Binding of Ringhals Venom Direct Hemolytic Factor to Erythrocytes and Osmotic Ghosts of Various Animal Species

Previous investigations[1,2] demonstrated that lysis of washed erythrocytes by cobra venoms is produced by the synergistic action of two venom components: a basic protein which is moderately hemolytic by itself and therefore named 'direct lytic factor' (DLF), and the venom phospholipase A (phosphatide-acyl hydrolase EC 3.1.1.4) which is non-hemolytic when applied alone. The DLF enables the venom phospholipase to split the red cell membrane phospholipids, the process resulting in enhanced hemolysis. A similar synergistic action has been demonstrated on platelets[3] and mitochondria[4]. Furthermore, evidence has been provided for the concept that the difference in sensitivity of washed red cells from various animal species to both the lytic and the phospholipid splitting actions of Ringhals venom is primarily a reflection of their susceptibility to the action of DLF[2].

These observations prompted the question as to whether erythrocytes of various animal species bind DLF, and if so, whether the binding is correlated with the particular red cell sensitivity. Guinea-pig, human, and sheep red cells were chosen as showing, in this sequence, decreasing susceptibility to direct hemolysis by Ringhals venom. The DLF fraction was labelled with I^{131} according to the method of TALMAYE et al.[5], and radioactivity determined in a well-type scintillation counter. The I^{131} activity of the labelled DLF varied between 1–3 mμc per μg protein. Protein concentration in the DLF solutions was determined according to the method of LOWRY et al.[6]. Other technical details have been given elsewhere[2].

As seen in Figure 1, binding of the I^{131}-labelled DLF varies with the type of erythrocyte. In contrast to sheep erythrocytes, binding of DLF by guinea-pig and human erythrocytes increases during incubation and is accompanied by progressive hemolysis.

When DLF-coated erythrocytes were repeatedly washed with saline and finally hemolysed by 25 vol of distilled water, about 90% of the total radioactivity was found associated with the sedimented red cell ghosts of each of the three types studied, showing that DLF is bound to the erythrocyte membrane.

The amount of DLF attached to ghosts obtained by osmotic hemolysis (Figure 2) is not time-dependent and is much higher than that attached to the corresponding erythrocytes. Presumably, new binding sites are uncovered by the osmotic hemolysis. Binding of DLF by the three types of osmotic ghosts was found, within the range tested (50 up to 400 μg DLF per ml), to increase linearly with its concentration in the suspension. Figure 3

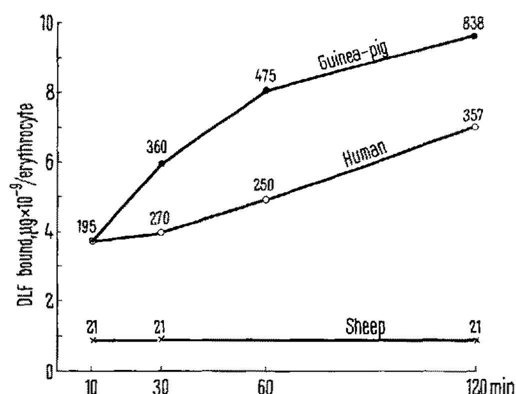

Fig. 1. Binding of DLF by red blood cells of various species. Incubation system: 100 μg of I^{131}-labelled DLF and 0.5 ml packed erythrocytes, brought to a final volume of 2 ml with Tris-buffered saline of pH 7.2, were incubated at 37°C. The amount of DLF bound by the erythrocytes was calculated from the I^{131} activity of 0.5 ml aliquots removed from the incubation system before and after sedimentation of the cells at 800 g. The activities of the cell-free aliquots were subtracted from those of the cell containing aliquots, previously corrected for hematocrit. All determinations were run in four parallel samples. Numbers indicate mg% hemoglobin in the suspending medium.

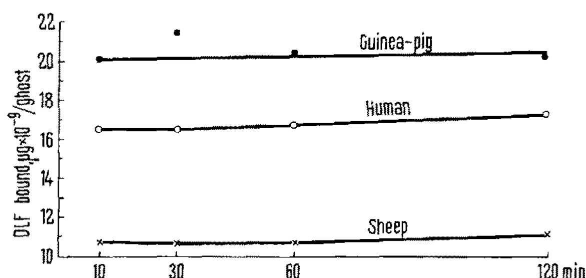

Fig. 2. Binding of DLF by osmotic ghosts. Experimental conditions as in Figure 1, except for using, instead of 0.5 ml packed cells, the corresponding amount of red cell osmotic ghosts. Ghosts were prepared by hemolysis of erythrocytes with 25 vol of distilled water and sedimentation at 14,000 g.

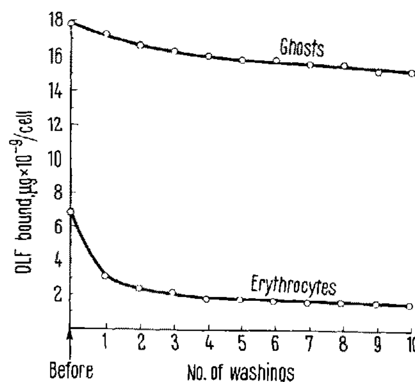

Fig. 3. Effect of repeated washing on the amount of DLF bound to human erythrocytes and osmotic ghosts. Incubation systems as in Figure 1 and Figure 2. After 15 min incubation, erythrocytes or ghosts were sedimented and repeatedly washed with 15 ml of Tris-buffered saline. I^{131} activity was determined on the sediments following each washing. Each value was corrected for the amount of I^{131} activity found in control test tubes containing no cells and washed in the same way.

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shows that, after repeated saline washings, the bulk of the bound DLF remains attached to both human red cells and osmotic ghosts. Similar results were obtained for the guinea-pig and sheep red cells and ghosts.

Calculation of the amounts of DLF bound at the end of 2 h incubation gave $1.2 \cdot 10^{-9}$ µg DLF per 100 μ^2 for the resistant sheep red cells (surface area 67 μ^2 [7]) and $4.3 \cdot 10^{-9}$ µg DLF per 100 μ^2 for the more sensitive human red cells (surface area 163 μ^2 [7]) (data on guinea-pig red cell surface area were not available). In contradistinction, the amount of DLF bound to the ghost surface, $16.6 \cdot 10^{-9}$ µg DLF per 100 μ^2 for sheep and $10.5 \cdot 10^{-9}$ µg DLF per 100 μ^2 for human, bears no relationship to the type of red cell sensitivity.

The above data would fit with the hypothesis that the red cell sensitivity to the direct lytic action of cobra venom is a function of its capacity to bind DLF. It was demonstrated in a previous study[2] that DLF enables phospholipase A to split phospholipids in the osmotic ghosts from sensitive red cells but not in those from the resistant ones. Since the amount of DLF bound per unit surface area to the latter exceeds that to the former, the degree of sensitivity of osmotic ghost phospholipids to DLF must be determined by a particular membrane structure, possibly deriving from different phospholipid composition, strength of phospholipid binding within the membrane, and fatty acid pattern[8–11].

Résumé. Le taux du facteur lytique (DLF) provenant du venin du serpent Ringhals, lié aux érythrocytes de différentes espèces animales, correspond à leur sensibilité à l'action du venin. Cette correspondence n'est plus valable pour les membranes érythrocytaires obtenues par hémolyse osmotique. La capacité de ces membranes de lier DLF est supérieure à celle des érythrocytes intacts. La cinétique de liaison du DLF aux membranes est différente de celle aux érythrocytes intacts.

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Einfluss von Psychopharmaka auf die nichtveresterten Fettsäuren im Blutplasma

Die Bestimmung der nichtveresterten Fettsäuren (NFS) im Blutplasma gibt einen Einblick in die Dynamik des Fettstoffwechsels. Ihre Konzentration ist abhängig vom Ernährungszustand, von den energetischen Bedürfnissen, von nervösen und hormonalen Einflüssen und nicht zuletzt von der verfügbaren Glucosemenge. Insulin, oral wirksame Antidiabetika und Salicylat verbessern die Glucoseverwertung und bewirken eine Abnahme der NFS im strömenden Blut[1,2]. Von blutzuckersteigernden Substanzen mit hemmendem Einfluss auf die Glucose-Utilisation ist dagegen zu erwarten, dass sie eine Zunahme der NFS im Plasma verursachen. Zu dieser Gruppe gehören auch Chlorpromazin, Chlorprothixen und einige andere Psychopharmaka[3,4]. In Versuchen an Ratten und Schweinen haben wir nach Verabreichung von Chlorpromazin tatsächlich einen bedeutenden Anstieg der zirkulierenden NFS festgestellt[1,5]. Diese Beobachtung wurde bestätigt. So fand z.B. HOLLISTER[6], dass Chlorpromazin auch beim Menschen eine beträchtliche Zunahme der NFS im Plasma verursacht. Über den Mechanismus dieser Wirkung ist freilich wenig bekannt. Nachstehend wird über den Einfluss folgender Substanzen auf die Konzentration der NFS im Blutplasma von Ratten berichtet: Promazinphosphat, Chlorpromazin · HCl, Chlorpromazinsulfoxid · HCl, Triflupromazin · HCl, Chlorprothixen · HCl, Chlorprothixensulfoxid · HCl, Prothipendyl · HCl Hydrat, Haloperidol, Imipramin · HCl, Amitriptylin · HCl, Chlordiazepoxid · HCl, Äthyltryptaminacetat (D,L-α-Äthyltryptaminacetat, Etryptamin), «Tradon-L» (2-Diäthylamino-4-oxo-5-phenyl-oxazolin) und Pipradol · HCl.

Als Versuchstiere dienten ausgewachsene männliche und weibliche Wistar-Ratten. Die in Wasser gelösten Substanzen wurden intramuskulär injiziert (2 ml/kg). Unmittelbar vor und 2 bzw. 4 h nach der Injektion wurden Blutproben aus einer Zungenvene der nicht narkotisierten Tiere entnommen. Die Bestimmung der NFS in 0,2 oder 0,5 ml Plasma erfolgte mit einer kolorimetrischen Methode[7]. Die Ergebnisse wurden statistisch bearbeitet (Student's *t*-Test).

Tabelle I zeigt, dass die untersuchten Pharmaka mit Ausnahme von Chlorprothixensulfoxid, Imipramin, Pipradol und Phenobarbital eine signifikante Zunahme der NFS im Plasma verursachen. Zur näheren Charakterisierung dieser Wirkung wurden einige zusätzliche Versuche mit Chlorpromazin (und Chlorprothixen) durchgeführt. Zunächst stellte sich heraus, dass Chlorpromazin keine weitere Vermehrung der NFS im Plasma bewirkt, wenn ihre Konzentration schon zu Versuchsbeginn durch Hunger oder Vorbehandlung mit Reserpin erhöht war (Tabelle II). Weiterhin wurde geprüft, ob sich die Wirkung der

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