Effect of calcium and β -adrenoceptor antagonists on β -adrenoceptor density and $G_{s\alpha}$ expression in human atria

C. R. JONES, P. FANDELEUR, B. HARRIS & F. R. BÜHLER¹

Merrell Dow Research Institute, 16 rue d'Ankara, Strasbourg 67084 France and ¹Department of Research, Kantonsspital, Basel Ch-4031, Switzerland

In 21 patients undergoing open heart surgery the effects of the β -adrenoceptor antagonists propranolol and sotalol and the calcium antagonist nifedipine (alone or in combination) on right atrial β -adrenoceptor density and G protein expression were studied. The non-selective β -adrenoceptor antagonists increased both right atrial β_1 - and β_2 -adrenoceptor density; this increase was not affected by nifedipine. There was no correlation between β -adrenoceptor density and expression of the G protein subunit $G_{s\alpha}$ suggesting independent regulation of the gene expression for G proteins and their coupled receptors.

Keywords β -adrenoceptor antagonist propranolol sotalol nifedipine

Introduction

The human heart expresses both β_1 - and β_2 -adrenoceptors that are coupled functionally to adenylate cyclase and mediate both inotropic and chronotropic responses (Brodde & Wang, 1988). The administration of β -adrenoceptor antagonists whether non-selective or β_2 -selective has been shown to increase β_2 -adrenoceptor density in human lymphocytes (Aarons *et al.*, 1980; Brodde & Wang, 1988; Whyte *et al.*, 1987; Wood *et al.*, 1982). However the effects of β -adrenoceptor antagonists on β_1 -adrenoceptor regulation are less clear, although in homogenate binding studies there is evidence for subtype selective regulation of human cardiac β_1 - and β_2 -adrenoceptors. We therefore used quantitative autoradiography to investigate cardiac β -adrenoceptor density in patients undergoing open heart surgery together with 'in situ hybridisation' techniques to quantify whether or not G protein expression changed in parallel with the change in β -adrenoceptor number.

Methods

Quantitative autoradiographic studies were performed on cryostat cut 10 μ m sections from

human right atrial appendages incubated with 50 pM (–)[¹²⁵I]-cyanopindolol in the presence or absence of the selective antagonists ICI 118,551 (β_2) and CGP 20712A (β_1) to define β_1 - and β_2 -adrenoceptor population as previously described (Buxton *et al.*, 1987). Films were analysed using a computerized image-analysis system (MCID, Image Research Inc., Ontario, Canada) and the optical density of the areas of interest converted to fmol mg^{–1} protein by comparison with optical densities of known standards from Amersham International (Cortes *et al.*, 1984). *In situ* hybridisation was performed using a single stranded DNA probe prepared from a Pst 1 fragment (nucleotide residues –13 to 93) of the human clone pHGs α which has been shown to have little sequence homology with other G proteins (Harris, 1988). The protocol followed that previously used by Mengod *et al.* (1988) as modified from the original procedure of Hafen *et al.* (1983) with the exception that the oligonucleotide probe was labelled using the 3' end labelling system with terminal transferase and S³⁵dATP (Dupont Nemour, France). Films were calibrated using standards made from the labelled oligonucleotide in brain paste and cut at 10 μ m.

Correspondence: Dr C. R. Jones, Merrell Dow Research Institute, 16 rue d'Ankara, 67084 Strasbourg Cedex, France

The results reported are from two independent experiments. All patients were NYHA class I–II and were age matched. Patients were divided into the following groups: controls ($n = 6$; mean age 60 ± 8 years) taking neither β -adrenoceptor nor calcium antagonists for the preceding month and undergoing cardiac surgery for either valvular or ischaemic heart disease; patients treated with β -adrenoceptor antagonists alone (propranolol 160 mg day^{-1} or sotalol 300 mg day^{-1} , $n = 4$, mean age 52 ± 7 years); or patients treated with β -adrenoceptor antagonists in combination with calcium antagonists (nifedipine $60\text{--}80 \text{ mg day}^{-1}$, $n = 6$, mean age 55 ± 8 years); patients treated with a calcium antagonist alone (nifedipine $40\text{--}80 \text{ mg day}^{-1}$, mean age 60 ± 5 years). Statistical analysis was with Student's t -test with the Bonferroni correction for multiple comparisons (a value of $P < 0.05$ taken as significant). To correct for the two-fold selectivity of the ligand [^{125}I]-CYP for β_2 relative to β_1 -adrenoceptors (Neve *et al.*, 1986) the receptor occupancy was calculated from the law of mass action for a competitive antagonism of binding to the β_1 - and β_2 -adrenoceptor populations

(β_1 occupancy = $K_1A/(K_1A + K_2B + 1)$, where $K_1 = K_D$ ICI 118,551 for β_1 -adrenoceptors, and B = the concentration of competitor ICI 118,551. The calculated occupancies were 59% for β_1 and 75% for β_2 -adrenoceptors using 100 nM ICI 118,551 and 100 nM CGP 20712A.

Results

The β -adrenoceptor densities found in the atria are shown in Figure 1. In the group treated with nonselective β -adrenoceptor antagonists β_1 -adrenoceptor concentration increased significantly ($P < 0.05$) from 118 ± 18 to 144 ± 14 (fmol mg^{-1} protein) and that of β_2 -adrenoceptors from 18 ± 1.7 to 30 ± 2.1 fmol mg^{-1} protein. There was no significant change in either β_1 - or β_2 -adrenoceptors in the group treated with calcium antagonists alone and in the group receiving both a calcium antagonist and a β -adrenoceptor antagonist the increase in β -adrenoceptor number was not modified. The level of G_{sa} subunit expression was not significantly different in any of the groups (Figure 1).

Figure 1 Histogram of β -adrenoceptor density in patients treated with no drug (Ct), β -adrenoceptor antagonists alone (β), calcium antagonists alone (CA) or in combination with β -adrenoceptor antagonists (CA/ β). Open bars represent levels of G_{sa} subunit mRNA as quantified by *in situ* hybridisation and filled bars are receptor densities (fmol mg^{-1} protein) ($\square$ β_1 , $\blacksquare$ β_2).

Discussion

In this study β -adrenoceptor density in the human heart was modified by prior treatment with β -adrenoceptor antagonists and effects were seen on both β_1 - and β_2 -adrenoceptors. However the lack of change in G_{sa} expression between the different patient groups suggests that gene expression of receptors and their G proteins is independently regulated. This contrasts with the case of calcium channel expression and β -adrenoceptor density which have been shown to increase in parallel under certain conditions (Ferry & Kauman *et al.*, 1987; Wagner *et al.*, 1989). Based on studies of β -adrenoceptor regulation following treatment with

β -adrenoceptor antagonists it has been proposed that β -adrenoceptor blockade may exert a beneficial effect in heart failure by reversing decreases in β -adrenoceptor number (Fowler & Bristow, 1985). There may be additional effects of β_1 -adrenoceptor antagonists on cardiac β_2 -adrenoceptors that could be beneficial in heart failure since cardiac β_2 -adrenoceptor, but not β_1 -adrenoceptor responses are enhanced by atenolol treatment (Hall *et al.*, 1988) yet β_2 -adrenoceptors are not increased by treatment with β_1 -selective adrenoceptor antagonists such as atenolol (Michel *et al.*, 1988). The lack of effect of β -adrenoceptor antagonists on G_s protein suggests that this effect is not secondary to changes in G_s protein levels.

References

- Aarons, R. D., Nies, A. S., Gal, J., Hecstrand, L. R. & Molinoff, P. B. (1980). Elevation of β -adrenergic receptor density in human lymphocytes after propranolol administration. *J. clin. Invest.*, **65**, 949–957.
- Brodde, O. E. & Wang, X. L. (1988). Beta-adrenoceptor changes in blood lymphocytes and altered drug responsiveness. *Ann. clin. Res.*, **20**, 311–323.
- Buxton, B. F., Jones, C. R., Molenaar, P. & Summers, R. J. (1987). Characterization and autoradiographic localization of β -adrenoceptor subtypes in human cardiac tissues. *Br. J. Pharmacol.*, **92**, 299–310.
- Cortes, R., Pazos, A., Probst, A. & Palacios, J. M. (1984). Quantitative light microscopic autoradiographic localization of cholinergic muscarinic receptors in the human brain. *Brainstem Neurosci.*, **43**, 1574–1581.
- Fowler, M. B. & Bristow, M. R. (1985). Rationale for beta-adrenergic blocking drugs in cardiomyopathy. *Am. J. Cardiol.*, **55**, 120D–124D.
- Ferry, D. R. & Kaumann, A. J. (1987). Relationship between β -adrenoceptors and calcium channels in human ventricular myocardium. *Br. J. Pharmacol.*, **90**, 447–457.
- Hall, J. A., Kaumann, A. J., Wells, F. C. & Brown, M. J. (1988). β_2 -adrenoceptor mediated inotropic responses of human atria: receptor subtype regulation by atenolol. *Br. J. Pharmacol.*, **93**, 116P.
- Harris, B. A. (1988). Complete cDNA sequence of a human stimulatory GTP binding protein a subunit. *Nucleic Acids Res.*, **16**, 3585–3589.
- Hafen, E., Levine, M., Garber, R. L. & Gehring, W. J. (1983). An improved in situ hybridization method for the detection of cellular RNAs in *Drosophila* tissue sections and its application for localising transcripts of the homeotic Antennapedia gene complex. *Embo. J.*, **2**, 617–623.
- Mengod, G., Palacios, J. M., Probst, A. & Harris, B. (1988). Regional distribution of the expression of a human stimulatory GTP-binding protein α -subunit in the human brain studied by in situ hybridization. *Mol. Brain Res.*, **4**, 23–29.
- Michel, M. C., Pingsmann, A., Beckeringh, J. J., Zerkowski, H. R. & Brodde, O. E. (1988). Selective regulation of β_1 - and β_2 -adrenoceptors in the human heart by chronic beta-adrenoceptor antagonist treatment. *Br. J. Pharmacol.*, **94**, 685–692.
- Neve, K. A., McGonigle, P. & Molinoff, P. B. (1986). Quantitative analysis of the selectivity of radioligands for subtypes of beta-adrenergic receptors. *J. Pharmacol. exp. Ther.*, **238**, 46–53.
- Wagner, J. A., Sax, F. L., Weisman, H. F., Porterfield, J., MacIntosh, C., Weisfeldt, M. L., Snyder, S. H. & Epstein, S. E. (1989). Calcium-antagonists receptors in the atrial tissue of patients with hypertrophic cardiomyopathy. *New Engl. J. Med.*, **320**, 755–761.
- Whyte, K., Jones, C. R., Howie, C. A., Deighton, N., Summer, D. J. & Reid, J. L. (1987). Haemodynamic, metabolic, and lymphocyte beta₂-adrenoceptor changes following chronic beta-adrenoceptor antagonism. *Eur. J. clin. Pharmacol.*, **32**, 237–243.
- Wood, A. J. J., Feldman, R. & Nadeau, J. (1982). Physiological regulation of beta-receptors in man. *Clin. exp. Hypertension*, **A4**, 807–817.