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# Effect of Long-Term Beta-Blockade with Alprenolol on Platelet Function and Fibrinolytic Activity in Patients with Coronary Heart Disease

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**Summary.** In 14 patients with coronary heart disease the effect of long-term treatment (mean 16 months, range 12–33) with alprenolol on platelet fuction and fibrinolytic activity was studied. While on the beta-blocker and two weeks after gradula withdrawal of it, the patients performed a bicycle-ergometer test and blood samples were obtained before and following exercise. Pre-exercise fibrinolytic activity, assessed by the euglobulin clot lysis time, was 183 ± 27 min (mean ± SEM) while on alprenolol as compared to 111 ± 18 min ($p < 0.01$) after its withdrawal. Activation of fibrinolysis following exercise was not significantly influenced by alprenolol. In patients treated with alprenolol, the pre-exercise threshold level of ADP, producing platelet aggregation was 3.3 μM (geometric mean) and 5.1 μM after stopping treatment ($p \leq 0.05$). In patients receiving the beta-blocker, the ADP- threshold value dropped from 3.3 μM before exercise to 2.3 μM immediately after exercise (not significant). The corresponding values after withdrawal of alprenolol were 5.1 μM and 2.7 μM ($p \leq 0.02$). Adrenaline – stimulated aggregation was not significantly influenced by alprenolol. Serotonin release from platelets following maximal ADP- and adrenaline stimuli was not significantly changed by exercise in patients on beta-blockade. After stopping treatment, ADP-induced serotonin release was 22 ± 4.1% before and 15 ± 4.7% after exercise ($p < 0.02$). The corresponding values using the adrenaline stimulus were 29 ± 5.7% and 17 ± 4.7% ($p < 0.05$). It is suggested that during physical stress alprenolol may protect platelets against aggregatory stimuli.

**Key words:** alprenolol, coronary heart disease, platelet aggregation; fibrinolysis, platelet serotonin release, exercise

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The use of beta-adrenoceptor blocking agents in secondary prevention of myocardial infarction (MI) is currently the subject of debate. While a number of trials have failed to demonstrate a significant effect on mortality in post MI-patients [1–6], three controlled, randomized, prospective trials showed a reduction in long-term mortality and sudden death among patients given a beta-blocker during a post-MI period of 1–2 years [7–9]. So far, how beta-adrenoceptor blockade may protect the myocardium in patients who have sustained acute MI is poorly understood. Presumably these drugs may be effective through several mechanisms: lowering myocardial oxygen demands [10], protection against repeated minor episodes of myocardial ischaemia, and an anti-arrhythmic effect may all be operative [11]. In addition, propranolol has been reported to decrease platelet aggregation in patients with coronary heart disease [12–14], and to increase fibrinolytic activity in normal subjects during physical exercise [15].

The present study was designed to investigate whether alprenolol, which in two controlled trials reduced long-term mortality in MI [7, 9], may influence platelet function and fibrinolytic activity in patients with coronary heart disease (CHD).

## Patients and Methods

### *Patients*

The patients in the present study were all attending an out-patient clinic for followup of patients who had been enrolled in a double blind controlled, trial of alprenolol in MI at Sundby Hospital, Copenhagen, Denmark. The design of this trial has been described in detail elsewhere [9]. In short between 1st March 1976 and 31st December, 1978 all patients admitted to our Coronary Care Unit with suspected acute MI

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were eligible for participation in the trial. Immediately upon admission the subjects included in the study were randomized to receive either alprenolol 200 mg b.i.d. or matching placebo; a sustained release preparation (Aptin Durules®) was used. After discharge from the hospital trial, patients were seen at regular follow-up visits in the out-patient clinic. After treatment for one year, alprenolol/placebo was gradually withdrawn over a two-week period, apart from those in need of beta-blockade because of angina pectoris or hypertension. These patients continued treatment with alprenolol. Fourteen patients, 11 men and 3 women, 43–66 years (mean 57 years), were included in the present investigation. They all met the following criteria. 1) treatment with alprenolol 200 mg b.i.d. for at least one year; 2) CHD as evidenced by a history of prior MI or a positive exercise tolerance test. Patients on anticoagulant therapy and subjects with known diabetes mellitus, hyperlipidaemia, haematological or endocrinological disorders were excluded from the study. 11 patients received alprenolol for 12–18 months, 2 patients for 20 and 26 months, respectively, and 1 patient for 33 months (mean duration of treatment 16 months). Informed consent was obtained from all patients.

*Experimental Design*

No aspirin, other non-steroidal antiinflammatory drugs, oestrogens or benzodiazepine derivates were permitted for one week prior to study. All studies took place between 11.00 h and 14.00 h. Patients fasted and did not smoke for 4 h before the first blood sample. This sample was obtained exactly 2 h after the final dose of alprenolol. A 17-gauge needle was inserted into an antecubital vein using light stasis, and a slow infusion of physiological saline was begun. After 30 min rest in the supine position, blood was sampled by free flow from the cubital vein into plastic tubes containing $^1/_{10}$ volume trisodium citrate, the first 5 ml of blood having been discarded. A maximum multistage graded exercise test was then performed using a bicycle ergometer (Elma Schönander). The starting work load was 200 k.p.m. per minute and this load was augmented by 200 k.p.m. every four minutes until fatigue or chest pain developed. A 6-leas ECG was recorded continously. Immediately after the patient stopped pedalling, a second blood sample was taken, and the final sample was obtained following 20 min rest in the supine position. Alpreonolol was then gradually withdrawn over a two-week period, and after a further two weeks, the above procedure was repeated.

*Platelet Function and Fibrinolysis Studies*

Citrated platelet-rich plasma and platelet-poor plasma were prepared as described elsewhere [16]. Platelet aggregation was studied turbidometrically in a Payton aggregometer. Aggregation was induced by ADP in final concentrations of 0.25, 0.50, 1.00, 2.00, 4.00, 8.00 and 16.00 μM, and by adrenaline in final concentrations of 0.01, 0.05, 0.10 and 1.00 μg/ml. The concentrations of ADP and adrenaline required to induce irreversible aggregation were determined [16]. 14C-serotonin release was measured after aggregation with ADP 16 μM and adrenaline 1.00 μg/ml, as described earlier [16]. Euglobulin lysis time (ELT) was measured by a modification of the method previously described [17], the lysis of the clot being estimated turbidometrically (Clot lysis time recorder, Medicon Ltd., Glasgow).

*Statistical Methods*

In ADP and adrenaline aggregation studies the geometric mean is presented. In calculating the mean values, threshold concentrations $> 16$ μM in ADP studies were put equal to 32 μM, and threshold concentrations above 1.00 μg/ml in adrenaline studies were put equal to 2.00 μg/ml. For all other variables the arithmetic mean and the standard error of the mean are presented. The Wilcoxon matched pairs signed rank sum test was exployed as the test of significance of differences.

## Results

Heart rate and blood pressure before exercise and total working capacity were not significantly influenced by the beta-blockade (Table 1). Maximum heart rate was 125 ± 4 beats per min on alprenolol and 148 ± 5 b.p.m. off treatment ($p < 0.01$; Table 1). During beta-blockade and in the post-alprenolol state the reasons for stopping pedalling were fatigue in 12 patients and chest pain in 2 patients.

Pre-exercise fibrinolytic activity, assessed by the ELT, was 183 ± 27 min (range 54–425 min) on alprenolol and 111 ± 18 min (range 48–327 min) following cessation of treatment ($p < 0.01$; Fig. 1). Physical stress decreased ELT to the same degree regardless of treatment. Twenty minutes after exercise, ELT had returned to near its pre-exercise level in patients on the beta-blocker, whereas in the untreated state this value was still significantly lower than the pre-exercise ELT ($p < 0.02$; Fig. 1).

The effec ct of alprenolol on ADP-induced aggregation is illustrated in Fig. 2. The beta-blocker

**Table 1.** Haemodynamic parameters and maximal working capacity in 14 patients during and following cessation of alprenolol treatment.

| | Alprenolol | No alprenolol | |
|---|---|---|---|
| Systolic blood pressure at rest [mmHg] | 145 ± 5.5 | 145 ± 5.7 | NS |
| Diastolic blood pressure at rest [mmHg] | 91 ± 2.7 | 91 ± 2.8 | NS |
| Heart rate at rest [beats per min] | 72 ± 3.1 | 70 ± 2.8 | NS |
| Heart rate at maximum exercise [beats per min] | 125 ± 4.1 | 148 ± 5.3 | $p < 0.01$ |
| Maximal working capacity [k.p.m. × min] | 6182 ± 106.5 | 6071 ± 103.0 | NS |

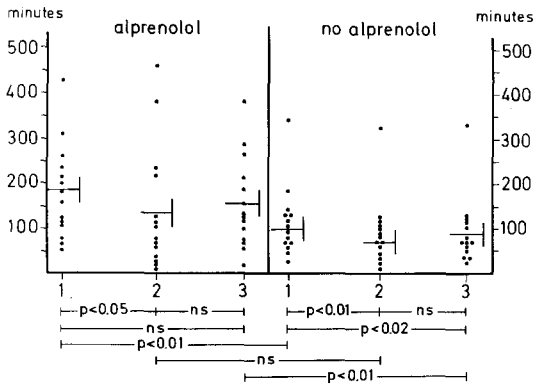


**Fig. 1.** Euglobulin clot lysis time (ELT) during and following cessation of alprenolol treatment in 14 patients; mean ± SEM; 1 30 min before exercise; 2 immediately after stopping exercise; 3 20 min after stopping exercise

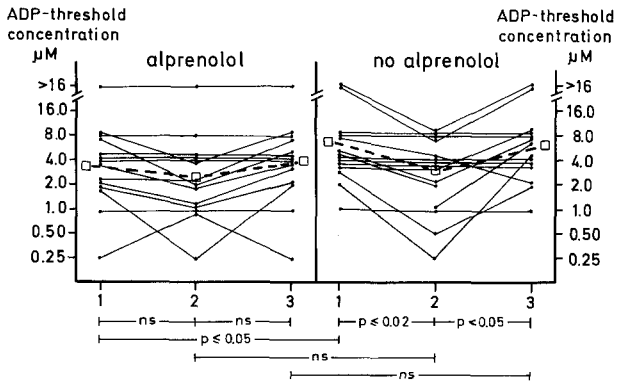


**Fig. 2.** ADP-threshold concentrations during and following cessation of alprenolol treatment in 14 patients; geometric mean. ADP-threshold values are shown on the ordinate axis in a logarithmic scale. 1 30 min before exercise; 2 immediately after stopping exercise; 3 20 min after stopping exercise

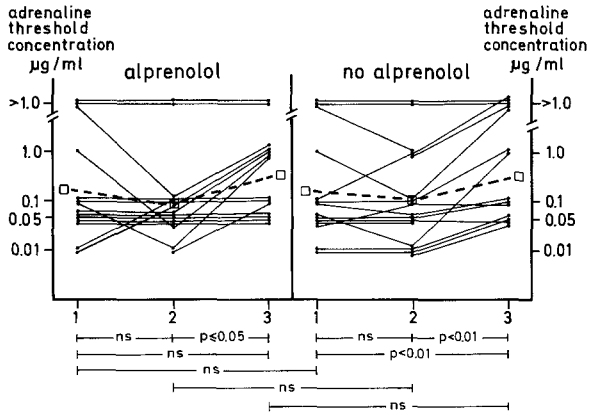


**Fig. 3.** Adrenaline-threshold concentrations in 14 patients during and following cessation of alprenolol treatment; geometric mean. Adrenaline-threshold values are shown on the ordinate axis in a logarithmic scale. 1 30 min before exercise; 2 immediately after stopping exercise; 3 20 min after stopping exercise

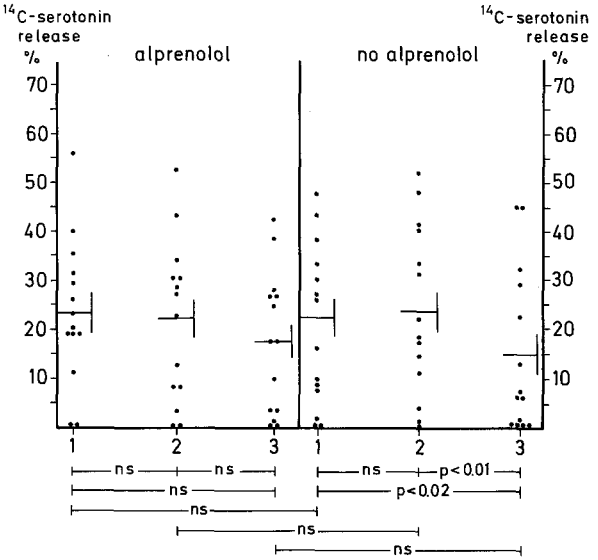


**Fig. 4.** Serotonin release (mean ± SEM) following maximal ADP-stimulus during and after cessation of alprenolol treatment in 14 patients.
1 30 min before exercise; 2 immediately after stopping exercise; 3 20 min after stopping exercise

caused a border-line significant decrease in the pre-exercise threshold value, from 3.3 µM on alprenolol to 5.1 µM off treatment ($p \leq 0.05$; Fig. 2). Physical stress did not cause a significant fluctuation in mean ADP-threshold level in patients on alprenolol, pre-exercise and post-exercise values being 3.3 µM and 2.3 µM, respecitvely (not significant; Fig. 2). The corresponding values in the off-alprenolol state were 5.1 µM and 2.7 µM ($p < 0.02$; Fig. 2). No significant effect of alprenolol on adrenaline-stimulated aggregation was recorded (Fig. 3).

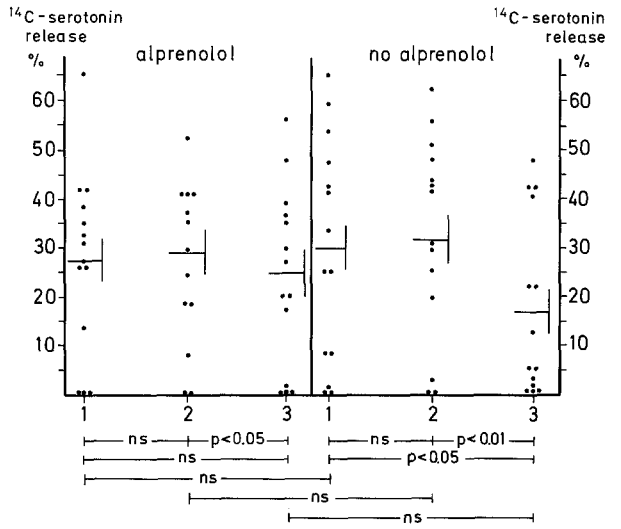


**Fig. 5.** Serotonin release (men ± SEM) following maximal adrenalinestimulus during and after cessation of alprenolol treatment in 14 patients.
1 30 min before exercise; 2 immediately after stopping exercise; 3 20 min after exercise.

Platelet function was further assessed by measuring serotonin release following maximal ADP and adrenaline stimuli. In ADP studies (Fig. 4), physical stress caused no significant change in serotonin release in patients on alprenolol, the pre-maximum-, and post-exercise values being 23 ± 3.8% (range 0–56%), 22 ± 4.2% (range 0–53%) and 18 ± 3.7% (range 0–43%, not significant). Following cessation of treatment, serotonin release decreased from the pre-exercise level of 22 ± 4.1% (range 0–47%) to 15 ± 4.7% (range 0–45%) 20 min after stopping exercise ($p < 0.02$; Fig. 4). In adrenaline studies (Fig. 5), serotonin release also decreased following exercise in the off-treatment state, the pre-exercise value being 29 ± 5.7% (range 0–57%) as compared to 17 ± 4.7% (range 0–47%) 20 min post-exercise ($p < 0.05$). The corresponding values while on beta-blocker were 27 ± 4.8% (range 0–65%) and 24 ± 4.8% (range 0–57%); not significant; Fig. 5).

## Discussion

In patients dying from an acute MI, post-mortem examination often fails to disclose occlusive arterial thrombi or erupted atherosclerotic plaques [18]. Especially in sudden death the role of platelet microthrombi has been emphasized [19]. Drugs influencing platelet aggregation may be effective therefore, in the secondary prevention of MI.

Adrenergic receptors are present in most tissues, platelets being no exception [20]. Several studies have established that adrenaline increases platelet adhesiveness-aggregation [21, 22]. This effect is mediated through alpha-adrenergic receptors [21–23]. Stimulating this receptor causes inhibition of prostaglanding ($PGE_1$)-induced formation of c-AMP and accordingly augments platelet-aggregability [24, 25]. Stimulation of the beta-adrenergic receptor increases intra-platelet c-AMP [24], thus tending to decrease aggregation. Adrenaline also activates the fibrinolytic system [26], a beta-2 receptor being claimed to be involved [27]. From a theoretical point of view, therefore, beta-blockade might adversely influence platelet function as well as fibrinolysis.

Several in-vitro an in-ivio studies have established that phentolamine, an alpha-receptor blocking agent, possesses platelet anti-aggregating properties [22, 23]. As for beta-blockers, they have been shown to decrease adrenaline- and ADP-induced aggregation in-vitro, but in most studies very high doses were required [20, 21, 28, 29]. In-vivo studies either showed no effect on platelet function of beta-blocking agents per se [30], or demonstrated clearcut inhibition of adrenaline and ADP-stimulated aggregation in patients with CHD [12, 14, 31]. The effect of beta-blockade on fibrinolytic activity has been studied in normal subjects. Alprenolol administered intravenously did not itself influence fibrinolysis, but adrenaline-induced activation of the fibrinolytic system was blocked [32]. In other studies, beta-blockers have accentuated fibrinolytic activity during physical stress [15].

In the present investigation patients with CHD were studied. Tablet intake was not controlled by checking the serum level of alprenolol, but a significant reduction in exercise heart rate was observed in patients on supposed to be on alprenolol.

Pre-exercise ELT was significantly longer in patients on alprenolol as compared to the post-beta-blocker state. However, all ELT-values reported in the present study were within the „normal" limits for age-matched patients with CHD in our laboratory. During exercise beta-blockade did not affect activation of fibrinolysis. Our data offer no explantation of the pre-exercise ELT prolongation in patients on chronic beta-blockade. This finding requires confirmation in further studies, in which both haemodynamics and plasma catecholamines should be monitored.

It is well established that physical stress is followed by a significant decrease in the threshold concentrations of ADP and adreanline causing aggregation [33]. A similar decrease was not recorded in our patients treated with alprenolol. A reduction in stress-induced fluctuations in the ADP threshold may well be beneficial in patients with CHD.

During physical stress platelets are activated [34, 35]. Platelets which have been exposed to aggregating stimuli show decreased sensivity to ADP and decreased serotonin release [36]. In the present study serotonin release following a maximal ADP stimulus was not significantly changed by exercise in patients on beta-blockade. Conversely, in the off-treatment state, exercise was followed by a significant decrease of serotonin release. Reduction of serotonin release following physical stress reflects exhaustion of platelets. Our findings may indicate that alprenolol protected platelets from stimuli during the physical exercise.

Several papers have stressed the fact that beta-blocking drugs (propranolol in particular has been studied) do not influence platelets by blocking beta-adrenergic receptors [12, 21, 29]. A non-specific membrane action, possibly affecting ion-fluxes, has been proposed [29]. Alprenolol, like propranolol, possesses so-called membrane stabilizing properties, which may be relevant in this respect.

In conclusion, in patients with CHD chronic beta-adrenoceptor blockade with alprenolol may cause a modest decrease in the resting fibrinolytic activity, as assessed by the ELT. However, the fibrinolytic capacity following physical exercise is not influenced by beta-adrenergic blockade. In addition, alprenolol may protect platelets against aggregating stimuli during exercise. The clinical significance, if any, of these findings awaits further study.

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