Altered platelet α_2 adrenoreceptor in acute myocardial infarction and its relation to plasma catecholamine concentrations

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SUMMARY Changes in platelet α_2 adrenoreceptors and their relation to plasma catecholamine concentrations were studied in 11 patients with acute transmural myocardial infarction. A radio-labelled α_2 adrenoreceptor antagonist, [^3H]-yohimbine, was used to assay α_2 adrenoreceptors on platelet membranes, and plasma catecholamine concentrations were measured by high performance liquid chromatography. The number of platelet α_2 adrenoreceptors, the dissociation constant, and plasma noradrenaline and adrenaline concentrations were studied 6.6 (3.3) (mean (SD)) hours after the onset of acute myocardial infarction and one month later. The mean (SD) number of adrenoreceptors increased significantly from 94.5 (50.5) fmol/mg protein immediately after infarction to 157.0 (65.7) fmol/mg protein one month later. The dissociation constant, however, did not change significantly (4.33 (1.40) nmol/l *vs* 4.37 (1.22) nmol/l). Raised noradrenaline (5.60 (4.37) nmol/l) and adrenaline (0.28 (0.14) nmol/l) concentrations had fallen significantly to normal values (1.21 (0.67) and 0.09 (0.05) nmol/l respectively) a month after infarction. The decrease in the number of α_2 adrenoreceptors soon after infarction may be beneficial because such a change will reduce the strength of various reactions to catecholamines, such as vasoconstriction.

Human platelets have been used to investigate the possible role of α_2 adrenoreceptors in disease because they are easily obtained and their membranes contain α_2 adrenoreceptors.¹ In patients with angina pectoris the number of platelet α_2 adrenoreceptors was reported to be significantly lower when patients had symptoms than when they did not.² The number of α_2 adrenoreceptors did not correlate with plasma noradrenaline concentrations. Changes in the number of α_2 adrenoreceptors in acute myocardial infarction have not been studied. This is likely to be an important factor because α_2 adrenoreceptors may affect coronary artery tone.

We used a radioligand binding technique to study

α_2 adrenoreceptors in patients with acute myocardial infarction and we investigated the possibility of a relation between changes in receptor number or affinity and plasma catecholamine concentrations.

Patients and methods

PATIENTS

We studied 11 patients (seven men) with a first acute transmural myocardial infarction. The infarction was anterior in five patients and inferior in six. They were selected from a consecutive series of 18 patients with acute myocardial infarction admitted to this hospital between May and September 1984. Two patients with cardiogenic shock and three with heart failure were excluded, as were two patients who underwent coronary artery bypass operation. The diagnosis of acute transmural myocardial infarction was based on the acute onset of chest pain that lasted for more than 30 minutes and was unresponsive to

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glyceryl trinitrate, and on ST segment elevation >2 mV in the standard 12 lead electrocardiogram. The diagnosis was subsequently confirmed by the evolution of typical electrocardiographic changes and an increase in creatine kinase MB isoenzyme.

One patient (case 4) had had angina before infarction and had been treated mainly with sublingual glyceryl trinitrate. One patient (case 9) had been taking a thiazide for hypertension. No patient had been given β receptor blocking agents. After admission all patients were given nitrates. Five patients (cases 3, 5, 7, 10, and 11) were treated with diltiazem. Catecholamines and β receptor blocking agents were not given to any patients. No patient had chest pain or showed signs of heart failure while in hospital. None had enzymatic or electrocardiographic evidence of a recurrence of acute myocardial infarction.

BLOOD SAMPLING AND ISOLATION OF PLATELET MEMBRANES

Blood was drawn from an antecubital vein immediately after admission (6.6 (3.3) hours after the onset of chest pain). Three patients (cases 1, 5, and 9) were initially in Forrester haemodynamic subset II and eight were in subset I. Patients in group I were given frusemide which improved their haemodynamic condition. Blood samples were taken a month after the onset of infarction. All medication was discontinued for at least 24 hours before the second blood samples were collected.

Platelet membranes were obtained by the method of Garcia-Sevilla *et al.*³ Blood (30–40 ml) was collected in acid citrate dextrose (8:1, vol/vol). The sample was centrifuged at 160 *g* for 10 minutes at 25°C, and the platelet rich plasma was titrated to pH 6.5 with the acid citrate dextrose solution. Plasma was then recentrifuged at 5100 *g* for 15 minutes at 25°C to obtain a platelet pellet. The pellet was washed twice by 5 ml of Tyrode's buffer (sodium chloride 137 mmol/l, potassium chloride 2.7 mmol/l, monobasic sodium phosphate 0.36 mmol/l, magnesium chloride 0.01 mmol/l, sodium bicarbonate 12.0 mmol/l, dextrose 0.56 mmol/l, pH 8.0) and recentrifuged for 15 min at 5100 *g*. The pellet was lysed by homogenisation in 3 ml of ice cold hypotonic buffer (TRIS edetic acid 5 mmol/l, pH 7.5). The platelet membranes were obtained by centrifugation at 39 000 *g* for 10 min and then resuspended in the TRIS incubation buffer (TRIS-hydrochloric acid 50 mmol/l, magnesium chloride 10 mmol/l, pH 7.5) used in the radioligand binding assay.

RADIOLIGAND BINDING ASSAY

We used [3 H]-yohimbine (New England Nuclear, Boston, Massachusetts), an α_2 adrenoreceptor

antagonist to measure total radioligand binding in 0.1 ml volumes of fresh platelet membranes (0.15 (0.1) mg protein). Samples were incubated in duplicate at 25°C for 20 minutes. Non-specific binding was measured by adding unlabelled yohimbine (10 μ mol/l) to other duplicate samples. Specific binding was calculated as the difference between total and non-specific binding. Incubations were terminated by adding 5 ml of the TRIS-incubation buffer to the samples. The membrane bound tritiated ligand was recovered by rapid filtration of the diluted sample under vacuum through Whatman GF/C glassfibre filters. The filters were washed twice with 10 ml of TRIS-incubation buffer, air dried, and counted for radioactivity as described by Smith *et al.*⁴ Protein concentrations were determined by the method of Lowry *et al.*⁵ We used Scatchard analysis of the saturation isotherms to determine the maximal number of binding sites (*x* intercept of a plot of specifically bound ligand *vs* bound/free ligand) and the dissociation constant (negative reciprocal of the slope of the regression line).⁶

PLASMA NORADRENALINE AND ADRENALINE

Plasma concentrations of noradrenaline and adrenaline were determined by the method of Yui *et al.*⁷

Fig. 1 Specific binding of [3 H]-yohimbine to platelet membranes from patients with acute myocardial infarction, soon after the onset (solid line) and one month later (dashed line) as a function of increasing concentrations (0.5 to 9 nmol/l) of the ligand. Inset: Scatchard plot showing the change in numbers of binding sites. B, specifically bound ligand; B:F, specifically bound ligand:free ligand.

Table Patient profile and summary of data

Case	Age/sex	Medication	* Angina	Kd (nmol/l)		Bmax (fmol/mg protein)		Noradrenaline/adrenaline (nmol/l)		Clinical course	
				Soon†	1 month	Soon	1 month	Soon	1 month	Forrester subset	Treatment after admission
Anterior myocardial infarction:											
1	62/M	None	(-)	3.57	4.01	36.4	118.8	6.27/0.33	1.95/0.05	II→I	GTNO, LISDN, frusemide
2	54/F	None	(-)	4.00	4.46	118.0	211.0	—	—	I	LISDN
3	38/M	None	(-)	6.58	6.10	135.1	136.5	1.83/0.11	0.89/0.11	I	LISDN, diltiazem
4	77/M	Sublingual GTN	(+)‡	6.71	6.71	112.9	250.0	4.56/0.33	2.31/0.05	I	LISDN
5	74/F	None	(-)	3.11	3.45	58.0	80.4	5.68/0.33	0.53/0.16	II→I	LISDN, diltiazem, frusemide
Inferior myocardial infarction:											
6	49/M	None	(-)	5.18	4.98	88.3	137.2	5.44/0.16	0.71/0.05	I	LISDN
7	73/F	None	(-)	3.60	3.86	93.3	267.0	16.34/0.49	1.83/0.05	I	LISDN, diltiazem
8	69/F	None	(-)	5.38	4.27	55.2	115.6	—	—	I	LISDN
9	78/M	Thiazide	(-)	3.53	4.48	61.4	62.4	5.68/0.44	1.42/0.05	II→I	LISDN, frusemide
10	57/M	None	(-)	3.17	2.66	219.2	181.2	2.49/0.11	0.59/0.16	I	LISDN, diltiazem
11	56/M	None	(-)	2.80	3.05	83.6	167.9	2.07/0.22	0.71/0.05	I	LISDN, diltiazem
Mean	62.5			4.33	4.37	96.5	157.0	5.60/0.28	1.21/0.09		
SD	13			1.40	1.22	50.5	65.7	4.37/0.14	0.67/0.05		

Bmax, maximal number of binding sites; Kd, dissociation constant; I and II, Forrester haemodynamic subset I and II; GTNO, glyceryl trinitrate ointment; LISDN, long acting isosorbide dinitrate.

*Before admission.

†Before onset of myocardial infarction.

‡Angina lasting for two weeks before onset of myocardial infarction.

Fig. 2 α_2 adrenoceptor number (Bmax; fmol/mg protein) and dissociation constant (Kd; nmol/l) in patients with acute myocardial infarction soon after and one month after onset.

Normal values are 1.24–0.89 nmol/l and 0.16–0.05 nmol/l respectively.

STATISTICAL ANALYSIS

Values are given as means and standard deviations. Statistical analysis was performed by Student's *t* test. Significance was defined as $p < 0.05$.

Results

BINDING DATA

Figure 1 shows that the specific binding of [3 H]-yohimbine to platelet membranes from patients was both saturable and of high affinity. The Table shows data for individual patients. The number of the adrenoceptors increased significantly from 96.5 (50.5) fmol/mg protein early (6.6 (3.3) hours) after infarction to 157.0 (65.7) fmol/mg protein one month later ($p < 0.01$) (Fig. 2). But there was no significant difference in the dissociation constant (4.33 (1.40) nmol/l vs 4.37 (1.22) nmol/l).

PLASMA CONCENTRATIONS OF NORADRENALINE AND ADRENALINE

Plasma concentrations of noradrenaline and adrenaline were measured in nine of 11 patients. They

were raised immediately after infarction, but they had fallen significantly one month later (noradrenaline from 5.60 (4.37) nmol/l to 1.21 (0.67) nmol/l, $p < 0.05$; adrenaline from 0.28 (0.14) nmol/l to 0.09 (0.05) nmol/l, $p < 0.05$). Changes in receptor number and concentrations of noradrenaline and epinephrine were not significantly correlated (noradrenaline $r = 0.588$, adrenaline $r = 0.10$, both NS).

Discussion

Alpha adrenergic stimulation has been shown to modulate coronary artery tone in dogs and patients with ischaemic heart disease.⁸⁻¹⁰ Alpha₂ adrenoreceptors play an important role in the coronary vasoconstriction elicited by sympathetic nerve stimulation and in regulating the tone of both large and small coronary arteries.⁹ Thus alterations in α_2 adrenoreceptors might affect the coronary artery tone. In patients with symptomatic angina Weiss *et al* found a decrease in platelet α_2 adrenoreceptor number that did not correlate with plasma catecholamine concentrations.² These findings raise the possibility that α_2 adrenoreceptor abnormalities may be associated with the pathogenesis of angina. There is, however, little information on the changes in α_2 adrenoreceptors in myocardial infarction. We found that soon after infarction the number of platelet α_2 adrenoreceptors was significantly lower than it was one month later. There was no alteration in affinity. This decrease in receptor number soon after infarction was accompanied by an increase in the plasma catecholamine concentrations.

In an experimental study plasma catecholamines increased rapidly within one minute of coronary occlusion.¹¹ This increase was considered to reflect enhanced release of noradrenaline from the postganglionic sympathetic nerve endings in the heart.¹² Adrenaline secretion from the adrenal medulla was reflexly induced by stimulation of cardiac receptors at the site and the boundary of the infarct.¹² The increase in plasma catecholamine concentrations was also demonstrated early in myocardial infarction in a previous clinical investigation.¹³ In the present study, catecholamine concentrations were significantly raised 6.6 (3.3) hours after infarction. The increase in our study, however, was less pronounced than that observed in previous investigations.^{12 13} This might be due to our excluding patients with cardiogenic shock or severe heart failure, which are both conditions that enhance sympathoadrenal responses.¹³

Receptor down regulation is believed to occur when the cell is exposed for some time to higher concentrations of an agonist. The results of experimental studies of down regulation of platelet α_2

adrenoreceptor are controversial.^{14 15} Previous clinical studies found an inverse relation between plasma catecholamine concentrations, especially noradrenaline concentrations, and the number of α_2 adrenoreceptors in various conditions such as pheochromocytoma,^{16 17} congestive heart failure,¹⁸ and idiopathic orthostatic hypotension.¹⁶ This suggests agonist induced down regulation as a possible underlying mechanism. Our results resemble these clinical investigations. The correlation between changes in the receptor number and catecholamine concentrations was not, however, statistically significant. Furthermore, the affinity of the receptor for catecholamines is much higher than the ranges of physiological concentrations.¹⁹ Therefore, it appears unlikely that down regulation of platelet α_2 adrenoreceptor occurs in vivo.

Some unknown mechanisms other than down regulation may be involved in the modulation of the receptor number. Further investigations are necessary to clarify the mechanisms which regulate α_2 adrenoreceptor in vivo. In any event this decrease in the receptor number when catecholamine concentrations are raised during the early phases of acute infarction may be beneficial because it may moderate catecholamine induced reaction such as vasoconstriction.

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