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RAPID COMMUNICATION

Plasma Triglycerides Cosegregate With Erythrocyte Sodium Content in F2 Rats of HTG x Lewis Cross

J. ZICHA, Z. DOBEŠOVÁ, J. KUNEŠ

Institute of Physiology, Academy of Sciences of the Czech Republic, Prague, Czech Republic

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Summary

The possible association of plasma lipids (triglycerides and cholesterol) with erythrocyte Na^+ content (Na^+_i) and/or with alterations in red cell Na^+ and K^+ (Rb^+) transport was studied in a population of F2 hybrids obtained by crossing hypertensive Prague hereditary hypertriglyceridaemic (HTG) rats with normotensive Lewis rats. The obtained data indicated a strong cosegregation ($p<0.001$) of plasma triglycerides with erythrocyte Na^+ content. This was the cause for the close correlation of plasma triglycerides with the Na^+-K^+ pump activity (measured as ouabain-sensitive Na^+ extrusion). On the contrary, there was only marginal association ($p<0.05$) of erythrocyte Na^+ content with plasma cholesterol which was significantly ($p<0.01$) related to bumetanide-sensitive Rb^+ uptake mediated by the Na^+-K^+ cotransport system. Na^+ leak (bumetanide-resistant net Na^+ uptake) correlated positively with blood pressure in female but not in male F2 rats. The close association between plasma triglycerides and erythrocyte Na^+ content suggests that ion transport alterations might contribute to mechanisms responsible for the cosegregation of blood pressure with plasma triglycerides in HTG x Lewis F2 hybrids.

Key words:

Hereditary hypertriglyceridaemic rats – Genetic hypertension – Plasma triglycerides – Plasma cholesterol – Erythrocyte Na^+ content – Na^+ leak – Na^+-K^+ cotransport – Na^+-K^+ pump

Ion transport alterations found in essential hypertension seem to be coupled to high blood pressure through the changes in lipid metabolism (Duhm and Behr 1986, Hunt *et al.* 1986, Pagnan *et al.* 1989). Augmented ouabain-resistant Na^+ influx (due to the enhancement of both Na^+ leak and Na^+-K^+ cotransport) was also described in hereditary hypertriglyceridaemic (HTG) rats (Kuneš *et al.* 1994). This strain, originally selected from Wistar rats for elevated plasma triglycerides (Vrána and Kazdová 1990), is also hypertensive (Štolba *et al.* 1992, Kuneš *et al.* 1994). Recently, Kuneš *et al.* (1995) demonstrated cosegregation of blood pressure with plasma triglycerides in F2 hybrids derived from the cross of HTG rats with normotensive Lewis animals. The aim of the present study carried out in the same F2 population was to search for the cosegregation of plasma triglycerides or cholesterol with red cell ion transport alterations characteristic for HTG rats.

One hundred and twenty-seven F2 hybrids that were obtained from the cross of normotensive Lewis females with hypertensive HTG males were studied at the age of 5 months. Rats were kept under standard laboratory conditions, were fed pelleted rat chow containing 0.4 % NaCl (ST-1 Velaz, Prague) and drank tap water *ad libitum*. Blood pressure was measured in conscious animals one day after catheterization of the carotid artery. All blood pressure measurements were performed between 0800 and 1200 h to avoid the influence of circadian variation in blood pressure. Haematocrit, haemoglobin and Na^+ content of fresh erythrocytes as well as plasma levels of triglycerides and cholesterol were determined from heparinized blood withdrawn from the abdominal aorta under ether anaesthesia. Erythrocytes were washed three times with a saline medium (in mmol/l: NaCl 140, glucose 5, phosphoric acid 2.5, MOPS 10, pH 7.4 at 37 °C, 310 mosmol/l) and incubated in this medium

containing 3.5 mmol/l RbCl under gentle shaking for 30 min at 37 °C (for details see Kuneš *et al.* 1994). Net Na^+ and unidirectional Rb^+ (K^+) fluxes were assessed at intracellular Na^+ and extracellular Rb^+ (K^+) concentrations which were close to those found *in vivo*. Ouabain (5 mmol/l) and bumetanide (100 lmol/l) were used as inhibitors of the Na^+-K^+ pump and the Na^+-K^+-$2Cl^-$ cotransport system, whereas cation leaks were defined as residual fluxes resistant to both ouabain and bumetanide. Erythrocyte cation contents and transport rates were expressed per mean cell haemoglobin content found in particular animals. The data were evaluated by linear regression analysis to assess the relationships between blood pressure, plasma lipids and ion transport parameters.

Table 1 summarizes correlation coefficients of the relationships between plasma lipids, blood pressure, erythrocyte Na^+ content and particular ion transport parameters. Blood pressure cosegregated with plasma triglycerides but not with plasma cholesterol. The most important finding is illustrated in Fig. 1 which shows a highly significant cosegregation of plasma triglycerides with erythrocyte Na^+ content. This association was further reflected by a significant correlation of plasma triglycerides with the activity of the Na^+-K^+ pump (measured as ouabain-sensitive Na^+ extrusion) which is dependent on erythrocyte Na^+ content. There was a borderline correlation of plasma cholesterol with the erythrocyte Na^+ content ($p<0.05$) but plasma cholesterol was positively related to bumetanide-sensitive Rb^+ uptake mediated by the Na^+-K^+ cotransport system (Table 1). None of the investigated parameters of red cell Na^+ and K^+ transport correlated significantly with blood pressure in the whole population of HTG x Lewis F2 hybrids. Nevertheless, bumetanide-resistant net Na^+ uptake (Na^+ leak) correlated positively with mean arterial pressure ($r=0.30$, $n=65$, $p<0.02$) in the subpopulation of female F2 rats in which highly significant correlations of plasma triglycerides with erythrocyte Na^+ content ($r=0.59$, $p<0.001$), ouabain-sensitive Na^+ extrusion ($r=0.49$, $p<0.001$) and ouabain-sensitive Rb^+ uptake ($r=0.32$, $p<0.01$) were observed.

Table 1
The relationship between plasma lipids, blood pressure, erythrocyte Na^+ content and red cell Na^+ or Rb^+ transport in HTG x Lewis F2 hybrids (n = 127).

	Triglycerides	Cholesterol
Mean arterial pressure	0.30**	−0.01
Erythrocyte Na^+ content	0.50***	0.22*
OS net Na^+ extrusion	0.41**	0.06
BS net Na^+ uptake	−0.03	0.10
BR net Na^+ uptake	−0.13	0.04
BS Rb^+ uptake	0.11	0.26**
BR Rb^+ uptake	−0.19	−0.02

*MAP – mean arterial pressure, OS – ouabain-sensitive, BS – bumetanide-sensitive, BR – bumetanide-resistant. Data are correlation coefficients r; their statistical significance: * $p<0.05$, ** $p<0.01$, *** $p<0.001$.*

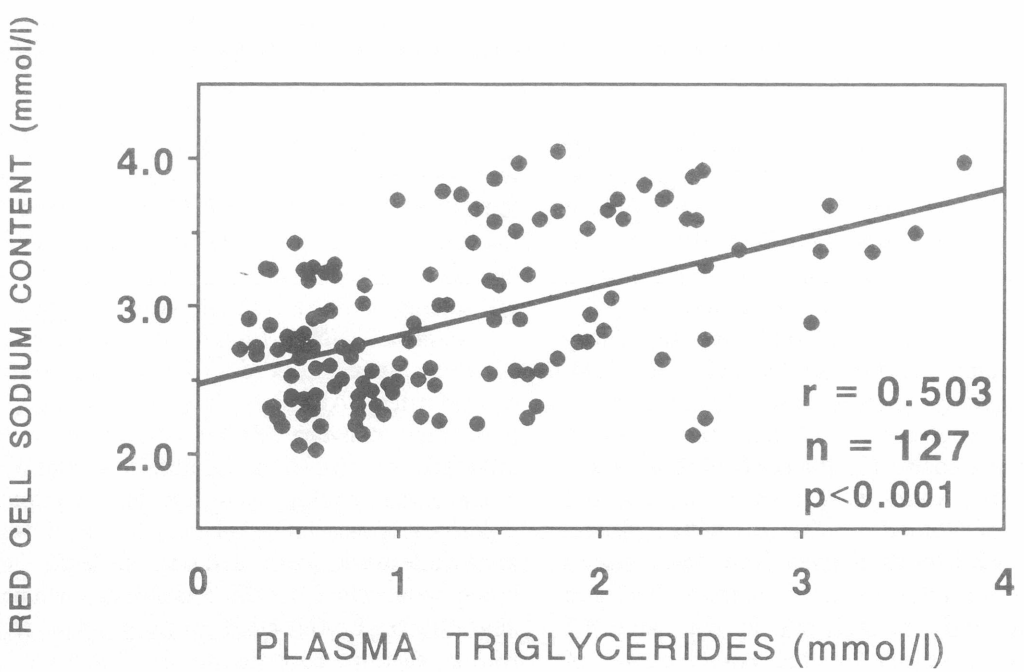

Fig. 1
The relationship between erythrocyte Na^+ content and plasma triglycerides in HTG x Lewis F2 hybrids.

Vincent *et al.* (1993) and Kuneš *et al.* (1995) were the first who demonstrated a cosegregation of plasma lipids with blood pressure of F2 hybrids derived from the cross of hypertensive rats characterized by abnormal lipid metabolism (Lyon hypertensive rats or Prague hereditary hypertriglyceridemic rats) with their normotensive, normolipidaemic controls. Though the possible mechanism(s) mediating this relationship are still not clear, alterations of ion transport might be involved. Human studies based upon epidemiological or intervention data (Duhm *et al.* 1986, Hunt *et al.* 1986, Hespel *et al.* 1988, Weder *et al.* 1991, Corrocher *et al.* 1992) indicate that alterations of plasma lipids might affect kinetic properties of particular ion transport systems through the changes of cell membrane lipid composition (Kelly *et al.* 1989, Pagnan *et al.* 1989, Duhm *et al.* 1993). The modified composition of molecular species of phosphatidylcholine and phosphatidylethanolamine in erythrocyte membrane seems to mediate the influence of hyperlipidaemia on the activity of several ion transporters (Duhm and Engelmann 1992). To our knowledge, the experiments on red cell ion transport in HTG rats (Kuneš *et al.* 1994) and their F2 hybrids (this paper) are the first studies confirming the influence of plasma lipids on Na^+ and K^+ transport in erythrocytes of rats with genetic hypertension and hyperlipidaemia. The observation of a significant correlation of blood pressure with Na^+ leak in our female F2 hybrids is in good agreement with our previous findings on the important role of Na^+ leak in the pathogenesis of hypertension in Dahl salt-sensitive rats (Zicha and Duhm 1990), spontaneously hypertensive rats (Bin Talib and Zicha 1993), Prague recombinant inbred strains (SHR x Brown Norway) (Bin Talib *et al.* 1992), Prague hereditary hypertriglyceridaemic rats (Kuneš *et al.* 1994) and Lyon hypertensive rats (Zicha *et al.* 1995). It should be noted that abnormalities of lipid metabolism were demonstrated in all above mentioned forms of experimental hypertension (Mondon *et al.* 1993, Reaven and Chang 1991, Vrána and Kazdová 1990, Sassolas *et al.* 1981).

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Reprint requests

Dr. J. Zicha, Institute of Physiology, Academy of Sciences of the Czech Republic, Vídeňská 1083, 142 20 Prague 4, Czech Republic.