Response of Fibrinolytic Activity to Venous Occlusion

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Summary

Resting fibrinolytic activity and the response of the fibrinolytic system to venous occlusion were studied in a group of healthy middle-aged men. All subjects showed increased fibrinolytic activity but of varying degrees. There was a linear relationship between resting and occluded levels of fibrinolytic activity but many subjects with lower levels of fibrinolytic activity showed an anomalous response. Responses over the expected level were more common than unexpectedly low levels of response. Fibrinogen and plasminogen concentrations were inversely correlated with fibrinolytic activity.

Introduction

It has been suggested that there is a dynamic equilibrium between blood coagulation and fibrinolysis and that disturbance of this balance may result in fibrin deposition, thrombus formation, and atherosclerosis.[1 2] Attempts to show a defective fibrinolytic system in people with atherosclerosis and its complications have produced conflicting results, possibly because of the practice of measuring fibrinolytic levels at rest. Some workers regard the fibrinolytic activity of the plasma of a resting subject as an inadequate measure of true fibrinolytic capacity,[3] and various techniques have been used to determine the levels of fibrinolytic activity which might be achieved on stimulation. Plasminogen activator in the blood is responsible for physiological plasma fibrinolysis and increased amounts of this activator are present after exercise, emotional stress, trauma, adrenaline administration, and nicotinic acid injection. Venous occlusion also increases the fibrinolytic activity of blood within the occluded segment.[4] Some people respond poorly to these stimuli and "poor responders" to one stimulus—for example, intravenous adrenaline—are likely to be poor responders to other forms of stimulation—for example, exercise.[5 6]

Our aim was to determine whether the response of the fibrinolytic system to stimulation—that is the fibrinolytic capacity—was related to plasma fibronolytic activity at rest and to examine the relation between the various factors within the fibrinolytic system—plasminogen activator, plasminogen, and fibrinogen.

Subjects and Methods

During 1968-70 many executive grade male civil servants aged 40-64 years recorded their leisure-time activities over two days for a study of exercise and coronary heart disease.[7] In September 1970 all the men in their 40s from two government offices in the London area who had participated in this large-scale questionnaire study were invited to take part in a study on the feasibility of lowering blood cholesterol levels by simple dietary recommendations.[8] During this study resting fibrinolytic activity was determined on four separate occasions over three years. Those men whose fibrinolytic activity, as measured by the dilute blood-clot lysis method[9 10] was less than nine hours or more than 18 hours on at least three occasions out of four were invited to participate in our study. As we could not study all the 150 men in the diet study we chose those men at the extremes of the population in terms of their resting fibrinolytic activity to determine whether they differed in their response to stress. Fifty-nine healthy men aged 45-53 years (mean 50 years) agreed to take part.

METHODS

All subjects attended in the fasting state and did not smoke on the morning of the investigation. They were specifically asked to avoid undue physical activity on that morning, and on arrival were allowed to relax in the waiting area. These men were unlikely to be unduly anxious as this was their ninth visit to us over three years and venepuncture had been carried out at each visit. Each man was weighed and his blood pressure recorded in the recumbent position, after which he remained recumbent for a further 10 minutes. An indwelling needle was then inserted into the antecubital vein and kept patent with 3·8% sodium citrate solution. An initial blood sample was obtained followed five minutes later by a second resting sample. The sphygmomanometer cuff around the upper arm was then inflated to a level midway between the systolic and diastolic blood pressure levels and this pressure was maintained for 10 minutes. A third blood sample was obtained just before the cuff was deflated ("occluded" level). Five minutes after release of the cuff pressure a fourth sample was collected ("post-occlusion" level).

Dilute Blood Clot Lysis.—The method of Fearnley *et al.*[9] as modified by Lackner and Goosen[10] was used. Tests on all four samples were carried out within one hour of collection.

Euglobulin Lysis Time.—The method described by Cash[11] was used. Lysis was recorded by an automatic camera at five-minute intervals for the first two hours and thereafter at 15-minute intervals. The time taken for the clots to disappear completely was regarded as the lysis time. The results were expressed in units of fibrinolytic activity using the reciprocal of the square of the clot lysis time[12] multiplied by 10^6 for convenience of expression.

Fibrin Plate Method.—20 µl of the resuspended euglobulin fraction was applied to fibrin plates and the lysis area recorded after 18 hours' incubation at 37°C.[13 14]

For all three methods the mean of the two measurements made before venous occlusion was used to represent the resting level of fibrinolytic activity.

Fibrinogen was estimated by the heat precipitation method on fresh blood collected in citrate with 2 M aminocaproic acid.[15] Plasminogen was measured on citrated plasma stored at −20°C[16 17] and expressed in casein units/ml.

Results

DILUTE BLOOD CLOT LYSIS TIME

Fibrinolytic activity is inversely proportional to lysis time—that is, subjects with low levels of fibrinolytic activity have long lysis times. Based on four separate dilute blood clot lysis time estimations made in duplicate over three years, resting fibrinolytic activity was regarded as "high activity" (<9 hours) in 31 men (group 1) and "low activity" (>18 hours) in 28 men (group 2).

Group 1.—A dilute blood clot lysis time of less than nine hours had been recorded on all four previous occasions in 19 men and on three out of four previous occasions in 12 men. The times in our study were less than nine hours in 20 men, nine to 18 hours in nine men, and over 18 hours in two men.

Group 2.—A dilute blood clot lysis time of over 18 hours had been recorded on all four previous occasions in 16 men and on three out of

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four previous occasions in 12 men. In our study 26 men had times greater than 18 hours and two had times less than 18 hours (16 hours and 10 hours).

RESTING, OCCLUDED, AND POST-OCCLUSION FIBRINOLYTIC ACTIVITY

Fig. 1 shows the resting, occluded, and post-occlusion levels of fibrinolytic activity as determined by the three methods used. For the dilute blood clot lysis time and euglobulin lysis time methods the fibrinolytic activity is shown in minutes. For the fibrin plate method it is shown in terms of the area of lysis (mm^2). These data are shown without conversion to more appropriate units of fibrinolytic activity in order to indicate "response" in the way in which it is actually observed and is usually reported by most observers.

In general, all three methods of assessing fibrinolytic activity showed similar patterns of response in both activity groups. In group 1 there was a fairly clear separation between resting and occluded levels with an incomplete return to resting levels in the post-occlusion period. In group 2 the separation between resting and occluded levels was less pronounced and a much wider range of levels was seen during the occluded period. In the post-occlusion period there was again an incomplete return towards the resting levels.

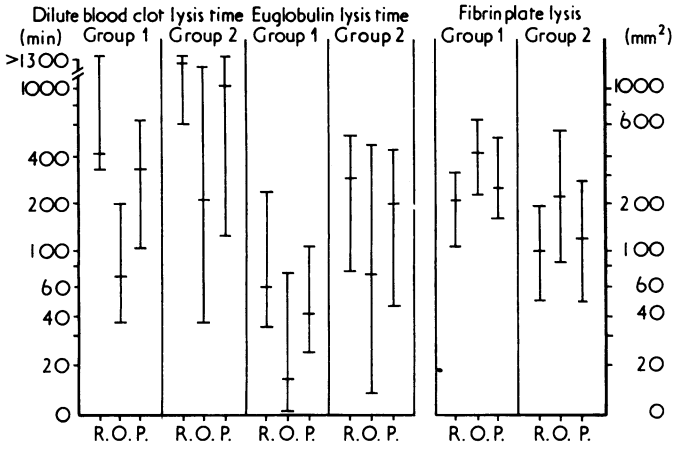

FIG. 1—Median (and range) resting (R.), occluded (O.), and post-occlusion (P.) levels of fibrinolytic activity determined by three methods. Group 1 = High activity. Group 2 = Low activity.

RELATION BETWEEN RESTING AND OCCLUDED LEVELS OF FIBRINOLYTIC ACTIVITY

Euglobulin Lysis Time.—Fig. 2 shows resting levels of fibrinolytic activity measured by the euglobulin lysis method and expressed in minutes plotted against the decrement in lysis time produced by venous occlusion—that is, the difference between resting and occluded lysis times. There was a close relation between the resting lysis time (log) and the decrement on occlusion (log) ($r=0{\cdot}91$). The relation between resting euglobulin lysis time (log) and occluded

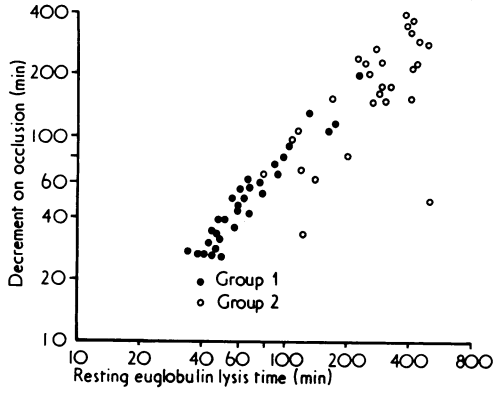

FIG. 2—Relation between resting euglobulin lysis time and decrement in lysis time after venous occlusion.

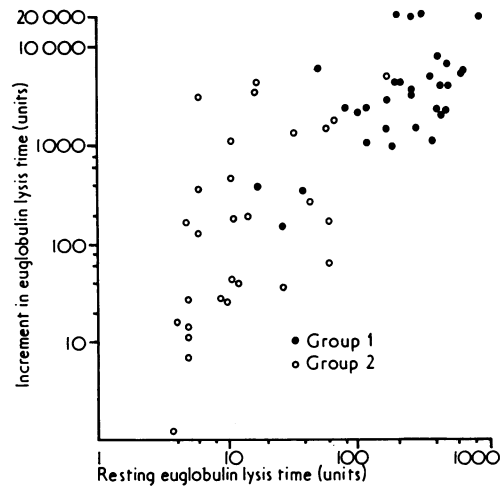

FIG. 3—Relation between resting euglobulin lysis time and increment in activity after venous occlusion.

euglobulin lysis time (log) was not as close as that for resting and decremental levels ($r=0{\cdot}81$). In fig. 3 the euglobulin lysis time in minutes has been converted to units of fibrinloytic activity using the reciprocal of the square of the lysis time $\times 10^6$. The increment on venous occlusion was the difference between units at rest and units on occlusion. There was a relationship between the resting fibrinolytic activity expressed in these units and the increment in units produced by venous occlusion (log/log) ($r=0{\cdot}78$). Men with low levels of fibrinolytic activity (long lysis times and therefore a small number of fibrinolytic units) had small increments in fibrinolytic activity in response to the stress of venous occlusion. Men with high levels of fibrinolytic activity (short lysis times and therefore a large number of units) had large increments in response to venous occlusion.

Fibrin Plate Method.—The resting levels of fibrinolytic activity measured by the fibrin plate method and expressed in mm^2 correlated well with the occluded levels (log/log) ($r=0{\cdot}77$). When the resting level was plotted against the increment in lysed area (log/log) there was a much lower degree of correlation ($r=0{\cdot}47$).

RELATION BETWEEN METHODS USED TO DETERMINE FIBRINOLYTIC ACTIVITY

There was a close correlation between resting levels of fibrinolytic activity measured by the euglobulin method and by the fibrin plate method ($r=0{\cdot}91$) (log/log). As the dilute blood clot lysis method may have no clear end-point—that is, it may be over 24 hours—correlation could not readily be expressed with resting levels assessed by euglobulin lysis time and the fibrin plate method. The correlations for occluded values on dilute blood clot lysis time with occluded values on euglobulin lysis time and on the fibrin plate method (log/log) were also high ($r=0{\cdot}92$ for euglobulin lysis and $r=0{\cdot}90$ for the fibrin plate method).

PLASMA FIBRINOGEN AND PLASMINOGEN

The packed cell volume (P.C.V.) increased from 44·1% to 51·2% (16%) during the period of venous occlusion. Plasma fibrinogen concentration rose by 20% (3·02 to 3·63 g/l) and plasma plasminogen concentration by 18% (1·65 to 1·94 casein units/ml). The correlation between the change in P.C.V. and the change in fibrinolytic activity (units) for all 59 subjects was low ($r=0{\cdot}19$). The increase in fibrinogen and plasminogen concentration in the occluded specimens seemed to be directly due to haemoconcentration and there seemed to be no increase in the absolute level after venous occlusion.

There was a significant correlation ($P<0{\cdot}01$) between fibrinolytic

Fibrinogen and Plasminogen Concentrations in Relation to Resting Levels of Fibrinolytic Activity

Resting fibrinolytic activity (units)	<12	12-70	71-260	>260
No. of men	16	15	14	14
Mean fibrinolytic activity (units)	7	36	178	462
Mean fibrinogen (g/l)	3·45	3·11	2·78	2·69
Plasminogen (casein units/ml)	1·72	1·74	1·63	1·51

activity (units) and fibrinogen and plasminogen concentrations ($r = -0.54$ and $r = -0.40$ respectively). When the men were ranked in order of fibrinolytic activity and divided into four groups of about equal size (see table) both fibrinogen and plasminogen decreased with increasing fibrinolytic activity and the relationship did not differ significantly from linearity ($P < 0.01$).

Discussion

Our interest in the fibrinolytic system arises from a concern with its possible relationship to atherosclerosis and the complications of atherosclerosis. Some investigators have found that low levels of fibrinolytic activity are more common among atherosclerotic patients (coronary heart disease, peripheral vascular disease) than controls, whereas others have been unable to show such differences. Inter-racial studies have shown that there are striking differences in fibrinolytic activity between communities prone to atherosclerosis and its complications and those communities which are relatively free from such problems.[1 8] Nevertheless, there is no clear evidence which relates a disturbed fibrinolytic system to the aetiology or pathogenesis of atherosclerosis or which indicates that established arterial disease is associated with a deficient fibrinolytic capacity.

Considerable caution has been exercised in extrapolating the results obtained on single measurements of fibrinolytic activity in resting subjects to the systemic circulation generally, and resting levels have been regarded as an inadequate measure of true fibrinolytic capacity.[3] Individuals may have low resting levels of fibrinolytic activity but respond sharply to the effects of stimulation (exercise, adrenaline, venous occlusion). We chose venous occlusion as a stimulus as it was more acceptable and convenient than adrenaline injection or exercise in a middle-aged group of men, some of whom were at increased risk of coronary heart disease. Our results are in general agreement with established findings, and all of the 59 subjects showed some increase in fibrinolytic activity after venous occlusion, though the degree of response differed considerably.

In those men with consistently high levels of fibrinolytic activity on the previous four visits (group 1) the response to venous occlusion was striking and only a few men remained within the range of the resting levels. In men with consistently low levels of fibrinolytic activity on the previous four visits (group 2) the response to venous occlusion was much more variable, and on both euglobulin lysis time and the fibrin plate method almost half the subjects were still within the resting range of values. Of those subjects with high resting levels of fibrinolytic activity (>100 units) none showed a low level of response; but in those with lower resting levels of fibrinolytic activity there was a wide range of incremental levels.

Clearly, there is a strong correlation between the resting level of fibrinolytic activity and the response elicited by venous occlusion, which gives considerable predictive value to the resting level. For euglobulin lysis time the resting level is related to the decrement in time consequent on the venous occlusion and for the fibrin plate method the resting area of lysis is related to the occluded area of lysis. The linear relationship between resting fibrinolytic activity and the increase in activity seen on stimulation has also been shown in exercise studies,[11 19] after adrenaline injection,[20] and after oral glucose.[21] The mechanisms by which plasminogen activator is made available after these stimuli are not known, and if there is some common pathway its nature remains speculative.

Many studies of the fibrinolytic system have failed to show a relation between fibrinolytic activity and such variables as fibrinogen and plasminogen. We found that both fibrinogen and plasminogen correlated significantly with fibrinolytic activity and both showed decreasing concentrations with increasing levels of fibrinolytic activity. It seems logical that where fibrinolytic activity is high the substrate from which the active enzyme plasmin is derived might be lowered. It also seems logical that an active fibrinolytic system would result in lower levels of fibrinogen in the plasma. Thus, the raised levels of fibrinogen often reported in patients with atherosclerosis and its complications might merely reflect diminished fibrinolytic activity.

Do measurements of the fibrinolytic activity in venous blood give a valid guide to arterial blood levels? Though activator level is significantly higher in venous blood[22] the difference is relatively small and the relation between arterial and venous levels is linear.[23] Furthermore, the relation between arterial and venous levels of other major components of the fibrinolytic system (plasminogen, fibrinogen, antiplasmin activity, anti-activator activity) are also constant within narrow limits, which strongly suggests that estimations on venous blood may be used to predict arterial levels.

It is tempting to speculate on the significance of the findings in fig. 3. Clearly, those subjects with lower resting levels of fibrinolytic activity (<100 units) include not only "poor responders"—that is, those whose response to stress is less than would be expected from the linear relationship shown—but also those whose response is better than would be predicted from their resting levels. The phenomenon is one of anomalous response rather than poor response or non-response, and indeed responses greater than expected are more common than the converse. If the level of plasminogen activator present in the blood vessels (or its ability to be released) is affected by atherosclerosis (or the factors producing atherosclerosis) then one might assume that people with resting levels over 100 units and with the predicted response to venous occlusion have healthy blood vessels (or low levels of atherogenic factors). Those with resting levels below 100 units and with an anomalous response to venous occlusion might be reflecting their degree of atherosclerosis or the presence of atherogenic factors, or both. Those falling short of their expected response would, therefore, have more disease than suggested by their resting levels of fibrinolytic activity; those exceeding their expected levels would have less disease than suggested by their resting levels. Unfortunately for the assessment of this hypothesis, we do not know the relative contribution of veins and arteries to the total systemic fibrinolytic capacity and we know remarkably little about the function of the veins in patients with atherosclerosis.

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