Niigata City, 950 Japan, [2]First Department of Internal Medicine, Niigata University School of Medicine, [3]Department of Pharmacology, Niigata College of Pharmacy

Summary. For seven days atenolol (a β-antagonist) and xamoterol (a β-partial agonist) were give orally to healthy volunteers at 50 mg once daily and 100 mg twice daily, respectively. Lymphocyte β-receptor density (Bmax) and affinity (Kd) were determined by radioligand binding assay by using ^{125}I-iodocyanopindolol.

Atenolol significantly increased Bmax from $1,566 \pm 297$ to $3,213 \pm 1,134$ sites/cell and Kd from 1.12 ± 0.13 to 4.32 ± 2.26 nM. Systolic blood pressure and heart rate were significantly decreased during the treatment with atenolol compared with the basal levels. Xamoterol markedly increased the Bmax and Kd from $1,466 \pm 373$ and 1.07 ± 0.14 to $7,169 \pm 3,768$ sites/cell and 6.01 ± 3.84 nM, respectively ($p < 0.01$). But, in contrast to atenolol, the systolic blood pressure increased slightly ($p < 0.05$), and the heart rate was unchanged. Following withdrawal of both drugs, both Bmax and Kd returned to the basal levels.

The results indicate that xamoterol up-regulates lymphocyte β-receptors without a decrease in systolic blood pressure, thus suggesting that xamoterol may be beneficial in the treatment of patients with heart failure.

Recent advances in radioligand binding assay and purification of receptor proteins have allowed quantitative and qualitative studies of receptors.[1,2] There is evidence that changes in β-receptors in myocardial membranes and lymphocytes are closely related to the pathophysiology and effects of treatment.[3]

When β-antagonists are administered to patients with hypertension or angina pectoris, the density (Bmax) of β-receptors increase, i.e., they are up-regulated.[4] On the contrary, when β-agonists are administered to patients with bronchial asthma or heart failure, the β-receptors are down-regulated.[5]

In patients with chronic heart failure, plasma norepinephrine levels are elevated, while the Bmax of β-receptors in the myocardium and lymphocytes decrease. Thus a β-blockade has been proposed in these patients to produce an up-regulation of β-receptors.[6,7] However, β-blockers are usually contraindicated in patients with heart failure as they may depress cardiac functions.[8] It is, therefore, postulated that a drug which increases β-receptor density without having a negative inotropic action may be of clinical benefit.

Atenolol is a β-adrenergic antagonist. Xamoterol (ICI 118,587, "Corwin®") is a β-adrenergic partial agonist which is currently being evaluated for the treatment of mild to moderate heart failure. The present study was performed to investigate the effects of atenolol and xamoterol on lymphocyte β-receptors in healthy volunteers. We now report that xamoterol therapy increases the number of β-adrenergic binding sites on the lymphocytes.

SUBJECTS AND METHODS

Eight healthy male volunteers (mean age 20 years, range 18-23 years) were used in the study. Informed consent was obtained from each subject after the volunteer had received a full explanation about the study. Atenolol (Tenormin®) was given orally (50 mg once daily). Heart rate, blood pressure, lymphocyte β-receptors, and plasma atenolol concentrations were obtained before treatment, on days 2, 5, and 7 of treatment, and 2 and 5 day after withdrawal of atenolol. One month after withdrawal of atenolol, xamoterol was given orally (100 mg twice daily) for 7

Table 1. Effects in healthy volunteers of atenolol and xamoterol on blood pressure, heart rate and lymphocyte β-receptors

	Before	Treatment			Withdrawal	
		2	5	7 (days)	2	5 (days)
(1) Atenolol (50 mg od)						
Syst BP (mmHg)	128±14	116±16*	110±18*	111±19*	115±17*	125±20
HR (beats/min)	73±8	59±10*	59±14*	57±9*	67±11	76±17
Bmax (sites/cell)	1566±297	3213±1134*	2943±1026**	2403±1053**	2030±1269*	1755±459
Kd (nM)	1.12±0.13	4.32±2.26**	3.58±1.04**	2.32±0.88**	0.88±0.34	1.22±0.35
Plasma levels (μg/ml)	—	240±80	187±60	254±81	<5	<5
(2) Xamoterol (100 mg bd)						
Syst BP (mmHg)	118±17	127±17*	133±19*	126±16*	122±16	120±15
HR (beats/min)	78±15	81±7	75±13	86±13	78±11	73±11
Bmax (sites/cell)	1466±373	7169±3768**	4946±2500**	5130±2889**	1998±214**	1420±383
Kd (nM)	1.07±0.14	6.01±3.84**	3.55±2.30**	2.61±1.15**	0.99±0.21	1.01±0.16
Plasma levels (μg/ml)	—	84±23	61±35	69±24	<5	<5

Values are mean±1SD. Syst=systolic and HR=heart rate.

*$p < 0.05$, **$p < 0.01$ when compared with values before administration of drugs.

days. Measurements similar to those for atenolol were made.

Measurements of heart rate and blood pressure were made 2 hours after the morning dose with the subjects in a supine position after 5 minute rest. Venous blood samples (20 ml) were then withdrawn. 15 ml of each sample was used to isolate lymphocytes, and 5 ml was used for assaying plasma concentrations of atenolol or xamoterol. Plasma concentration of atenolol was determined by high-liquid performance chromatography, and that of xamoterol by radioimmunoassay.

The Bmax and affinity (Kd) of lymphocyte β-adrenergic receptors were determined from the binding of ^{125}I-iodocyanopindolol (ICYP) as a radioligand. Heparinized 15 ml blood samples were placed on a Ficoll conray by Böyum's method and centrifuged at 1,500 rpm for 30 min to isolate the lymphocytes.[4,9] These were washed twice with a phosphate-buffered saline at 1,000 rpm for 10 min and then suspended in a mixture of 120 mM Tris-HCl and 40 mM MgCl$_2$ at pH 7.4. The lymphocytes (at least 90% small lymphocytes, <10% monocytes or polymorphonuclear leukocytes) were incubated at 23°C for 60 min with ICYP (0.01–1.0 nM) in 60 mM Tris-HCl and 20 mM MgCl$_2$ at pH7.4. Incubation was stopped by filtration through a glass fiber filter (GF/C, Whatman). Radioligand bound to lymphocytes was determined with an Autowell gamma counter (ARC-251, Aloka). The lymphocytes were incubated with and without 10

μM l-propranolol, and the radioligand binding suppressed by propranolol was defined as a binding specific to β-receptors. Bmax and Kd of the β-receptors were calculated by Scatchard plot.[10,11] Proteins were assayed by Lowry's method,[12] and the number of β-receptors per lymphocyte cells was calculated by the method of Galant et al.[5]

Statistical Analysis

The data were statistically analysed by using Student's t-test. All data are expressed as the mean± 1SD. Values of $p < 0.05$ were considered significant.
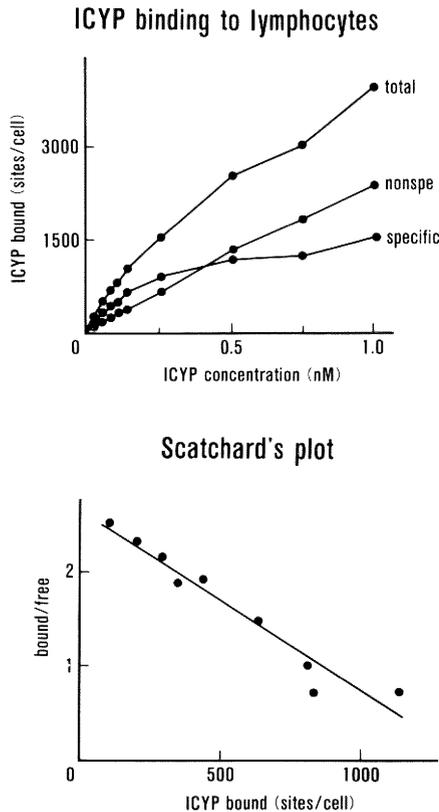
RESULTS

(1) Blood pressure and heart rate (Tab.)
Systolic blood pressure and heart rate were decreased significantly by atenolol and returned to the pre-treatment level after withdrawal of the drug. During treatment with xamoterol, systolic blood pressure increased slightly but significantly and after withdrawal of the drug returned to the pre-treatment level. Heart rate was not changed by xamoterol.
(2) Lymphocyte β-receptors (Figs. 1, 2 and Tab.)
ICYP bound to lymphocytes reached the peak level after about 15 min; then a constant level was maintained for up to 60 min. When 10 μM propranolol was added, it was dissociated from the

ICYP binding to lymphocytes


Scatchard's plot

Fig. 1. Results of a representative binding isotherm. Upper panel shows a saturation-binding curve. Intact lymphocytes were incubated with various concentrations of the β-adrenergic antagonist ^{125}I-iodocyanopindrol (ICYP), and the binding was determined with filtration to separate bound and unbound radioactivity. The experiment was performed both in the presence of 10 μM propranolol to determine nonspecific binding (nonspe) and in its absence to determine total binding (total). The difference between the two values represents specific binding (specific) to the receptors.

The lower panel shows a Scatchard's plot. The specific binding results from the upper panel are transformed in order to plot the ratio of bound-to-free radioactivity against the amount bound. In this liner plot, the intercept yields the maximum number of binding sites (Bmax), and the slope is the negative reciprocal of the dissociation constant (Kd).[13]

lymphocytes, thus indicating that the binding was reversible. When the dose of ICYP was increased in incubation, ICYP bound to the lymphocytes increased accordingly. However, the binding was saturated at a certain level, and the Scatchard plot showed a straight line.[13]

Atenolol significantly increased the Bmax and Kd of the β-receptors, the peak increase appearing on day 2 of treatment (from $1,566 \pm 297$ and 1.12 ± 0.13 to $3,213 \pm 1,134$ sites/cell and 4.32 ± 2.26 nM, respectively). The values remained significantly high during treatment, but fell to pre-treatment levels after atenolol was withdrawn.

Xamoterol increased both variables significantly from $1,466 \pm 373$ and 1.07 ± 0.14 to $7,169 \pm 3,768$ sites/cell and 6.01 ± 3.84 nM, respectively. During xamoterol treatment, the values of Bmax and Kd remained higher than during atenolol treatment. However, both returned to the pre-treatment levels after withdrawal of xamoterol.

Atenolol 50mg/day

Xamoterol 200mg/day
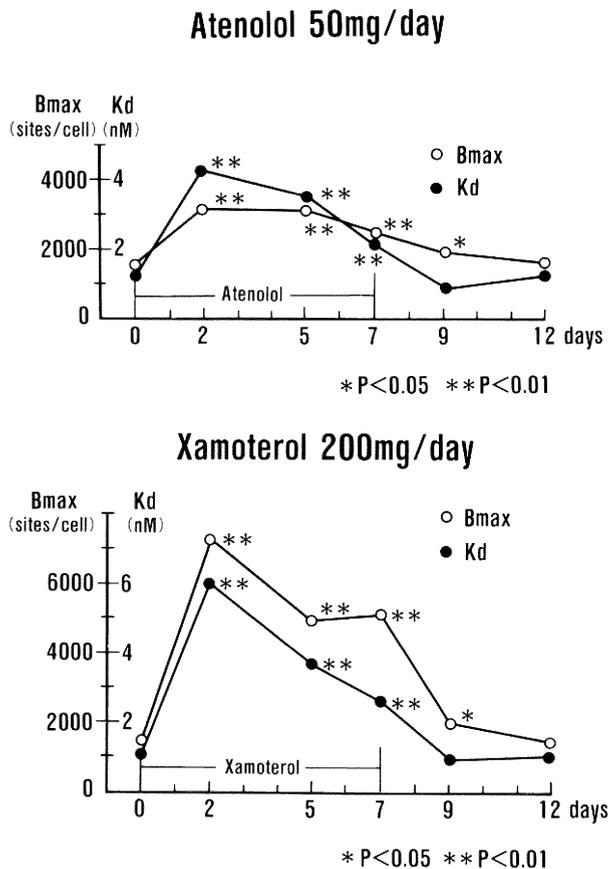
Fig. 2. Effects of atenolol and xamoterol administration on the density (Bmax) and affinity (Kd) of β-adrenergic receptors in lymphocytes. Each point is the mean of values from all subjects. *$p < 0.05$, **$p < 0.01$ when compared with values before drugs.

(3) Plasma concentrations of atenolol and xamoterol (Tab.)

Plasma concentrations of atenolol and xamoterol were in the therapeutic ranges and were eliminated from the plasma within two days of withdrawing treatment.

DISCUSSION

β-antagonists are widely used to treat hypertension and angina pectoris. If the β-antagonist is suddenly discontinued in patients with angina pectoris, anginal attacks or myocardial infarction may develop due to a "β-blocker withdrawal phenomenon", and sudden death has been reported.[14,15] The present study has demonstrated that the values of Bmax and Kd of lymphocyte β-receptors were elevated during treatment with β-antagonists and decreased after withdrawal of these drugs. The "β-blocker withdrawal phenomenon" has been reported to be related to increased sympathetic tone or to rebound hypersensitivity of β-receptors to sympathetic stimuli. On the contrary, if β-agonists are given to asthmatic patients for extended periods, bronchodilation and responsiveness of leukocyte cyclic AMP to catecholamines often decrease. This seems, therefore, due to a reduction in density and affinity of lymphocyte β-receptors, i.e., β-receptors are down-regulated.[16,17] It is now postulated that β-receptors are down-regulated by β-agonists and up-regulated by β-antagonists.

Xamoterol is a β-partial agonist with intrinsic sympathomimetic activity of 43% at the level of isoprenaline.[18] When the sympathetic tone is low, it acts as a β-agonist ($\beta_1 : \beta_2 = 1,000 : 1$), and when the sympathetic tone is high, it acts as a β-antagonist; the cardioselectivity ratio ($\beta_1 : \beta_2$) as an antagonist is 13:1 in the dog and 40:1 in the rat and rabbit.[19,20] Thus xamoterol will stabilize the cardiac function against changes in sympathetic tone. Xamoterol may be useful in patients with heart failure who show abnormally elevated sympathetic tone and whose β-receptors are down-regulated.[21]

In our previous study, the acute and chronic effects of xamoterol were evaluated in patients with heart failure (NYHA class II–III).[21] When xamoterol was given intravenously, blood pressure, heart rate, cardiac index, and pulmonary wedge pressure were all acutely unaffected. Nevertheless, exercise tolerance and haemodynamics improved when the drug was given for 3 months. These results may indicate that xamoterol did not improve the cardiac functions of

patients with heart failure through β-agonism and did through up-regulation of β-receptors.

In the present study, we measured lymphocyte β-receptors for convenience, although studies of β-receptor down-regulation should ideally involve cardiac β-receptors. Lefkowitz et al. reported that the effects of β-antagonists on β-receptors do not differ in nature between the heart and lymphocytes. [22,23] In this study, atenolol and xamoterol increased the value of the Bmax of lamphocyte β-receptors in healthy volunteers. This suggests that atenolol and xamoterol up-regulated β-receptors in healthy volunteers, and this tendency appeared to be related to the β-antagonist effects of both drugs. Atenolol decreased both systolic blood pressure and heart rate while xamoterol increased systolic blood pressure and did not affect heart rate. These haemodynamic changes reflected the β-antagonist effect of atenolol and the β-agonist effect of xamoterol. It is interesting that xamoterol up-regulated lymphocyte β-receptors without showing antagonist action to haemodynamics different from atenolol. This finding suggests that xamoterol may improve cardiac functions by an appropriate effect of antagonist activity (preventing the cardiotoxic effect of catecholamines, normalizing down-regulated β-adrenergic pathways, and suppressing exercise-induced tachycardia) and an agonist activity (increasing ventricular contractility).

In the present study, treatment of both drugs caused an increase in the value of Kd in the lymphocyte β-receptors. β-receptor agonists influenced the values of Kd in insulin receptor sites, and prostaglandines caused changes in the signaling systems of β-receptors.[24-26] The signaling system using cyclic AMP as second messengers may be the best known, and β-receptors conformed to this system.[27] It is possible that xamoterol caused the changes of the coupling systems between β-receptors, GTP-binding protein, and adenylate cyclase.

A possible limitation of this study is that cardiac β_1-receptors which seem to be related to down-regulation in heart failure were not measured. Further studies are needed to define the effect of xamoterol on cardiac β_1-receptors, and the relation between its effect on β-receptors and cardiac function in patients with heart failure.

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