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Fluidity gradient of erythrocyte membranes in diabetics: the effect of resorcylidene aminoguanidine

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Abstract

We estimated in vitro membrane fluidity gradient in erythrocytes (RBC) from diabetic patients, using a fluorescent dye 1,6-diphenyl-1,3,5-hexatriene (DPH). The rate constant of DPH incorporation (k) into the membranes was determined by fitting experimental data to an exponential equation. Four important findings were made. First, membrane fluidity in the hydrocarbon region of RBC from diabetic patients is decreased compared with control cells ($P<0.01$). Second, the rate constant k of DPH incorporation into the membranes of RBC from diabetic patients was lower ($P<0.01$), which indicates an altered fluidity gradient in the membranes. Third, resorcylidene aminoguanidine (RAG) decreased significantly ($P<0.001$) the anisotropy values in RBC membranes from diabetic patients, which means that it apparently acted as a fluidizing agent. Lastly, no significant differences in the rate constants k were found between the control membranes (from RAG untreated RBC) and the membranes isolated from RAG pretreated blood from diabetic patients, as well as between the control membranes and those from RAG pretreated control blood. In conclusion, RAG affects lipid–protein interactions in RBC membranes, which results in membrane lipid bilayer fluidization and leads to the restoration of natural physiological membrane dynamic parameters in RBC from diabetic patients.

Keywords: Diabetes mellitus; Membrane fluidity; Diphenylhexatriene; Resorcylidene aminoguanidine

1. Introduction

Diabetes is characterized by numerous alterations in cell membrane properties, such as enhanced rigidity, permeability for cations, and transmembrane potential in its absolute magnitude [1–4]. It has been occasionally pointed out in some studies concerning RBC and platelet membranes from diabetic patients that the alterations in membrane fluidity depended upon the depth of membrane lipid bilayer [5], and that such a peculiar gradient in membrane lipid fluidity might underlie some phenomena crucial for cell membrane functioning, like transmembrane transport or triggering of signal transduction [6]. Such reasoning was challenging, not only because it has been briefly evidenced by the use of some modulators of membrane fluidity [6], and more even so, because it prompted the search for new potential therapeutic agents [2,7,8].

In the present study, we focused on the use of one selected aromatic aminoguanidine derivative: RAG. This compound has been shown to reduce membrane rigidity [2,9,10], hyperpolarization [4], and lipoperoxidation [7]. Moreover, RAG has been claimed to also have anti-glycation activity, which justifies the interest in RAG in prevention of late diabetic sequelae [2,8,9]. In the present study, we further explored the idea of the pharmacological modulation of the dynamic properties of RBC membranes in diabetes mellitus. Our particular aim was to characterize the observed rigidity of these membranes in more detail, i.e. in terms of the fluorescence anisotropy decay of DPH.

2. Experimental

Aliquots of whole blood from diabetic patients ($n=28$, type 1 and type 2 diabetes mellitus) and healthy donors ($n=10$) were incubated with resorcylidene aminoguanidine (2,4-dihydroxybenzaldehyde aminoguanidine, RAG·HCl) at the concentration of 0.25 mmol/l for 30 min at 37 °C. The

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samples were then extensively washed with cold phosphate-buffered saline (0.15 mol/l NaCl, 1.9 mmol/l NaH_2PO_4, 8.1 mol/l Na_2HPO_4; pH 7.4) and subjected to a moderate haemolysis in cold hypotonic buffer (10 mmol/l Tris·HCl; pH 7.4) in order to prepare RBC membrane ghosts.

The degree of DPH fluorescence anisotropy, which is in reciprocal proportion to membrane lipid fluidity, was measured in isolated erythrocyte membranes at room temperature with the Specord M-40 spectrophotometer. DPH was added to membrane suspension to the final concentration of 10^{-6} mol/l [9,10].

The incorporation of DPH into the membranes was characterized by fluorescence anisotropy decay in the 1–60 min range. The experimental data were fitted to an exponential curve using the least square method [11]. The rate constant of DPH incorporation (k) was calculated according to the equation:

$$r(t) = r + (R_0 - R_S)\exp(-kt)$$

where t is the time measured from the DPH addition, R_0 and R_S are respectively the minimized values of the fit at the beginning and at the completion of DPH passage through the membrane into the hydrophobic core.

3. Results and discussion

The following observations deserve particular attention as the outcome of the present study. First, we observed significantly increased steady-state anisotropy values in the cells from diabetic patients (0.293 ± 0.012 in the group of diabetic patients vs. 0.259 ± 0.012 in the control group, $P < 0.01$; Fig. 1), which indicates decreased erythrocyte membrane fluidity.

Second, the rate constant k of DPH incorporation into the membranes was significantly lower (0.066 ± 0.017 min^{-1}) in RBC from diabetic patients compared to that in the control group (0.098 ± 0.014 min^{-1}), which indicates an altered fluidity gradient in the membranes from diabetic patients ($P < 0.01$, Fig. 2). Third, RAG decreased the steady-state anisotropy values in RBC membranes (by $8.9 \pm 1.6\%$ in the diabetic patients and by $4.6 \pm 1.7\%$ in the control group; Fig. 1), which means that it apparently acted as a fluidizing agent ($P < 0.001$). The reduction of the values was more pronounced in the group of diabetic patients ($P < 0.01$). Upon the action of RAG, the degree of steady-state anisotropy in the membranes from diabetic patients returned to the level relevant to that observed in the control group (0.259 in control $_{RAG(-)}$ vs. 0.267 in diabetic $_{RAG(+)}$, NS). Lastly, RAG was not the only efficient membrane fluidizer in RBC membranes from diabetic patients; it also restored the rate constant k of DPH incorporation into these membranes to the natural physiological values observed in control RBC from healthy donors (Fig. 2). We revealed that no significant differences in the rate constant k were found between the membranes of untreated RBC (control membranes), and the membranes isolated from RAG pretreated blood from diabetic patients (0.096 ± 0.025 min^{-1}), as well as between control membranes and those from RAG pretreated control blood (0.103 ± 0.012 min^{-1}) (Fig. 1).

Overall, diabetes was associated not only with the reduced RBC membrane fluidity, but also with significantly lower rate constants of the incorporation of the rod-like fluorescent label into the membrane lipid bilayer. The latter observation might be interpreted in terms of the altered fluidity gradient in the RBC membranes from diabetic patients. We feel it is necessary to particularly emphasize that the different time courses of DPH incorporation in RBC membranes from healthy control subjects and diabetic patients bear some important methodological consequences. It seems reasonably certain to conclude that any extrapolation(s) of steady-state DPH anisotropy values, often reported in literature, on the overall RBC membrane dy-

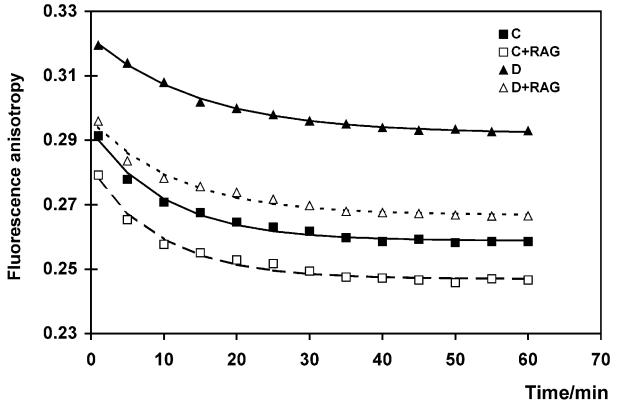

Fig. 1. Effect of resorcylidene aminoguanidine (RAG) on the time courses of the incorporation of 1,6-diphenylhexatriene-1,3,5 (DPH) into the membranes of red blood cells from control subjects (C) and diabetic patients (D).

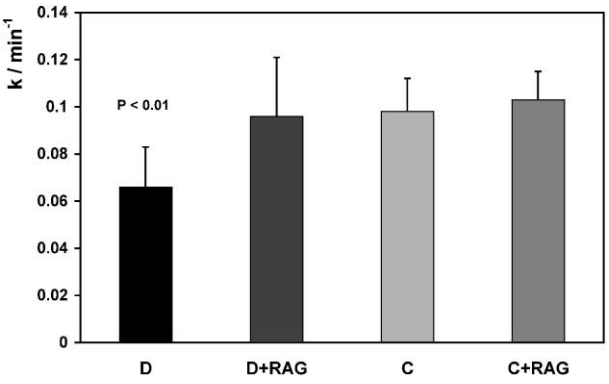

Fig. 2. Effect of resorcylidene aminoguanidine (RAG) on the rate constant k of 1,6-diphenylhexatriene-1,3,5 (DPH) incorporation into the membranes of red blood cells from control subjects (C) and diabetic patients (D).

namic parameters have to be evaluated with caution. We further revealed that anti-glycation agent RAG is likely to affect lipid–protein interactions in RBC membranes. This results in membrane lipid bilayer fluidization and possibly also lowering of structural order in the hydrophobic interior of the less fluid membranes in diabetes. Interestingly, when pretreated with RAG, the membranes from diabetic patients showed characteristics close to that found in the control RBC.

4. Conclusion

We confirmed that membrane fluidity in the hydrocarbon region of erythrocytes in diabetic patients is significantly decreased. The differences in the DPH rate constants between the diabetic and control groups could be attributed to the alterations in the membrane fluidity gradient. The aromatic aminoguanidine derivative RAG is able to restore the natural physiological membrane dynamic parameters in RBC from diabetic patients approximating to those revealed for control RBC. The ameliorating effect of RAG on the structural order in the hydrophobic interior of RBC membrane lipid bilayer and on the diabetes-associated attenuated fluidity gradient might be beneficial in diabetes due to the improvement of impaired membrane functions controlled by membrane fluidity.

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