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Differences in Haemolytic Action of Hg^{2+} in Relation to Its Concentration and Ionic Strength of Incubating Solutions

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Summary

The haemolytic action of different concentrations of $HgCl_2$ on rat red blood cells (RBC) was studied *in vitro*. The concentrations of $HgCl_2$ in incubating media were 0.15, 0.25 and 0.50 mmol.l^{-1}. The ionic strength of the media varied from 0 to 154 mmol.l^{-1} NaCl. Isotonicity of solutions was also compensated using isotonic glucose in different concentrations (287 – 0 mmol.l^{-1}). Osmolarity of solutions varied from 287 to 308 mOsm. Besides these solutions the haemolysis in Krebs-Ringer solution was also studied. Haemolysis was characterized with two maxima in all concentrations of Hg^{2+}. The first maximum was observed at low ionic strength and the second one at high ionic strength. In relation to the increased concentrations of Hg^{2+}, the first maximum of haemolysis progressively declined towards the higher ionic strength. In the Krebs-Ringer solutions, the increased concentration of Hg^{2+} was followed by reduced haemolysis. The haemolytic concentration of 0.15 mmol.l^{-1} was found to be optimal.

Key words

Erythrocytes – $HgCl_2$ – Haemolysis – Ionic strength

Introduction

The mechanism of the haemolytic action of mercury ions has been intensively studied. It was found that mercury compounds influence the transport of ions, water and other nonelectrolytes in different cells (Nordlin 1990). The target organelle of mercurials is the plasma membrane (Brookes 1988). Active and passive transport processes of ions and water are closely connected with membrane enzymes activity. Their inhibition by Hg^{2+} ions suppresses or totally blocks membrane transport. The most sensitive enzymes are those which contain –SH groups (Rothstein 1970, Sokol *et al.* 1981). In consequence, active ion and water transport across the membrane is blocked (Macey and Farmer 1970, Sha'afi and Feinstein 1977). However, the passive permeability of ions and water remains unchanged and results in colloid-osmotic haemolysis (Pasow 1964). The haemolysis can occur using organic as well as inorganic mercury compounds (Benga 1988).

Because the intensity of membrane protein inhibition depends on the Hg^{2+} ion concentration, this study was carried out to establish its optimal haemolytical concentration.

Material and Methods

Blood from the tail vein was used for *in vitro* experiments. Each group consisted of 15 male rats of the Wistar strain, 3 months old. Fifteen μl of whole blood were added into 3.0 ml of incubating medium and incubated for 60 min at 37 °C. The incubating solutions were isotonic and contained standard amounts of NaCl and isotonic glucose. Concentrations of NaCl were as follows: 0.0, 15.4, 30.8, 46.2, 61.6, 77.0, 92.4, 107.8, 123.2, 138.6 and 154.0 mmol.l^{-1}. The concentrations of glucose were: 287.0, 258.3, 229.6, 200.9, 172.2, 143.5, 114.8, 86.1, 57.4, 28.7 and 0.0 mmol.l^{-1}. Besides this, haemolysis was also carried out in the Krebs-Ringer solution. Its composition was as follows (in mmol.l^{-1}): NaCl 119.48, $CaCl_2$ 0.65, $MgSO_4$ 2.26, Na_2HPO_4 16.39 and glucose 5.5. Three different concentrations of $HgCl_2$ were used: 0.15, 0.25 and 0.50 mmol.l^{-1}. After incubation, the samples were centrifuged for 15 min at 700xg. Haemolysis was measured spectrophotometricaly at 540 nm. The results were expressed in percentage of the haemolysis occurring in distilled water. The results were expressed as the means ± S.D.

Results

The results of the haemolytic action of $HgCl_2$ in all three concentrations can be seen in Fig. 1. At the concentration of 0.15 mmol.l^{-1} $HgCl_2$ (full triangles) in isotonic glucose haemolysis occurred around 45 %. In the concentrations between 15.4 to 61.6 mmol.l^{-1} NaCl haemolysis was substantially higher (above 82 %). But in the concentrations between 77.0 to 107.8 mmol.l^{-1} NaCl haemolysis became lower. The haemolysis again increased in concentrations from 123.2 to 154.0 mmol.l^{-1} NaCl. In last two concentrations of NaCl haemolysis was greater than 85 %.

The haemolytic action of 0.25 mmol.l^{-1} $HgCl_2$ (open squares) in isotonic glucose is lower in comparison to the haemolysis in 0.15 mmol.l^{-1} $HgCl_2$ (15 % on the average). When the concentration of NaCl was raised, haemolysis became higher and the maximum was observed between 46.2 to 77.0 mmol.l^{-1} NaCl. The further course of the haemolysis was similar as in 0.15 mmol.l^{-1} $HgCl_2$.

In the concentration of 0.50 mmol.l^{-1} $HgCl_2$ (asterisks), the haemolysis is substantially different in comparison with the first two concentrations of $HgCl_2$. Lowest haemolysis was observed in solutions from 0.0 to 46.2 mmol.l^{-1} NaCl. The maximum of haemolysis clearly declined at higher concentrations of NaCl (77.0-92.4 mmol.l^{-1}). Low haemolysis was found between 107.8 to 123.2 mmol.l^{-1} NaCl. In the last two concentrations of NaCl (138.6-154.0 mmol.l^{-1}), haemolysis did not show differences in comparison with the two foregoing haemolytical curves.

The haemolysis in Krebs-Ringer solution shows different intensity in relation to the concentration of $HgCl_2$. Haemolysis was very high (around 80 %) in the lowest concentration (at 15 mmol.l^{-1}). However, in higher concentrations (0.25 and 0.50 mmol.l^{-1}) haemolysis significantly decreased. In the highest concentration of $HgCl_2$ haemolysis was minimal (10 % on the average).

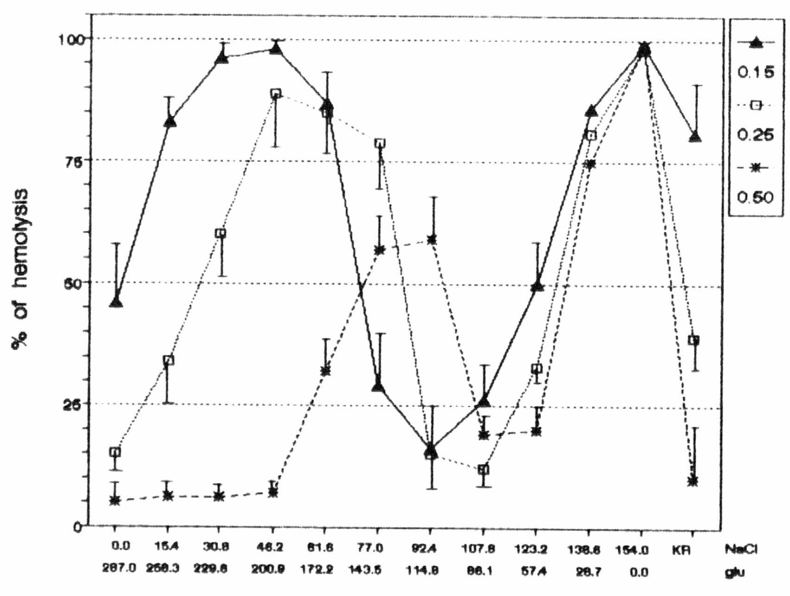

Fig. 1
The haemolytic action of different concentrations of $HgCl_2$ in relation to the concentrations of NaCl (ionic strength) and glucose. *Ordinate:* haemolysis in %. *Abscissa:* concentrations of NaCl and glucose (in mmol.l^{-1}) Full triangles: 0.15 mmol.l^{-1} $HgCl_2$; open squares: 0.25 mmol.l^{-1} $HgCl_2$; asterisks: 0.50 mmol.l^{-1} $HgCl_2$.

Discussion

Colloid-osmotic haemolysis as a consequence of Hg^{2+} ions at various ionic concentrations makes it possible to determine the biophysical state of the membrane skeleton from integral membrane proteins. In isotonic solutions with a low ionic strength, the spectrin-actin complex reveals the degree of lability or dissolution (Pinder *et al.* 1992). Under these conditions we observed the first maximum of haemolysis. Increased ionic strength of the incubating medium resulted in a cellular overload with ions and water.

After reaching the critical volume haemolysis of the cells occurs. The consequence of this event is the second maximum of haemolysis.

At elevated concentrations of Hg^{2+} ions their haemolytical action is different. At higher concentrations (0.25 and 0.50 $mmol.l^{-1}$ $HgCl_2$) the first maximum of haemolysis declined in relation to the higher concentrations of NaCl. This fact could be explained on the basis the solubility effect of the spectrin-actin complex and then by the antagonizing effect on the denaturation activity of $HgCl_2$. The higher solvatation which is connected to the increased NaCl concentration, diminished denaturation and therefore enhanced haemolysis. On the other hand, the second maximum of haemolysis which decreased with increasing Hg^{2+} concentration could be explained on the basis of a direct denaturating effect of Hg^{2+} ions on integral membrane proteins and diminution of haemolysis. A similar decrease of haemolysis was also observed in Krebs-Ringer solution.

These experiments show that the optimal haemolytic concentration of $HgCl_2$ was 0.15 $mmol.l^{-1}$ at which concentration the first and second haemolytic maxima could be clearly distinguished in relation to the ionic strength.

Application of the described method in clinical research brought about very interesting results. In patients after acute cerebral ischemia, the haemolytic action at the first maximum as well as at the second one was significantly lower in comparison with healthy donors (unpublished data).

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