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In Vitro Haemolysis from Adrenochrome in the Blood of Schizophrenic Patients, Revised

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Summary. The recently reported abnormal in vitro haemolysis from catecholamine metabolites in schizophrenia was tested. We did not find the postulated increase in haemolysis, though the exchange of plasma for Ringer solution renders some patients' erythrocytes more susceptible to the effects of adrenochrome. The possibility of a subgroup of schizophrenia with a defect in membrane stability is discussed.

Key words: Adrenochrome – Haemolysis – Schizophrenia

Zusammenfassung. Die früher beschriebene erhöhte Hämolyse im Blut schizophrener Patienten durch Katecholaminmetaboliten wurde nachgeprüft. Wir konnten die erhöhte Hämolyse nicht bestätigen, allerdings schienen nach Austausch des Plasmas durch Ringer-Lösung die Erythrozyten der Patienten etwas anfälliger gegen den Adrenochromeinfluß zu sein. Die Möglichkeit einer Störung der Membranstabilität in einer Untergruppe von Schizophrenen wird diskutiert.

Schlüsselwörter: Adrenochrom – Hämolyse – Schizophrenie

Introduction

Hegedus and Altschule described how rheomelanins are produced from catecholamines in the blood plasma [1]. The authors mentioned that the erythrocytes of many chronic schizophrenic patients were exceedingly susceptible to the haemolytic effects of catecholamine derivatives [3]. They developed a method which allowed them to distinguish between an increased in vitro haemolysis in chronic schizophrenic patients and the haemolysis in the blood of controls [4].

We have tested this method by examining a group of our patients and a control group, and then by studying the influence of plasma substitution by Ringer solution in our incubation samples.

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Materials and Methods

Adrenochrome was supplied by Sigma, all other testing reagents by Merck. Ringer solution was prepared as follows: 0.8 g NaCl, 0.02 g KCl, 0.02 g $CaCl_2 \times 2\,H_2O$, 0.1 g Na_2CO_3 were filled up to 100 ml with demineralized water, left for a day at 4° C, and precipitated calcium carbonate was decanted.

Venous blood was taken with plastic syringes, and each 10 ml were mixed in 50 ml Erlenmeyer flasks with 0.1 ml of 25% potassium oxalate and gently shaken. The following samples were incubated in a shaker incubator for 24 h at 38° C:

1. Blood, without further manipulations.
2. Blood, the plasma of which was substituted for by Ringer solution; 10 ml blood were centrifuged for 15 min at 1500 *g*. The plasma plus buffy coat were removed, substituted for by Ringer, and the suspension was carefully stirred with a glass stick. The samples were designated 'Ringer-substituted.' More, or less, stirring did not influence haemolysis.
3. Blood was again centrifuged as in 2. and Ringer solution substituted for the supernatant. After mixing, further centrifuging, and repeated substitution of Ringer solution for the supernatant, and mixing again, the samples were used as 'washed samples.'

Two methods were used for incubation:

a. 5 ml blood and suspension, respectively, were shaken in an Erlenmeyer flask under sterile conditions at a frequency of 92/min and a displacement of 2.5 cm. The 'blanks' contained no adrenochrome, the test samples 2.0 mg adrenochrome.
b. 2 ml blood were pipetted into centrifuge tubes under sterile conditions and shaken at a frequency of 100—104/min and at a displacement of 2.5 cm. To the blanks, 0.4 ml of Ringer were added, to the test samples 0.8 mg adrenochrome in 0.4 ml ringer solution.

After incubation, 3 ml of the samples prepared according to a. and 2 ml of the samples according to b. were centrifuged for 20 min at 1500 *g*. Of the supernatant of each sample, we diluted 0.01 ml with 3 ml water, then measured with a Zeiss PMQ2 spectral photometer at 280 nm and 400 nm wavelength, using 1 cm quartz cuvettes. For a. the values were run as single test, for b. we designed a double test.

To analyze the haemolysis of blood from membrane-damaging agents other than adrenochrome, succinic anhydride and sodium metaperiodate were placed into centrifuge tubes and, after adding 1 ml oxalate blood, shaken for 24 h. Succinic anhydride was dissolved in chloroform. The respective amount was placed into test tubes and the chloroform evaporated. Sodium metaperiodate was used, dissolved in 20 µl water. The respective blank consisted only of 20 µl water. We used 0.5 and 1.0 mg of succinic anhydride, and 0.1 and 0.2 mg sodium metaperiodate, respectively. The following neuroleptics were studied in how much they protect membranes against succinic anhydride and sodium metaperiodate: chlorpromazine, haloperidol, fluspirilene, fluphenazine, and prothipendyl, in concentrations of about 10^{-7} M, 10^{-6} M, and 10^{-5} M.

Our patients were in-patients. According to the evaluation of at least two independent psychiatrists they were diagnosed schizophrenic, showing inappropriate affect, looseness of associations, or personality disorders according to Bleuler or first-rank symptoms according to Schneider. This group comprised chronic as well as acute forms of schizophrenia, with a course of between 2 months and 40 years. All of them were on neuroleptics. A total of 62 schizophrenic patients participated in the study; 38 were males, 24 females, aged between 17 and 61, with a median of 35 years; 39 were patients in our hospital, 23 in the psychiatric division of the Allgemeines Krankenhaus Hamburg-Ochsenzoll. Blood of three patients was run both in 5 and 2 ml assays. We defined chronic schizophrenia on the basis of two different guiding principles: Psychoses of more than 3 years duration, whereby this point of separation was chosen arbitrarily ('chronic 1'). Psychoses without florid signs of the illness, e.g., without vivid hallucinations, restlessness, or catatonic behaviour. That means these patients were not *symptomproduktiv* at the time of blood drawing ('chronic 2'). The control group consisted of staff members and blood donors with no history of mental illness. The groups were comparable in age and sex.

Results

Using Hegedus' method [4] for the incubation of the blood with adrenochrome, taking 5 ml blood without any manipulation (method 1.a.), or 2 ml blood (method 1.b.), we did not find any difference between patients and controls at 280 and 400 nm wavelength. In our graphs (Figs. 1 and 2, left columns), we depicted only the absorbances at 400 nm, which are supposed to represent mainly the products of haemolysis. The cases which were diagnosed as being chronic in two different aspects likewise revealed no significant difference from the control group. The readings scattered between very high and very low values in both methods and showed the same distribution as those of the acute psychoses. Substituting Ringer solution for plasma (method 2.a. and b.) there was a remarkable difference (Figs. 1 and 2, middle columns). Some of the patients' marks were considerably higher than those of the control group. Statistically, the mean values were significantly different ($P<0.001$ in method 2.a. and $P<0.01$ in method 2.b.;

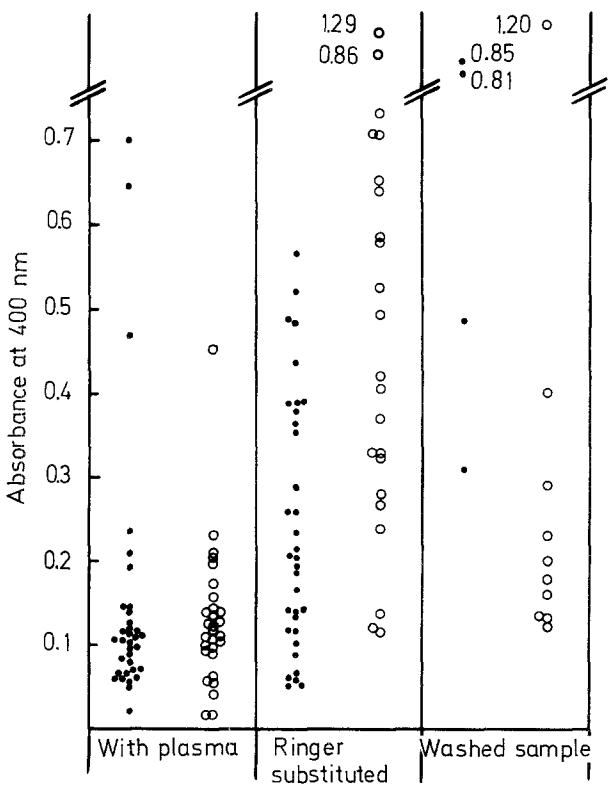

Fig. 1. Absorbance values obtained with blood from a control group and schizophrenic patients. Plotted are the readings after subtraction of blanks of 5 ml assays with adrenochrome. ● = control. ○ = patients. *Left column:* Control $n=34$, patients $n=29$ (16 'chronic 1', 15 'chronic 2'). *Middle column:* Control $n=34$, patients $n=23$ (13 'chronic 1', 14 'chronic 2'). *Right column:* Control $n=18$, patients $n=14$ (7 'chronic 1', 5 'chronic 2'). For washed samples are plotted values with low blanks only

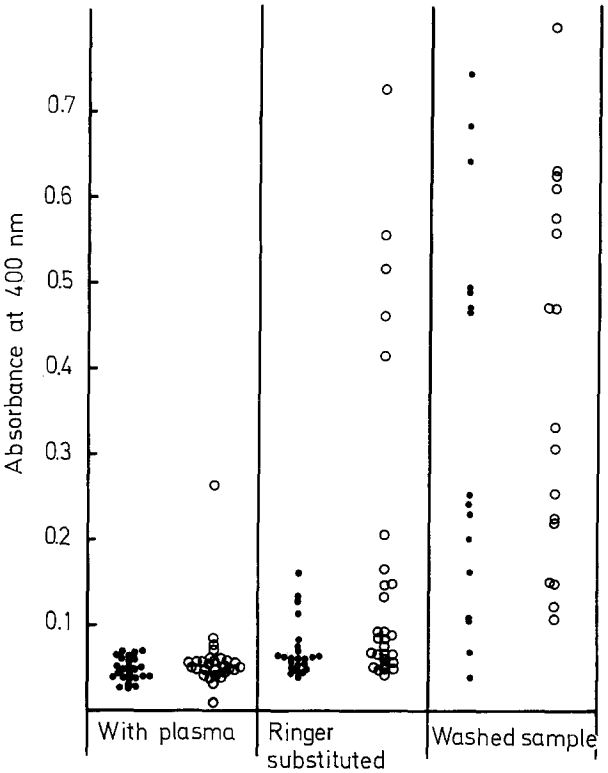

Fig. 2. Absorbance values obtained with blood from a control group and schizophrenic patients. Plotted are the readings after subtraction of blanks of 2 ml assays with adrenochrome. • = control. o = patients. *Left column:* Control $n = 25$, patients $n = 36$ (27 'chronic 1', 22 'chronic 2'). *Middle column:* Control $n = 23$, patients $n = 28$ (21 'chronic 1', 15 'chronic 2'). *Right column:* Control $n = 24$, patients $n = 30$ (18 'chronic 1', 14 'chronic 2'). For washed samples are plotted values with low blanks only

U-test of Wilcoxon, Mann and Whitney). Of the blind values, 80% were accumulated in a bulk, whereas the rest showed some haemolysis. The difference between the groups was even more evident in the case of those 80% of the values with low blanks. The values of the patients were not correlated with age, sex, duration of illness, or acuteness, nor with the quantity of neuroleptics applied.

Method 3.a. and b.—washing of the erythrocytes with Ringer solution—did not show a great difference between patients and controls, because the results were inconsistent. Sometimes all control results were higher than patients' results, partly the reverse happened. However, if control and patients' values run in the same assay are compared, patients' values more often exceed the absorbance of the controls. With increasing blanks, haemolysis from adrenochrome becomes more extensive. If the erythrocytes have been damaged before, the effects of adrenochrome are intensified, so that a potential difference between patients and controls cannot be found. Regarding only the values with low blanks, no difference between patients and controls can be shown (Figs. 1 and 2, right columns).

Haemolysis from succinic anhydride and sodium metaperiodate was not any different in patients and controls. The drugs did not show a protective effect against succinic anhydride, whereas there seemed to be a slightly protective effect against sodium metaperiodate.

Discussion

Hegedus et al. have described a dose-dependent haemolysis from adrenochrome [4]. They suppose that there is a chemical mechanism that protects the erythrocytes of healthy people against the haemolytic effects of rheomelanins more effectively than in schizophrenics. Using this method we did not find a difference between the control group and the schizophrenics. Substituting Ringer for most of the plasma, however, resulted in a significant difference, but even then most patients' results were in the same range as those of the control group.

As all patients were receiving neuroleptics we could interpret our findings as follows: Removal of the plasma diminishes the drug effect which prevents the haemolysis that we actually expect. Also, Hegedus et al. say that, in their results (without giving further details), patients on phenothiazines are closer to the control group [4].

If the production of a rheomelanin in the plasma is responsible for the haemolysis, removing the plasma should result in a general decrease of haemolysis. Our findings (cf. left with middle columns in Figs. 1 and 2) prove that this is not the case. It seems improbable that the membranes of the erythrocytes should be less stable in patients: in contact with membrane-damaging agents like succinic anhydride and sodium metaperiodate, the blood samples of controls and patients read the same, no matter which of our methods we use.

The reported findings allow different models of interpretation to be tested in further studies. For instance, there could be a catecholamine transport system in the membranes of the erythrocytes, which by binding of aminochromes is changed so that the membrane stability is affected. The capacity to bind aminochrome could be slightly higher in some schizophrenics and thus cause the higher rate of haemolysis. Hegedus and Altschule say that normally a little less than 50% of a set amount of 4 mg adrenolutin per 10 ml blood are bound by precipitation with trichloro-acetic acid, whereas the percentage is higher in schizophrenics [2]. Minimal amounts of aminochrome from the breakdown of catecholamines could be bound too massively in the brains of schizophrenics, thus destroying the membranes in the long run. Membrane functions being restricted, an increased liberation of catecholamines and further production of aminochrome would lead to a snowballing effect. Psychotropic drugs competing with adrenochromes would prevent this instability of the membranes. These concepts about a vicious circle would help to explain the relationship between endogenous and environmental factors in schizophrenia in the light of a stress-catecholamine concept. The radicals originating from the catecholamines can combine with many biological macromolecules, thus transforming them into antigens which excite the immune system. Findings of auto-antibodies in schizophrenics, as discussed by Matthysse and Matthysse [6], could be explained by this theory.

If findings concerning the dopamine hypothesis of schizophrenia [5] were, in a subgroup, the result of a slightly altered aminochrome metabolism, erythrocytes would be a suitable object in which to study the underlying disorders in this subgroup of schizophrenics.

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