Diffuse structural abnormalities in cell membranes from genetically hypertensive rats: a fluorescence polarization study

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Summary

1. Diphenylhexatriene was used as a fluorescence probe to detect structural differences between membranes from genetically hypertensive rats and those from their normotensive controls. Both isolated membranes (erythrocyte ghosts) and whole cells (platelets) were studied.

2. In the Okamoto-Aoki strain, the fluorescence polarization of diphenylhexatriene and consequently the 'equivalent microviscosities' were altered in all membranes, even in those of young normotensive SHR. These abnormalities varied with age (in whole cells) and were not detected in females.

3. These alterations were not restricted to the SHR strain and were also observed in the Sabra hypertensive strain (SBH). The 'equivalent microviscosity' of hypertensive Sabra erythrocyte ghosts was higher than that of the original Sabra rats. Salt loading of the Sabra rats promoted an increase in the 'equivalent microviscosity' of their erythrocyte membranes, which nearly reached the Sabra hypertensive level.

4. These results support the hypothesis of a genetic change in hypertensive rats leading, directly or indirectly, to diffuse alterations of cell membrane structure, which seem to be similar to those caused by high salt intake.

Key words: diphenylhexatriene, fluorescence polarization, high salt intake, plasma membrane, spontaneously hypertensive rat.

Abbreviation: DPH, diphenylhexatriene.

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Introduction

Functional alterations have been described recently in erythrocytes from genetically hypertensive rats and patients with primary hypertension. These modifications mainly concern the Na⁺, K⁺ and Li⁺ transmembrane movements [1-9]. Changes in phospholipid metabolism have also been reported [10-12]. Such functional alterations may either result from the presence of abnormal circulating or intracellular substances, or reflect structural changes in the membrane itself. Structural alterations have indeed been observed by using fluorescent or spin label probes embedded in the membrane [13-14]; the density of sites able to bind calcium with high affinity was reported to be lower in SHR than in WKY rat erythrocyte membrane [15-16]. These structural alterations are not restricted to the erythrocyte membrane but also exist in plasma membranes isolated from other cells [17]. Here we confirm our previous observations made on isolated SHR membranes with diphenylhexatriene as a fluorescent probe, and further extend the analysis to platelets and to the influence of sex and aging. In order to know whether the observed changes are specific for the Okamoto-Aoki hypertensive strain or may reflect a more generalized alteration linked to hypertension, structural differences in the erythrocyte membrane from hypertension-prone and resistant Sabra rats [18] were also investigated, before and after high salt intake.

Methods

Male and female Okamoto spontaneously hypertensive rats (SHR) and the corresponding Kyoto normotensive strain (WKY) were studied between 3 and 16 weeks of age.
Hypertension-prone (SBH) and sodium-resistant (SBN) Sabra rats were compared with the original Sabra rats. Male animals were studied at the age of 14 weeks, under two different conditions of sodium intake: a 0.3%-sodium diet and tap water to drink or 2%-sodium diet and 1% NaCl as beverage for 3–4 weeks. Systolic blood pressures were measured by sphygmomanometry.

Blood was sampled by cardiac puncture on stunned animals and collected with heparin or citrate as anticoagulant. Platelets were prepared from platelet-rich plasma and washed in the following medium (mmol/l): NaCl 103, citric acid 36, glucose 5, KCl 5, CaCl₂ 2, MgCl₂ 1; pH 6.5. Erythrocytes were washed twice with a pH 8 buffer containing phosphate (5 mmol/l) and NaCl (150 mmol/l), the white buffy layer was carefully discarded and lysis performed in 40 vol. of a phosphate buffer (5 mmol/l), pH 8.0, containing MgSO₄ (1 mmol/l). Ghosts were collected by centrifugation at 48,000 g and washed with the same buffer.

Diphenylhexatriene (DPH) dissolved in freshly distilled tetrahydrofuran (2 mmol/l) was dispersed in the membrane buffer at a concentration of 2 μmol/l. After mixing with an equal volume of the membrane preparation, the solution was incubated for 30 min at 37°C. In all cases the extinction due to light scattering of the membrane suspension was adjusted to 0.15 in square 5 mm optical pathlength cuvettes at 456 nm. Emission from DPH (1 μmol/l) dispersed in the buffer was always less than 3% of the emission of DPH in the membranes. The polarization ratio from DPH in the membranes was checked to be independent of the emission and excitation wavelength with Glan–Thomson polarizers on a Fica 55,000 absolute differential spectrofluorimeter. The polarization ratios were measured on a Elscint MVI spectrometer.

**Results**

**Okamoto rat strain**

The higher polarization ratios previously demonstrated for male SHR erythrocyte ghosts within the temperature range studied (10–40°C) were confirmed. However, when measured in female rats, no significant difference was observed (Fig. 1) either in young (3 weeks old) or in older rats (12 weeks old). The decrease in activation energies detected in male SHR ghosts did not exist in female SHR ghosts, and activation energies were identical in male WKY rat and female SHR and WKY rat erythrocyte membranes.

The difference in DPH fluorescence polarization ratios between male SHR and WKY rat platelets was greater than that between erythrocyte ghosts, and varied with age (Fig. 1): SHR platelets had a lower polarization ratio in young animals; this difference decreased as age increased and became null at about 7 weeks. In older rats, SHR platelets exhibited higher polarization ratios than those of age-matched WKY platelets. When female rats were compared, no significant difference was observed between the platelets of the two substrains, in agreement with the results of erythrocyte study.

**Sabra rat strain**

To determine whether the observed differences were restricted to the Okamoto substrain or reflected a general property of hypertensive rats, the same study was repeated with the hypertension-prone and resistant Sabra rats. When fed with the standard diet, both SBH and SBN rats differed from the original Sabra rats with regard to the polarization ratio of DPH-containing ghosts (Fig. 1b). High salt diet induced substantial differences in the structural characteristics of the membrane between the three substrains: high salt intake promoted an increase in the polarization ratio of the Sabra ghosts which nearly reached the SBH level, whereas in the latter only a slight increase of polarization ratio was obtained from high salt load. In contrast, polarization values of the hypertension-resistant rats slightly decreased. This high sodium diet increases systolic blood pressure by 13 ± 11, 19 ± 6 and 8 ± 8 mmHg in the original Sabra, the hypertension-prone and -resistant rats respectively.

**Discussion**

DPH polarization measurement provides information about the restriction of DPH rotation in the membrane and can be considered as an index of the structure of its local environment. This gives a measure of the ‘equivalent microviscosity’ of the membrane. The observed differences in DPH polarization values thus reflect alterations in the membrane structure.

Both in male spontaneously hypertensive rats of the Okamoto substrain and in the hypertension-prone Sabra rats, enhanced ‘equivalent microviscosities’ were observed in erythrocyte membranes. These differences may
Fig. 1. (a) Influence of sex and age on the difference in fluorescence polarization ratios of DPH for erythrocyte ghosts and platelets from spontaneously hypertensive rats and their normotensive controls (WKY rats). (b) Differences in fluorescence polarization ratios of DPH embedded into erythrocyte ghosts from hypertension-prone (SBH) and -resistant (SBN) Sabra rats and the original Sabra strain (SB). Animals were studied under two different conditions of sodium intake in (b). Values were measured at 35°C. Significance by Student’s paired t-test: * P < 0.05; ** P < 0.01; *** P < 0.001.

reflect genetic differences of these two substrains as they exist in animals in which hypertension is yet not well developed. In addition, environmental factors are able to induce similar membrane changes since a high sodium intake increases the 'equivalent microviscosity' of Sabra erythrocyte membrane up to the level characteristic of the hypertension-prone substrain. In salt-resistant Sabra rats, high sodium intake produces only a minor change in the membrane microviscosity.

Changes in the membrane structure of erythrocytes and platelets probed with DPH did not appear in female rats of the Okamoto strain, which are known to reach lower blood pressures than males. This study of fluorescence polarization of DPH embedded in cellular membranes from rats genetically selected with respect to the development of high blood pressure, confirms the existence of membrane alterations, which antedate hypertension. The role of these structural changes in the control of cellular functions is unknown but it is noteworthy that structural modifications similar to those observed in genetically hypertension-prone rats may be induced by salt loading in normotensive rats.

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