Binding studies of platelet α_2 - and lymphocyte β_2 -adrenoceptors in patients with cirrhosis

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Radioligand binding studies were performed on 10 patients with cirrhosis and 10 healthy subjects. $B_{\max}$ and K_D of platelet α_2 -adrenoceptors, studied using [^3H]-yohimbine, were similar in both groups ($B_{\max}$ 24.9 vs 22.1 fmol/ 10^9 platelets, $P = 0.47$; K_D 4.6 vs 5.5 nmol l^{-1} , $P = 0.56$). $B_{\max}$ and K_D of lymphocyte β_2 -adrenoceptors, studied using [^{125}I]-iodocyanopindolol, were also similar in both groups ($B_{\max}$ 24.0 vs 27.2 fmol mg^{-1} protein, $P = 0.55$; K_D 49.6 vs 55.3 pmol l^{-1} , $P = 0.65$). In this model there is no evidence of adrenoceptor down-regulation in cirrhosis despite the increased sympathetic activity in this condition.

Keywords adrenoceptors cirrhosis platelet α_2 lymphocyte β_2

Introduction

Patients with decompensated cirrhosis have diminished peripheral vascular resistance (Murray *et al.*, 1958) despite increased activity of the sympathetic nervous system (Henriksen *et al.*, 1981; Nicholls *et al.*, 1985). This implies interference with sympathetic control of vascular tone. There is reduced cardiovascular reactivity to sympathetic pressor stimuli in cirrhosis (Lunzer *et al.*, 1973, 1975; MacGilchrist *et al.*, 1988). Therefore the number and/or function of adrenoceptors may be abnormal in cirrhosis. α_1 -adrenoceptors on vascular smooth muscle are inaccessible to study in man. We therefore studied α_2 -adrenoceptors on platelets, and also β_2 -adrenoceptors on circulating lymphocytes.

Methods

We studied 10 patients with histologically-proven decompensated cirrhosis (Pugh's grade B or C; seven males; 41–78 years, median 56.5) and 10 healthy subjects (seven males; 39–77 years, median 55). The aetiology of the cirrhosis was alcohol (7 cases), chronic active hepatitis (2) and cryptogenic (1). Two patients had ascites. Alcohol was stopped for 1 month, diuretics for 3 days and all other medication for 1 day

prior to study. The studies were performed on age- and sex-matched pairs. 90 min after breakfast and 30 min after insertion of a peripheral intravenous cannula, supine blood pressure (BP) and heart rate (HR) were measured by semi-automatic sphygmomanometer. Steady state was determined when systolic BP varied by < 5 mm Hg on five consecutive readings. Blood (150 ml) was then drawn for plasma bilirubin, albumin, prothrombin time, noradrenaline (NA) and for the receptor studies.

Platelet studies

A platelet suspension was prepared from citrated whole blood (Jones *et al.*, 1986), and recentrifuged and resuspended twice more to remove any retained NA. The final platelet concentration was $10^9 \mu\text{l}^{-1}$. Binding studies were then performed according to Motulsky *et al.* (1980) as modified by Jones *et al.* (1986). Aliquots of the platelet suspension were incubated with 6 concentrations of [^3H]-yohimbine (Amersham, 90 Ci mmol^{-1}) 1.2–18 nM in duplicate for 20 min at 25°C. Non-specific binding was determined using 1 μM phentolamine. Incubations were terminated by rapid vacuum filtration with 20 ml ice cold Tris HCl (50 mM, pH 7.4). The filters were dried

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overnight and [^3H] bound radioactivity determined by liquid scintillation at 30% efficiency. Saturation binding isotherms were analysed by plotting free/bound radioactivity vs free using least squares fitting to obtain values for the antagonist dissociation constant, K_D and the maximum number of binding sites, $B_{\max}$.

Lymphocyte studies

After collection of the platelet-rich plasma, lymphocytes were isolated using Ficoll (Pharmacia) as the layering medium (Boyum, 1968), membranes were prepared as described by Aarons *et al.* (1983) and the pellet stored at -70°C in incubation buffer. Aliquots (100 μl) of the lymphocyte membrane preparation were incubated with eight concentrations of [^{125}I]-iodocyanopindolol (Amersham) 10–150 pM according to Brodde (1983). 60 min incubations at 25°C were terminated by 10 ml of incubation buffer and vacuum filtration. Previous experiments confirmed that equilibration was achieved by this method (Deighton, 1988). The radioactivity of the wet filters was determined by gamma counting at 80% efficiency. Non-specific binding was determined using 1 μM propranolol. K_D and $B_{\max}$ were estimated by a modified Hane plot analysis.

The results were compared by Student's unpaired *t*-test. The power of the study to detect a difference of one standard deviation exceeds 0.8.

All subjects gave written informed consent and the study was approved by Greater Glasgow Health Board Research and Ethics Committee.

Results

In the cirrhotic group the median bilirubin was $34\text{ }\mu\text{mol l}^{-1}$ (range 12–123), albumin 31 g l^{-1} (21–37) and prothrombin time 4 s prolonged (0–11). HR, BP, plasma NA, and $B_{\max}$ and K_D for both receptor studies are listed in Table 1. The cirrhotics had higher plasma NA, faster HR and marginally lower BP than the controls. However $B_{\max}$ and K_D were unchanged for both platelet α_2 - and lymphocyte β_2 -adrenoceptors.

Discussion

The small number of patients studied means that these results have to be regarded as preliminary, with the caveat that minor differences might not have been detected. However the confidence intervals show no trend towards a difference and the negative result is likely to be valid.

This is the first study to examine the binding capacity of α -adrenoceptors in cirrhosis, although we previously demonstrated reduced pressor responses to both α_1 - and α_2 -adrenoceptor agonists (MacGilchrist *et al.*, 1988). The similar $B_{\max}$ values in both groups indicates that in decompensated cirrhosis, sympathetic activation, indicated by elevation of plasma NA, does not lead to generalised down-regulation of α -adrenoceptors. A similar lack of effect of sympathetic stimulation on α_2 -adrenoceptor number has been reported previously (Jones *et al.*, 1985; Pfeifer *et al.*, 1984).

The normal platelet receptors do not neces-

Table 1 Heart rate, blood pressure, plasma noradrenaline and $B_{\max}$ and K_D for both platelet α_2 - and lymphocyte β_2 -adrenoceptors. Analysis was by unpaired Student's *t*-test.

	Cirrhosis		Controls		95% confidence intervals of difference	P
	Mean	s.e. mean	Mean	s.e. mean		
Heart rate (beats min^{-1})	80	4.7	61	2.2	7.6 to 30.4	< 0.0005*
Systolic BP (mm Hg)	117	2.8	127	5.4	-21.9 to 1.9	0.11
Diastolic BP (mm Hg)	64	2.8	76	2.8	-18.7 to -1.3	< 0.02*
Plasma noradrenaline (mmol l^{-1})	4.4	0.5	2.6	0.3	0.1 to 3.5	< 0.02*
<i>Platelet α_2-adrenoceptors</i>						
$B_{\max}$ (fmol mg^{-1} protein)	24.9	3.4	22.1	1.9	-4.0 to 9.6	0.47
K_D (nmol l^{-1})	4.6	1.1	5.5	0.9	-3.6 to 2.6	0.56
<i>Lymphocyte β_2-adrenoceptors</i>						
$B_{\max}$ (fmol/ 10^9 platelets)	24.0	2.4	27.2	3.9	-12.6 to 6.2	0.55
K_D (pmol l^{-1})	49.6	4.5	55.3	9.4	-26.9 to 15.5	0.65

sarily reflect normal vascular receptors. If so, it would place the interference with sympathetic control of vascular function at the post-receptor level. This hypothesis is supported by evidence of a blunted pressor response to both sympathetic and non-sympathetic agonists in cirrhosis, both in rats (Murray & Paller, 1985) and in man (MacGilchrist *et al.*, 1988).

The lack of any reduction in the affinity of platelet α_2 -adrenoceptors, as indicated by the normal K_D values, does not support the hypothesis that the haemodynamic disturbance in cirrhosis is due to interference at the receptor binding site by some catecholamine-like substance (Bernardini *et al.*, 1982). However, platelet receptors again may not be representative of vascular receptors. Also, K_D represents the affinity of receptors *in vitro* after washing and does not exclude some weakly-bound substance having an effect *in vivo*.

We found no reduction in number or affinity of lymphocyte β_2 -adrenoceptors in decompensated cirrhosis. This is consistent with a normal cardiovascular response to β -adrenoceptor agonists in these same cirrhotic patients (MacGilchrist *et al.*, 1988). In rabbits, chronic

NA infusion had no effect on β_2 -adrenoceptor number (Deighton *et al.*, 1988).

Gerbes *et al.* (1986) also found no change in density or affinity of β_2 -adrenoceptors in cirrhosis. However a reduction in B_{max} in a subgroup of four patients with severe ascites was interpreted as evidence of down regulation of β_2 -adrenoceptors in severe cirrhosis. Since only two patients in our study had ascites, these studies are not necessarily contradictory, although neither of our ascitic patients had low B_{max} values. In contrast to our study, the cirrhotic patients reported by Gerbes did not discontinue their diuretics, which are known to reduce lymphocyte β -adrenoceptor number in hypertensive patients (Middeke *et al.*, 1985). Our study provides evidence against the hypothesis that the reduced efficacy of propranolol in preventing variceal rebleeding in decompensated cirrhosis is due to β -adrenoceptor down-regulation (Gerbes *et al.*, 1986).

In summary, using α_2 -adrenoceptors on platelets and β_2 -adrenoceptors on lymphocytes as our models, we find no adrenoceptor down-regulation in decompensated cirrhosis despite sympathetic activation.

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