A study of erythrocyte membrane proteins and urinary polypeptides in conga drumming haemoglobinuria

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Summary

1. The erythrocyte membrane proteins and glycoproteins and urinary polypeptides have been examined in a patient exhibiting intermittent pigmenturia associated with conga drumming.

2. Significant excretion of haemoglobin, albumin and probably erythrocyte carbonic anhydrase but not myoglobin occurred during the acute phase of the conga drumming-induced pigmenturia. This usually ceased within 24-48 h.

3. We found no evidence of aberrant erythrocyte membrane components on electrophoresis with either protein staining or a range of labelled lectins used for detection.

Key words: conga drumming pigmenturia, erythrocyte membrane, haemoglobinuria, march haemoglobinuria.

Introduction

An instance of drumming-induced haemoglobinuria was recorded by Kaden [1] in a man who had previously indulged in running with no ill effects. Similar cases have been described [2-4]; one presented with haemoglobinuria and myoglobinuria after conga drumming [2] and another with haemoglobinuria after karate hand exercises [3]. A third group presented with haemoglobinuria after playing Basque tennis and striking the pelota with the hand [4].

Very little is known about the cause of the haemolysis in drumming or sport-induced haemoglobinuria and it is usually ascribed to traumatic mechanical damage of the erythrocytes, perhaps associated with some unidentified, intrinsic, erythrocyte fragility. Studies made on cases of march haemoglobinuria [5, 6], a phenomenon associated with running and walking, may throw some light on this problem. SDS-polyacrylamide gel electrophoresis of erythrocyte membranes revealed that a component of molecular size 29 000 was absent in three patients [6]. The exact mechanism by which this defect leads to the haemolysis is not clear, but the observations point to the involvement of some anomalous membrane structure in these cases.

We have studied a patient who exhibited haemoglobinuria after conga drumming and dancing. Because of the tentative suggestion that there is an association between structurally altered membrane components and membrane fragility we have paid particular attention to the analysis of erythrocyte membrane proteins and glycoproteins.

Methods

Patient

In 1976 a 43 year old Ghanaian professional musician, resident in the U.K. since 1963, developed pigmenturia half an hour after energetic nightclub dancing. There was no associated myalgia and the urine was macroscopically normal after 12 h. A year later, after conga drumming for 1-4 h, pigmenturia recurred and there were further episodes associated with drumming at 3-4 months intervals. During the next 3 years the patient could recall only four instances when his drumming
had a history of extrinsic asthma with a positive skin test for grass pollen. As an adult he had received penicillin treatment for a venereal disease.

He presented for investigation of 'haematuria'. Blood and urine were sampled for routine clinical tests and the patient was investigated by abdominal and chest X-rays and an intravenous urogram. A deliberate experiment during which the patient drummed vigorously for 1 h was also carried out. Blood and urine were collected before, during and after drumming and were analysed for urinary protein and erythrocyte membranes.

**Biochemical methods**

Urine samples were concentrated between two- and ten-fold by using an Amicon pressure ultrafiltration apparatus. Erythrocyte membranes were prepared by the method described by Fairbanks et al. [7]. The membranes and urines were analysed by SDS-polyacrylamide gel electrophoresis in gradient (5-15%) polyacrylamide gels [8]. The carbohydrate-containing components were visualized by probing with 

\[ ^{125}I \text{-labelled lectins and autoradiography} \]

[9]. Eleven lectins with a range of specificities were employed (complete details are given by Goldstein & Hayes [10]). [Lectin agglutinin, principal specificity: concanavalin A \( \alpha \)-d-mannose; Lens culinaris \( \alpha \)-d-mannose; wheat germ sialic acid \( \beta \)-d-GlcNAc; Ulex europaeus I \( \alpha \)-L-fucose (group O-specific); soybean \( \alpha \)-d-GalNAc; Helix pomatia \( \alpha \)-d-GalNAc (group A-specific); peanut \( \beta \)-d-Gal(1,3)-d-GalNAc; Ricinus communis I \( \beta \)-d-galactose; Phaseolus vulgaris erythro-\( \beta \)-d-Gal -(1,4)GlcNAc; Sophora japonica \( \beta \)-d-GalNAc; succinylated wheat germ \( \beta \)-d-GlcNAc.]

In some experiments the membrane preparations were treated with a bacterial sialidase (\( Clostridium perfringens \) type VI) [11].

The isoenzymes of glucose 6-phosphate dehydrogenase (G6PD), lactate dehydrogenase (LDH) and phosphogluconate dehydrogenase (PGD) in the erythrocyte lysate were analysed by starch gel electrophoresis by using the electrophoretic systems and staining procedures given by Harris & Hopkinson [12]. The haemoglobin content of urine samples was determined by the cyanmethaemoglobin method [13].

Serum haptoglobin was assayed by rate reaction nephelometry.

**Results**

Chest and abdominal X-rays and the intravenous urogram were normal. Levels of serum electrolytes including calcium and phosphate, urea, creatine kinase, liver function enzymes, albumin, transferrin and haptoglobin in the samples taken at the time the patient presented with haematuria were all normal. Qualitative electrophoresis showed that the serum haptoglobin was type 2 and the transferrin type C. The platelet count was normal and there was no evidence of sickle haemoglobin trait. There were no atypical antibodies on blood group screening and he was blood group O, Rh positive. The TPHA and FTA-Abs tests were weakly positive, suggestive of previous treponemal infection. The isoenzyme patterns and relative activities of erythrocyte glucose 6-phosphate dehydrogenase, lactate dehydrogenase and phosphogluconate dehydrogenase were indistinguishable from control samples.

Comparison of the polypeptide profiles of the patient's urine with molecular weight standards, samples of purified myoglobin and extracts of soluble muscle protein (Fig. 1a) showed that the pigment in the urine was entirely attributable to haemoglobin. None of the major soluble muscle proteins including myoglobin were observed. However, certain unusual proteins were seen in these samples, which presumably derived from erythrocytes. One of these is carbonic anhydrase (CA), a major polypeptide of 29 000 (marked with an asterisk in Fig. 1a), which occurs at appreciable levels in adult erythrocytes (CA I 11.6 ± 2.3 mg/g of haemoglobin and CA II 1.8 ± 0.3 mg/g of haemoglobin) and next to haemoglobin is the most prominent erythrocyte protein [14]. It is unlikely that the carbonic anhydrase component derives from the kidney tubules since only CA II (0.6-1.3 mg/g of tissue) is found in this tissue and the enzyme is not a prominent component of the kidney soluble protein profile [15]. Significant quantities of serum albumin were also detected. The Tamm-Horsfall (TH) urinary polypeptide was not a major feature of the pigmented urine polypeptide profile. The urinary haemoglobin concentration reached a maximum of 6.5 g/l 2 h after drumming and fell to 0.069 g/l 24 h after drumming.

Haemoglobinuria was not induced by deliberate drumming exercise performed by the patient for 1 h, but some haemolysis was indicated by the presence of large amounts of carbonic anhydrase in samples collected immediately after the exercise and 40 min later (Fig. 1b). Although serum haptoglobin levels were within the normal range during the entire period they fell from 1.1 g/l before drumming to 0.4 g/l 90 min after the exercise. This decrease in serum haptoglobin, although not marked, is consistent with the binding and clearance of haemoglobin via the liver and presumably accounts for the absence of
FIG. 1. (a) Polypeptide profiles of urine from the patient, and of control specimens after SDS-polyacrylamide gel electrophoresis of urine samples. Patient’s urine: 1, obtained during an acute bout of pigmenturia associated with conga drumming; 2, 3, obtained during symptom-free follow-up 24 and 48 h later. At 30 min intervals: 4, before, 5, 6, during, and 7, 8, after the deliberate drumming exercise. The polypeptides indicated by * and arrowed are erythrocyte carbonic anhydrase and haemoglobin respectively. TH, Tamm-Horsfall protein; RBC, erythrocyte. (b) and (c) Erythrocyte membrane preparations from the patient and various control specimens separated on SDS-polyacrylamide gels and stained with Coomassie Blue (b) or 125I-labelled peanut agglutinin (c). + and − indicate specimens treated and not treated with bacterial sialidase respectively. *Components showing minor variation.
free haemoglobin passing through the kidney (normal range 0.3-1.9 g/l). Serum creatine kinase was normal at the start (80 units/l) but was elevated about two-fold during and after the exercise (up to 195 units/l; normal range 10-120 units/l). The urinary polypeptide profile was normal in specimens collected 5 h after drumming. Analysis of the erythrocyte membrane protein profiles revealed the same polypeptide patterns in the patient and controls. In particular there was no evidence that any of the components in the M₄ 25 000-35 000 range were weak or absent, as described in three cases of march haemoglobinuria by Banga and colleagues [6]. A minor difference in components of molecular size of about 150 000 was found (Fig. 1b). The patient appears to have a double band, whereas the control samples (n = 4) have only a single component of this size. Analysis of the membrane glycoproteins with a range of ¹²⁵I-labelled lectin probes did not reveal any major differences between the patient and control (n = 4) samples, apart from some minor variation in the mobility and intensity of certain components, which is probably within the range of normal sample to sample variation [9].

Discussion

The case reported here exhibits intermittent haemoglobinuria associated with the physical stress of conga drumming and other vigorous activity. It is noteworthy that experimental drumming did not completely reproduce the proteinuria experienced previously and that the patient felt that the experimental conditions failed to engender the same psychological responses as the live performance. Detailed analysis of the polypeptide profiles or urine samples showed that the pigmenturia was exclusively attributable to haemoglobin. There was no evidence of severe skeletal muscle damage and associated myoglobinuria and this case is therefore unlike that reported by Furie & Penn [2]. Furthermore, protein analyses and lectin probing techniques have shown that this patient is quite distinct from those cases of march haemoglobinuria reported by Banga et al. [6], which showed anomalous erythrocyte membrane proteins. Thus it appears that the exertional haemoglobinurias represent a heterogeneous collection of conditions, some of which are probably of genetic origin and others may be secondary to an acquired disorder.

Many of the erythrocyte membrane proteins are hydrophobic and insoluble and methods for their analysis are largely restricted to procedures such as SDS-polyacrylamide gel electrophoresis, which solubilize and denature the proteins. These studies would reveal the absence of deficiency of erythrocyte membrane polypeptides or any change in their molecular size or glycosylation. However, more subtle alterations such as amino acid substitutions and losses of small numbers of residues which could drastically affect membrane fragility, would not be detected.

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References