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EFFECTS OF LAMININ, PROTEOGLYCAN AND TYPE IV COLLAGEN, COMPONENTS OF BASEMENT MEMBRANES, ON PLATELET AGGREGATION

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Summary

The aggregatory properties of zone-specific basement membrane components, laminin, proteoglycan and type IV collagen were studied in platelet-rich plasma. Laminin and proteglycan of the subendothelial lamina rara of vessel walls did not cause aggregation of platelets or release of thromboxane B_2 in contrast to type IV collagen present in the deeper lamina densa zone. The results indicate the nonthrombogenic nature of lamina rara and furthermore that platelet aggregation and microthrombosis are initiated only when vessel wall injuries become deep enough to allow platelets to reach collagen in the lamina densa.

INTRODUCTION

The interaction between activated platelets and injured vessel walls is supposed to be the key event in the development of the atheromatic plaque (1) and changes in the production of antiaggregatory prostacyclin by the endothelial cells (2) or proaggregatory thromboxane A_2 by platelets (3) may be involved in this process (4). The basement membrane of the vessel wall is of special interest in this connection, since it becomes exposed to platelets in the case of endothelial injury. This structure consists of morphologically distinct regions, well identified in capillaries and small arteries. The lamina rara is the region upon which the endothelial cells rest, and the lamina densa the region beneath the lamina rara (5). The structural components of these regions appear to be

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zone-specific. Laminin (6), a glycoprotein, is found in the subendothelial lamina rara (7), a heparan sulfate proteoglycan (8) in the lamina rara zone adjacent to the lamina densa (G.R. Martin, personal communication) and type IV (basement membrane) collagen is present in the lamina densa alone (5,9). Type V (A,B) collagen is codistributed with the type IV collagen in some but not all basement membranes (10). The ultrastructure of the subendothelial basement membrane in large arteries is not known in detail, but like all basement membranes, it contains both laminin and type IV collagen as judged by immunofluorescence (unpublished results).

Previous reports have shown that type IV (11) and V (11,12, 13) collagens and interstitial collagen types I and III (14) in the dermis elicit platelet aggregation. The lamina rara, however, is the first subendothelial region exposed to platelets upon loss of endothelial integrity but the interactions of its components with platelets have not been studied. Here, we report how lamina rara -specific components, laminin and proteoglycan, and lamina densa -specific type IV collagen, affect platelet aggregation *in vitro* and compare them with known aggregatory agents.

MATERIAL AND METHODS

Laminin, proteoglycan and type IV collagen were extracted from basement membrane -forming mouse tumor (15) and purified as described elsewhere (6,8,15). Antibodies to these components have previously been shown to react with human basement membrane (6,8,9,16). Laminin and type IV collagen were pure as determined by amino acid analysis and SDS gel electrophoresis (17). The type IV collagen contained intact 160 000 and 140 000 dalton polypeptide chains (15,18,19) along with minor amounts of cross-linked components. The proteoglycan was purified from the tumor matrix with sodium $|^{35}S|$ sulphate- and $|6\text{-}^{3}H|$-glucosamine-labeled tumor proteoglycan as a marker (8) and it had the properties reported for basement membrane proteoglycan (8). Type I collagen was obtained from calf skin. The proteins were dissolved both in a 0.02 M sodium phosphate buffer, pH 7.4, and 0.05 Tris-HCl buffer, pH 7.4, containing 0.2 M NaCl.

Both buffers were used because it has been reported that type I collagen which is soluble in Tris-HCl buffer is poorly aggregatory in comparison with its fibrillar form in the phosphate buffer (14). Type IV collagen does not form fibrils in vivo (5) as do type I and III collagens but it has similar solubility properties in the two buffers used here.

Platelet-rich plasma containing 200,000 to 400,000 platelets per µl was prepared from blood of healthy volunteers, using sodium citrate (0.1 volume of 3.8 % w/v) as an anticoagulant. The blood donors had not ingested drugs known to interfere with platelet aggregation or prostaglandin synthesis for at least 10 days. Platelet aggregation was studied in a volume of 1.0 ml at 37° C in an aggregometer (Payton Dual Channel Aggregation Module connected to a Goerz RE recorder) following the addition of laminin, proteoglycan, type IV and I collagens (2 - 100 µg/ml final concentration), adrenaline (1 µg/ml) or ADP (1 µg/ml). At the end of each experiment acetylsalicylic acid was added to a final concentration of 0.4 mM to inhibit the activity of platelet cyclo-oxygenase. Thromboxane A_2 released during aggregation was determined by measuring its stable metabolite thromboxane B_2 (4) by radioimmunoassay (20).

RESULTS AND DISCUSSION

Laminin was soluble in both buffers whereas proteoglycan dissolved only in the Tris-HCl buffer. Neither one caused aggregation of platelets nor release of thromboxane B_2 at final concentrations ranging between 2 and 100 µg/ml (Fig. 1A, Table I), regardless of the buffer used. The intact type IV collagen in the phosphate buffer elicited aggregation and consequent thromboxane B_2 release, the reaction being dependent on the amount of collagen added (Fig. 1A and B, Table I). Concentrations below 12 µg/ml did not cause platelet aggregation. The type IV collagen, soluble in 0.05 M Tris-HCl buffer, pH 7.4, did not cause aggregation (not shown). Fibrillar type I collagen induced aggregation at a concentration as low as 2 µg/ml similarly as adrenaline (1 µg/ml) and ADP (1 µg/ml). Preincubation of the platelets with laminin or proteoglycan did not prevent the aggregatory effect of type IV and I collagens.

Interstitial type I and III collagens in fibrillar form are known to be equally potent inducers of platelet aggrega-

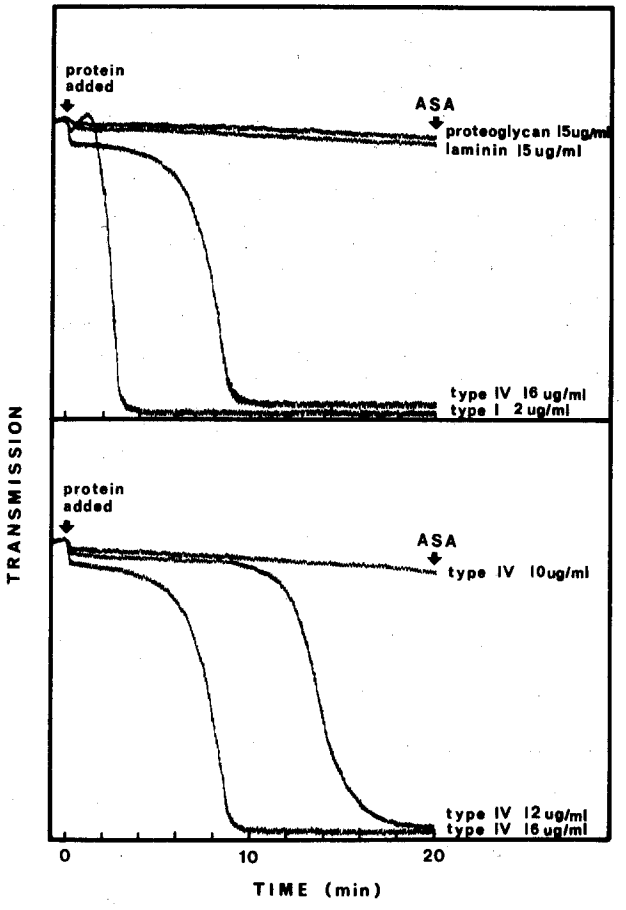

Figure 1. Effect of basement membrane zone proteins, laminin, proteoglycan and intact type IV collagen, and interstitial tissue type I collagen on platelet aggregation. The proteins were dissolved in a 0.02 M sodium phosphate buffer, pH 7.4, at concentrations of 1 mg/ml. Platelet-rich plasma was prepared as described in Materials and Methods and the aggregation was studied in an aggregometer. At the end of each experiment acetylsalicylic acid (ASA) was added to a final concentration of 0.4 mM to inhibit the platelet cyclo-oxygenase activity. (A) Aggregation of platelets following exposure to laminin, proteoglycan and type IV and I collagens. Incubation with laminin or proteoglycan at concentrations up to 100 µg/ml concentration did not elicit aggregation. (B) Aggregation of platelet with varying concentrations of type IV collagen. Concentrations below 12 µg/ml did not elicit aggregation.

tion in vitro (21). In the present study type IV collagen was not as active as type I. This suggests that there are differences between basement membrane and interstitial collagens in inducing platelet aggregation in vivo.

TABLE I

Platelet aggregation and thromboxane B_2 release in platelet-rich plasma following exposure to basement membrane components, type I collagen, adrenaline and ADP[a)]

Agent	Final concentration (µg/ml)	n[b)]	Platelet aggregation	Average thromboxane[c)] B_2 release (pg) / 400 x 10^6 platelets
Laminin	15 - 100	(11)	-	349±53
Proteoglycan	15 - 100	(4)	-	348±51
Type IV collagen	2 - 10	(3)	-	335±53
Type IV collagen	12 - 32	(4)	+	24,888±2,713
Type I collagen	2 - 10	(9)	+	20,520±896
Adrenaline	1	(7)	+	25,581±2,670
ADP	1	(4)	+	20,066±3,897

a) Platelet-rich plasma containing 200 000 to 400 000 platelets per µl was prepared from healthy volunteers using sodium citrate (0.1 volume of 3.8 % w/v) as an anticoagulant. Platelet aggregation was studied at 37°C in an aggregometer. At the end of each experiment acetylsalicylic acid was added to the reaction cuvettes to a final concentration of 0.4 mM to inhibit the activity of cyclo-oxygenase, and the amount of thromboxane A_2 released during aggregation was determined by measuring its stable metabolite thromboxane B_2 (4) by radioimmunoassay (20).

b) n = number of determinations

c) The amount of thromboxane B_2 is calculated as pg per 400 x 10^6 platelets (mean ± SEM).

This study provides the first evidence that components of the subendothelial lamina rara of basement membranes do not cause aggregation of platelets in contrast to collagenous components of the deeper lamina densa or underlying dermis. This indicates that the depth of the injury of the vessel wall ultrastructure is important with respect to the triggering of local platelet aggregation. Accordingly, normal turnover and minor injuries of the antiaggregatory endothelial cell layer of vessel walls will not cause microthrombosis if the lamina rara remains intact. Only when the injury becomes deep enough to allow platelets to reach type IV or V (A,B) collagens or type I and III collagens in the interstitial connective tissue, does platelet aggregation result. This may be important in the etiology of atherogenesis (1). This hypothesis is compatible with studies (22) showing that the "nonthrombogenic" lamina rara becomes thinner and the "proaggregatory" lamina densa thicker with advancing age, when the incidence of atherosclerosis increases.

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