Lack of effect of chronic calcium antagonist treatment on β_1 - and β_2 -adrenoceptors in right atria from patients with or without heart failure

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- 1 We studied the effects of chronic calcium antagonist (calcium entry blocker, CEB; nifedipine, verapamil, diltiazem) treatment on β -adrenoceptor density (assessed by (–)-[¹²⁵I]-iodocyanopindolol [ICYP] binding) and subtype distribution in right atria from 65 patients without apparent heart failure undergoing elective coronary artery bypass grafting (CAD-patients) and from 13 patients with moderate heart failure (NYHA class III to class III-IV) undergoing mitral valve replacement (MVD-patients).
- 2 In CAD-patients atrial β -adrenoceptor density was 79.3 ± 7.9 fmol ICYP bound mg^{-1} protein ($n = 18$), the β_1 : β_2 -adrenoceptor ratio 69:31%. Chronic CEB-treatment did not affect either atrial β -adrenoceptor density or β_1 : β_2 -adrenoceptor ratio.
- 3 In contrast, in CAD-patients chronically treated with β_1 -adrenoceptor antagonists (atenolol, bisoprolol, metoprolol) and CEB, atrial β -adrenoceptor density was significantly increased (108.6 ± 10.5 fmol ICYP bound mg^{-1} protein, $n = 21$); this increase was due to a selective increase in β_1 -adrenoceptors.
- 4 In MVD-patients atrial β -adrenoceptor density (55.5 ± 8.7 fmol ICYP bound mg^{-1} protein, $n = 7$) was significantly lower ($P < 0.05$) than in CAD-patients; β_1 : β_2 -adrenoceptor ratio, however, was not changed (67:33%). Chronic CEB-treatment of MVD-patients did not prevent the decrease in atrial β -adrenoceptors.
- 5 We conclude that chronic CEB-treatment does not affect human right atrial β -adrenoceptor density, either in patients without apparent heart failure or in patients with moderate heart failure. In addition, the increase in atrial β_1 -adrenoceptors caused by chronic β_1 -adrenoceptor antagonist treatment seems not to be affected by simultaneous CEB-treatment.

Keywords calcium antagonists human right atrium human cardiac β_1 - and β_2 -adrenoceptors β_1 -adrenoceptor antagonists heart failure

Introduction

Calcium antagonists (calcium entry blocker, CEB) are commonly used in many kinds of cardiovascular diseases such as in hypertension, angina pectoris, myocardial infarction and arrhythmia. In recent years evidence has accumulated that in the heart β -adrenoceptors and L-type calcium channels might be regulated by a common pathway (Ferry & Kaumann, 1987; Marsh, 1989; Skattebol & Triggle, 1986; Yonemochi *et al.*, 1990).

However, only a very few studies on the effects of chronic CEB-treatment on β -adrenoceptor number and subtype distribution in the human heart have been performed, and divergent results have been reported: Hedberg *et al.* (1985) found that in human right atria derived from patients undergoing elective coronary artery bypass grafting chronic treatment with nifedipine resulted in a significant increase in β -adrenoceptor

density. On the other hand, Jones *et al.* (1990) showed that in a limited number of patients with coronary artery disease chronic treatment with nifedipine had neither effect on right atrial β -adrenoceptor density nor on the increase in β -adrenoceptor density caused by propranolol or sotalol. Similarly, Wagner *et al.* (1989) did not observe any effect of chronic treatment with CEB (verapamil, diltiazem, nifedipine) on right atrial β -adrenoceptor density in patients with hypertrophic obstructive cardiomyopathy (HOCM) or coronary artery disease.

Because of these divergent results in the present study we have investigated the effect of chronic CEB-treatment in patients without apparent heart failure undergoing elective coronary artery bypass grafting on right atrial β -adrenoceptor density and the relative amount of β_1 - and β_2 -adrenoceptors. Furthermore we studied in these patients, whether CEB might affect the increase in right atrial β_1 -adrenoceptor density caused by chronic β_1 -adrenoceptor antagonist treatment (Michel *et al.*, 1988; Motomura *et al.*, 1990). To find out whether CEB might prevent the decrease in β -adrenoceptor density that usually occurs in chronic heart failure (for recent reviews see Bristow *et al.*, 1990; Brodde, 1991; Jones *et al.*, 1989) we also studied the effects of chronic CEB-treatment on right atrial β -adrenoceptor density and subtype distribution in a small group of patients with moderate heart failure (NYHA class III to class III-IV) undergoing mitral valve replacement.

Methods

Human right atrial appendages were obtained from 65 patients (50 males, 15 females, mean age: 57.4 ± 2.1 [range: 22–78] years) without apparent heart failure (no apparent clinical signs of forward- or backward heart failure like peripheral oedema or pulmonary congestion; no dyspnoea at rest or during moderate exercise) undergoing elective coronary artery bypass grafting (CAD-patients), and from 13 patients (5 males, 8 females, mean age: 58.6 ± 4.6 [range: 43–78] years) with moderate heart failure (NYHA class III to class III-IV) undergoing mitral valve replacement (MVD-patients) after having given informed written consent. No patient suffered from acute myocardial failure; none of the patients had been treated with catecholamines for at least 3 weeks before operation. The CAD-patients were subdivided into three groups according to the chronic pretreatment with CEB and β_1 -adrenoceptor antagonists: the first group (15 males, 3 females, mean age: 53.6 ± 2.9 years) had received neither CEB nor β -adrenoceptor antagonists for at least 6 weeks and was taken as control group; the second group (17 males, 9 females, mean age: 61.1 ± 1.5 years) was on treatment with the CEB nifedipine ($1-4 \times 10-20$ mg day⁻¹, mean daily dose 35.8 ± 3.8 mg, $n = 18$), verapamil (3×80 mg day⁻¹, mean daily dose 240 mg, $n = 2$) or diltiazem ($2-3 \times 60$ mg day⁻¹, mean daily dose 168 ± 13.4 mg, $n = 6$); and the third group (18 males, 3 females, mean age: 56.1 ± 2 years) was on combined CEB (nifedipine $1-4 \times 10-20$ mg day⁻¹, mean daily dose: 34 ± 5.2 mg, $n = 20$ or gallopamil 3×50 mg day⁻¹, $n = 1$) and a β_1 -adrenoceptor antagonist (metoprolol $1-3 \times 50$ mg day⁻¹, mean

daily dose: 93.8 ± 12 , $n = 11$; bisoprolol $1-2 \times 5-10$ mg day⁻¹, mean daily dose 7.5 ± 1.2 mg, $n = 7$; atenolol 1×50 mg day⁻¹, $n = 3$) treatment. CAD-patients were in addition treated with nitrates ($n = 45$) and occasionally with diuretics ($n = 3$), cardiac glycosides ($n = 2$), and ACE-inhibitors ($n = 2$), alone or in combination.

Similarly, the MVD-patients were subdivided into two groups according to the chronic treatment with CEB: the first group (3 males, 4 females, mean age 56.7 ± 4.4 years) had received no CEB for at least 6 weeks and was taken as control group; the second group (2 males, 4 females, mean age: 61.1 ± 4.9 years) was on treatment with the CEB verapamil ($1-3 \times 80-120$ mg day⁻¹; mean daily dose: 210 ± 34.6 mg, $n = 4$) or nifedipine ($2-3 \times 20$ mg day⁻¹; mean daily dose: 50 ± 14 mg, $n = 2$). Additional treatment of the MVD-patients was: cardiac glycosides ($n = 12$), nitrates ($n = 7$), diuretics ($n = 8$) and occasionally ACE-inhibitors ($n = 3$).

Premedication consisted of 2 mg flunitrazepam given orally in the evening before and on the morning of surgery. The operation was done under modified neurolept anaesthesia with flunitrazepam and fentanyl; controlled ventilation was performed, using pancuronium as muscle relaxant, by a 1:1-mixture of oxygen and N₂O; isoflurane was added up to 1.0% (v/v). Isoflurane was usually avoided in the MVD-patients. In all patients the atrial appendages were removed under normothermic conditions during installation of cardiopulmonary bypass. Immediately after removal, all specimens were placed in sealed vials containing ice-cold 0.9% w/v NaCl solution and transported to the laboratory.

Preparation of tissues was begun within 5–15 min after surgical removal. Membranes were prepared and the density of β -adrenoceptors in atrial membranes was determined by (–)-[¹²⁵I]-iodocyanopindolol (ICYP) binding at six concentrations of ICYP ranging from 5–200 pM essentially as recently described (Motomura *et al.*, 1990). Non-specific binding of ICYP was defined as binding to membranes, that is not displaced by a high concentration of the non-selective β -adrenoceptor antagonist ($\pm$)-CGP 12177 (1 μ M). Specific binding of ICYP was defined as total binding minus non-specific binding; it amounted usually to 70–80% at 50 pM of ICYP. To determine the relative amount of β_1 - and β_2 -adrenoceptors in right atria, membranes were incubated with ICYP (100 pM) in the presence or absence of 21 concentrations of the selective β_2 -adrenoceptor antagonist ICI 118,551 (Bilski *et al.*, 1983) and specific binding was determined as described above. ICI 118,551 competition curves were analyzed by fitting the experimental data to mono- or biphasic sigmoid curves using the iterative curve fitting program InPlot (GraphPAD software, San Diego, CA). Statistical analysis was performed using the *F*-ratio test to measure the goodness of fit of the competition curves for either one or two sites.

Lymphocytes were prepared from 30 ml venous EDTA-treated blood (withdrawn immediately before the removal of the right atrial appendages) by the method of Böyum (1968). Lymphocyte β_2 -adrenoceptor density was determined in intact cells by ICYP binding as described by Brodde *et al.* (1985).

Plasma catecholamine concentrations were determined by an h.p.l.c.-method with electrochemical detection (Bauch *et al.*, 1986), plasma renin activity and plasma aldosterone concentrations by commercially available radioimmunoassays (Sorin, Turin, Italy and Isotopen Diagnostik CIS, Dreieich, FRG, respectively).

The protein content of the samples was determined by the method of Bradford (1976) using bovine immunoglobulin G as a standard.

Statistical evaluations

The experimental data given in text and figures are means $\pm$ s.e. mean of n experiments. The equilibrium dissociation constant (K_D) and the maximal number of binding sites (B_{max}) were calculated from plots according to Scatchard (1949). The significance of differences in the number of binding sites in two different groups was estimated by unpaired Student's t -test. The relation between two parameters was assessed by linear regression analysis. A P value smaller than 0.05 was considered to be significant.

Drugs used

ICI 118,551 hydrochloride (erythro-($\pm$)-1-(7-methylindan-4-yloxy)-3-isopropylaminobutan-2-ol) was kindly donated by ICI Pharma (Plankstadt, FRG), and ($\pm$)-CGP 12177 hydrochloride (4-(3-tertiarybutylamino-2-hydroxypropoxy)-benzimidazole-2-on) by Ciba-Geigy (Basel, Switzerland). ($-$)[125 I]-Iodocyanopindolol (specific activity 2200 Ci mmol $^{-1}$) was obtained from New England Nuclear (Dreieich, FRG). All other chemicals were of the highest purity grade commercially available.

Results

Patients without apparent heart failure

The mean right atrial β -adrenoceptor density in the control group (i.e. CAD-patients without apparent heart failure who have not been treated with either CEB or β -adrenoceptor antagonists) was 79.3 ± 7.9 fmol ICYP specifically bound mg $^{-1}$ protein ($n = 18$); the K_D -value of ICYP was 24.3 ± 3.8 pM ($n = 18$). This value was not significantly different from that obtained in the group of CAD-patients chronically treated with CEB (Figure 1); the mean density in this group was 84.5 ± 7.0 fmol ICYP specifically bound mg $^{-1}$ protein ($n = 26$), the K_D -value was 20.7 ± 2.3 pM ($n = 26$). In the group of CAD-patients chronically treated with CEB and β_1 -adrenoceptor antagonists, however, the mean density of right atrial β -adrenoceptors (108.6 ± 10.5 fmol ICYP specifically bound mg $^{-1}$ protein, $n = 21$) was significantly higher ($P < 0.05$) than in the control group. The K_D -value of ICYP, however, was not significantly changed ($K_D = 25.5 \pm 4.1$ pM, $n = 21$).

On the other hand, neither chronic CEB-treatment nor chronic treatment with CEB and β_1 -adrenoceptor antagonists had any influence on lymphocyte β_2 -adrenoceptor density (Figure 1).

In all atria investigated, inhibition of ICYP binding by

Figure 1 Effect of chronic calcium antagonist treatment on right atrial and lymphocyte β -adrenoceptors in patients without apparent heart failure undergoing elective coronary artery bypass grafting. For details see **Methods**. Ordinate, from top to bottom: total β -adrenoceptor density in right atrial membranes in fmol ICYP specifically bound mg $^{-1}$ protein; β_1 - and β_2 -adrenoceptor density in right atrial membranes in fmol ICYP specifically bound mg $^{-1}$ protein; β_2 -adrenoceptor density in intact lymphocytes in ICYP binding sites/cell. Control = patients undergoing elective coronary artery bypass grafting who have been not treated with calcium antagonists or β -adrenoceptor antagonists; CEB = patients undergoing elective coronary artery bypass grafting who have been chronically treated with calcium antagonists; CEB + β_1 -adrenoceptor blocker = patients undergoing elective coronary artery bypass grafting who have been chronically treated with calcium antagonists and β_1 -adrenoceptor antagonists. Results are means $\pm$ s.e. mean; number of patients investigated at the bottom of the columns. * $P < 0.05$ vs the corresponding values in the control group.

the selective β_2 -adrenoceptor antagonist ICI 118,551 resulted in shallow competition curves that fitted significantly better to interaction with two classes of binding sites than to a one-site model. Using the iterative curve fitting program InPlot the relative amount of β_1 - and β_2 -adrenoceptors in these atria could be calculated. In the control group the β_1 : β_2 -adrenoceptor ratio was $69.2 \pm 2.1/30.8 \pm 2.1\%$ ($n = 16$); while this ratio was not changed in the group of patients chronically treated with CEB ($69.5 \pm 2.1/30.5 \pm 2.1\%$ [$n = 24$]), it was shifted significantly towards β_1 -adrenoceptors in the group of patients chronically treated with CEB and β_1 -adrenoceptor antagonists ($73.3 \pm 2.0/26.7 \pm 2.0\%$ [$n = 21$]). Thus, the increase in total β -adrenoceptor density in this group was solely due to an increase in β_1 -

adrenoceptor density, while β_2 -adrenoceptor density was not changed (Figure 1).

In all three groups of CAD-patients plasma catecholamine concentrations were not significantly different. Moreover, plasma aldosterone concentration and plasma renin activity were similar in control and in the group of patients chronically treated with CEB, whereas both tended to be lower in the patients chronically treated with CEB and β_1 -adrenoceptor antagonists (data not shown). None of these neurohumoral factors was (even not weakly) significantly related to right atrial β -adrenoceptor density, neither to total β -adrenoceptors nor to β_1 - or β_2 -adrenoceptors (data not shown).

Patients with moderate heart failure

We also studied the effects of chronic CEB-treatment on right atrial β -adrenoceptors and subtype distribution in a small group of MVD-patients with moderate heart failure (NYHA class III to class III-IV). In the MVD-patients not treated with CEB (control) right atrial β -adrenoceptor density (55.5 ± 8.7 fmol ICYP specifically bound mg^{-1} protein, $n = 7$; K_D -value for ICYP: 17.4 ± 3.9 pM, $n = 7$) was significantly lower ($P < 0.05$) than in the control group of CAD-patients without apparent heart failure (see above and Figure 2). This decrease was obviously due to a concomitant decrease of β_1 - and β_2 -adrenoceptors (Figure 2), since in the MVD-patients the β_1 : β_2 -adrenoceptor ratio ($67.1 \pm 4.5/32.9 \pm 4.5\%$, $n = 7$) was not significantly different from that in the atria of CAD-patients without apparent heart failure (see above). On the other hand, lymphocyte β_2 -adrenoceptor density in the MVD-patients (788 ± 101 ICYP binding sites/cell, $n = 7$, Figure 2) was quite similar to that in the CAD-patients without apparent heart failure (865 ± 94 ICYP binding sites/cell, $n = 17$, c.f. Figure 1).

Chronic treatment of the MVD-patients with CEB had no significant influence on right atrial β -adrenoceptors, neither on total β -adrenoceptor density nor on the β_1 : β_2 -adrenoceptor ratio (Figure 2). Similarly, lymphocyte β_2 -adrenoceptor density in these MVD-patients was not affected by the chronic CEB-treatment (Figure 2).

While in both groups of MVD-patients plasma catecholamine concentrations were not different from those in the CAD-patients without apparent heart failure, plasma renin activity and plasma aldosterone concentrations were markedly higher than in the corresponding groups of CAD-patients without apparent heart failure (data not shown).

Discussion

In recent years evidence has accumulated that in the heart β -adrenoceptor number and the number of L-type calcium channels may be coregulated. Thus, Ferry & Kaumann (1987) have shown that in HOCM-patients ventricular β -adrenoceptor density and the density of calcium channels (determined by [^3H]-nimodipine binding) were significantly correlated. Furthermore, Marsh (1989) demonstrated in cultured chick embryo ventricular cells that a 4 h exposure of the cells to $1 \mu\text{M}$ isoprenaline

Figure 2 Effect of chronic calcium antagonist treatment on right atrial and lymphocyte β -adrenoceptors in patients with moderate heart failure undergoing mitral valve replacement. For details see **Methods**. Ordinate, from top to bottom: total β -adrenoceptor density in right atrial membranes in fmol ICYP specifically bound mg^{-1} protein; β_1 - and β_2 -adrenoceptor density in right atrial membranes in fmol ICYP specifically bound mg^{-1} protein; β_2 -adrenoceptor density in intact lymphocytes in ICYP binding sites/cell. CAD-control: patients undergoing coronary artery bypass grafting who have been not treated with calcium antagonists or β -adrenoceptor antagonists (taken from Figure 1 for reasons of comparison). MVD-control = patients undergoing mitral valve replacement who have not been treated with calcium antagonists; MVD + CEB = patients undergoing mitral valve replacement who have been chronically treated with calcium antagonists. Results are mean $\pm$ s.e. mean; number of patients investigated at the bottom of the columns. * $P < 0.05$, $^+0.1 > P > 0.05$ vs the corresponding values in CAD-controls.

caused a concomitant decrease in the number of β -adrenoceptors and calcium channels (assessed by [^3H]-(+)-PN 200-110 binding). Moreover, in rat heart both β -adrenoceptor and calcium channel number was found to be increased following 6-hydroxy-dopamine treatment (Skattebol & Triggle, 1986). And finally, Yonemochi *et al.* (1990) recently reported, that incubation of cultured cardiac myocytes isolated from neonatal rat ventricle with different CEB (verapamil, diltiazem and nicardipine) induced a time- and concentration-dependent increase in β -adrenoceptor number.

As chronic β -adrenoceptor antagonist treatment increases cardiac β -adrenoceptor density (for reviews see Brodde, 1991; Stiles *et al.*, 1984), it might be possible that chronic CEB-treatment might do the same especially as *in vitro* experiments have shown that CEB might

prevent isoprenaline-induced desensitization of β_2 -adrenoceptors in circulating human lymphocytes (Hui & Yu, 1987, 1988). In fact, Hedberg *et al.* (1985) reported that in human right atria derived from patients undergoing elective coronary artery bypass grafting chronic treatment with nifedipine resulted in a significant increase in β -adrenoceptor density. In the present study, however, neither in CAD-patients without apparent heart failure nor in MVD-patients with moderate heart failure chronic treatment with CEB did affect right atrial β -adrenoceptor density or the relative amount of β_1 - and β_2 -adrenoceptors. These results, therefore, are not in agreement with those previously published by Hedberg *et al.* (1985) but confirm data published by Jones *et al.* (1990) who showed that in CAD-patients chronic treatment with nifedipine had no effect on right atrial β -adrenoceptor density, and by Wagner *et al.* (1989) who did not observe any effect of chronic treatment with CEB (verapamil, diltiazem, nifedipine) on right atrial β -adrenoceptor density in HOCM- or CAD-patients.

Moreover, in the present study in MVD-patients with moderate heart failure right atrial β -adrenoceptor number was significantly lower than in CAD-patients without apparent heart failure. Thus, the fact that chronic CEB-treatment of these patients had no effect on right atrial β -adrenoceptor number indicates that CEB may not be capable of inhibiting β -adrenoceptor down-regulation that usually occurs in chronic heart failure (for references see Bristow *et al.*, 1990; Brodde, 1991; Jones *et al.*, 1989).

We have recently shown that in CAD-patients chronic treatment with β -adrenoceptor antagonists without intrinsic sympathomimetic activity caused an up-regulation of right atrial β -adrenoceptor density (Michel *et al.*, 1988; Motomura *et al.*, 1990), but in a subtype-selective fashion: non-selective β -adrenoceptor antagonists such as propranolol or sotalol increased both right atrial β_1 - and β_2 -adrenoceptors, while β_1 -adrenoceptor selective antagonists such as metoprolol, atenolol or bisoprolol increased only β_1 -adrenoceptor number leaving β_2 -adrenoceptor number unchanged. In the present study, right atrial β -adrenoceptor density was significantly increased in CAD-patients without apparent heart failure chronically treated with the combination of CEB and β_1 -adrenoceptor selective antagonists; this increase was, however, due to a significant increase in β_1 -adrenoceptors only, while β_2 -adrenoceptor density was not changed. These data are compatible with the view that also in the presence of CEB chronic β_1 -adrenoceptor antagonist treatment

causes a selective up-regulation of right atrial β_1 -adrenoceptors. Similar to these results Jones *et al.* (1990) recently found that in CAD-patients chronically treated with a combination of non-selective β -adrenoceptor antagonists (propranolol or sotalol) and nifedipine right atrial β -adrenoceptor density was significantly higher than in non-treated patients or patients treated with nifedipine alone. From these and the present results we, therefore, feel justified to conclude that chronic CEB-treatment does not affect the increase in cardiac β -adrenoceptors following chronic treatment with β -adrenoceptor antagonists.

Finally the present results show that in all five groups of patients investigated in this study β -adrenoceptor number in circulating lymphocytes (containing exclusively β_2 -adrenoceptors, for review see Brodde & Wang, 1988) were nearly identical. While the lack of any effect of chronic β_1 -adrenoceptor antagonist treatment on lymphocyte β_2 -adrenoceptor number confirms recently reported data from several laboratories and is due to the fact that lymphocyte β_2 -adrenoceptors are subtype-selectively modulated only by drugs having a reasonable affinity to β_2 -adrenoceptors (for references see Brodde & Wang, 1988) the present results show in addition, that also in patients with moderate heart failure having a reduced cardiac β -adrenoceptor number lymphocyte β_2 -adrenoceptors are not changed. Thus, the present results add an additional example to the growing list of studies demonstrating that lymphocyte β -adrenoceptors are not a suitable model to monitor human cardiac β -adrenoceptor function (for recent review see Brodde *et al.*, 1989).

In conclusion: the results of the present study clearly demonstrate that chronic CEB-treatment does not affect human right atrial β -adrenoceptor density or the relative proportion of β_1 - and β_2 -adrenoceptors in patients without apparent heart failure. Furthermore, chronic CEB-treatment does not prevent the decrease in right atrial β -adrenoceptors in patients with moderate heart failure. And finally, the increase in right atrial β_1 -adrenoceptors caused by chronic β_1 -adrenoceptor antagonist treatment seems not to be affected by chronic CEB-treatment.

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References

- Bauch, H. J., Kelsch, U. & Hauss, W. H. (1986). Einfache, schnelle, selektive und quantitative Bestimmung von Adrenalin und Noradrenalin im Plasma durch Kombination von Flüssigextraktion, h.p.l.c.-Trennung und elektrochemischer Detektion. *J. clin. Chem. clin. Biochem.*, **24**, 651–658.
- Bilski, A. J., Halliday, S. E., Fitzgerald, J. D. & Wale, J. L. (1983). The pharmacology of a β_2 -selective adrenoceptor antagonist (ICI 118,551). *J. cardiovasc. Pharmacol.*, **5**, 430–437.
- Böyum, A. (1968). Isolation of mononuclear cells and granulocytes from human blood. *Scand. J. clin. Lab. Invest.*, **21** (suppl. 97), 77–89.
- Bradford, M. M. (1976). A rapid and sensitive method for quantitation of microgram quantities of protein utilizing the principle of protein dye binding. *Anal. Biochem.*, **72**, 248–264.
- Bristow, M. R., Hershberger, R. E., Port, J. D., Gilbert, E. M., Sandoval, A., Rasmussen, R., Cates, A. E. & Feldman, A. M. (1990). β -adrenergic pathways in non-

- failing and failing human ventricular myocardium. *Circulation*, **82** (suppl. I), I-12-I-25.
- Brodde, O.-E. (1991). β_1 - and β_2 -adrenoceptors in the human heart: properties, function, and alterations in chronic heart failure. *Pharmac. Rev.*, **43**, 203-242.
- Brodde, O.-E., Brinkmann, M., Schemuth, R., O'Hara, N. & Daul, A. (1985). Terbutaline-induced desensitization of human lymphocyte β_2 -adrenoceptors. Accelerated restoration of β -adrenoceptor responsiveness by prednisone and ketotifen. *J. clin. Invest.*, **76**, 1096-1101.
- Brodde, O.-E., Michel, M. C., Gordon, E. P., Sandoval, A., Gilbert E. M. & Bristow, M. R. (1989). β -Adrenoceptor regulation in the human heart: can it be monitored in circulating lymphocytes? *Eur. Heart J.*, **10** (Suppl. B), 2-10.
- Brodde, O.-E. & Wang, X.-L. (1988). Beta-adrenoceptor changes in blood lymphocytes and altered drug responsiveness. *Ann. clin. Res.*, **20**, 311-323.
- Ferry, D. R. & Kaumann, A. J. (1987). Relationship between β -adrenoceptors and calcium channels in human ventricular myocardium. *Br. J. Pharmac.*, **90**, 447-457.
- Hedberg, A., Kempf, F., Josephson, M. E. & Molinoff, P. B. (1985). Coexistence of beta-1 and beta-2 adrenergic receptors in the human heart: effects of treatment with receptor antagonists or calcium entry blockers. *J. Pharmac. exp. Ther.*, **234**, 561-568.
- Hui, K. K. & Yu, J. L. (1987). Effects of calcium blockers on beta adrenoceptor regulation in intact human lymphocytes. *Proceedings 12th IUPHAR congress Sydney/Australia*, Abstract P 724.
- Hui, K. K. & Yu, J. L. (1988). The effects of calcium channel blockers on isoprenaline induced cyclic adenosine 3', 5'-monophosphate generation in intact human lymphocytes. *Life Sci.*, **42**, 2037-2045.
- Jones, C. R., Fandeleur, P., Harris, B. & Bühler, F. R. (1990). Effect of calcium and β -adrenoceptor antagonists on β -adrenoceptor density and G_{sa} expression in human atria. *Br. J. clin. Pharmac.*, **30** (Suppl. 1), 171S-173S.
- Jones, C. R., Molenaar, P. & Summers, R. J. (1989). New views of human cardiac β -adrenoceptors. *J. Mol. Cell. Cardiol.*, **21**, 519-535.
- Marsh, J. D. (1989). Coregulation of calcium channels and beta-adrenergic receptors in cultured chick embryo ventricular cells. *J. clin. Invest.*, **84**, 817-823.
- Michel, M. C., Pingsmann, A., Beckeringh, J. J., Zerkowski, H.-R., Doetsch, N. & Brodde, O.-E. (1988). Selective regulation of β_1 - and β_2 -adrenoceptors in the human heart by chronic β -adrenoceptor antagonist treatment. *Br. J. Pharmac.*, **94**, 685-692.
- Motomura, S., Deighton, N. M., Zerkowski, H.-R., Doetsch, N., Michel, M. C. & Brodde, O.-E. (1990). Chronic β_1 -adrenoceptor antagonist treatment sensitizes β_2 -adrenoceptors, but desensitizes M_2 -muscarinic receptors in the human right atrium. *Br. J. Pharmac.*, **101**, 363-369.
- Scatchard, G. (1949). The attraction of proteins for small molecules and ions. *Ann. N.Y. Acad. Sci.*, **51**, 660-672.
- Skattebol, A. & Triggle, D. J. (1986). 6-Hydroxydopamine treatment increases β -adrenoceptors and Ca^{2+} channels in rat heart. *Eur. J. Pharmac.*, **127**, 287-289.
- Stiles, G. L., Caron, M. G. & Lefkowitz, R. J. (1984). β -Adrenergic receptors: biochemical mechanisms of physiological regulation. *Physiol. Rev.*, **64**, 661-743.
- Wagner, J. A., Sax, F. L., Weisman, H. F., Porterfield, J., McIntosh, C., Weisfeld, M. L., Synder, S. H. & Epstein, S. E. (1989). Calcium-antagonist receptor in the atrial tissues of patients with hypertrophic cardiomyopathy. *New Engl. J. Med.*, **320**, 755-761.
- Yonemochi, H., Saikawa, T., Takakura, T., Ito, S. and Takaki, R. (1990). Effect of calcium antagonists on β -receptors of cultured cardiac myocytes isolated from neonatal rat ventricle. *Circulation*, **81**, 1401-1408.

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