Alterations in Induced Potassium Calcium Efflux in the Erythrocytes of Patients with Autosomal Dominant Polycystic Kidney Disease and Hypertension

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Dear Sir,

Autosomal dominant polycystic kidney disease (ADPKD) can be frequently complicated by chronic renal failure and hypertension [1]. In the literature there is little consensus on the pathogenesis of hypertension in patients with ADPKD. In fact, although it would seem obvious that the incidence of hypertension would increase as renal insufficiency becomes more severe, it has also been demonstrated that there is no correlation between the severity of hypertension and the severity of renal involvement, and that 15-20% of subjects with ADPKD without renal function impairment have hypertension and that blood pressure alterations can be found in ADPKD patients without renal function impairment before puberty [2].

Importantly, the genetic trait appears to be dominant. The main gene locus responsible for ADPKD is PKD-1, localized on the short arm of chromosome 16, which presents a 100% penetration [3]. However, another locus is involved in the disease. Called PKD-2, it is linked to chromosome 4, which is altered in 10% of patients, and modifications in it could give rise to an altered cellular membrane structure, and therefore to anomalous functioning of the ionic transport systems [4, 5]. Recently Vareesangthip et al. [6] identified altered Na/Li countertransport in ADPKD patients, and Rutherford et al. [7] found that patients with essential hypertension had an altered ionic transport system. However, this alteration is present in the erythrocytes of all ADPKD patients, and so it does not explain why hypertension develops in only 15-20% of such patients without renal function impairment. We therefore evaluated induced $\text{Ca}^{2+}/\text{K}^{+}$ flow in the erythrocytes of ADPKD subjects in order to ascertain whether ADPKD patients with hypertension but without renal function impairment also had alterations in other ionic transport systems.

Our study was conducted on 40 subjects divided into four groups that were matched for age and gender as follows; 10 healthy controls (5 M, 5 F; mean age 52 ± 7 years); 10 with essential

hypertension (5 M, 5 F; mean age 51 ± 8 years; mean systolic blood pressure 172 ± 15 ; mean diastolic blood pressure 105 ± 11); 10 normotensives with ADPKD (5 M, 5 F; mean age 48 ± 10 years); 10 with ADPKD and hypertension (5 M, 5 F; mean age 50 ± 6 years; mean systolic blood pressure 168 ± 23 ; mean diastolic blood pressure 103 ± 7). None of the hypertensive subjects was on diuretic or Ca antagonist therapy. The subjects enrolled had creatinine clearance values ranging from 95 to 150 ml/min/1.75 m². At 8.00 a.m. blood samples were drawn from all the subjects for the evaluation of K⁺/Ca²⁺ flow induced by the Gardos effect [8].

Some of the blood was made unclottable by means of Na⁺ heparin; the remaining blood was washed three times with a physiological NaCl solution buffered with Tris-HCl, at pH 7.4; temperature was kept at 37 °C. The incubation medium for the erythrocytes was 107 mM NaCl and 20 mM Tris-HCl at pH 7.4, to which we added 0.2 mM of propranolol (final concentration), which gradually increased the intracellular Ca²⁺. To this solution we added 1 mM EGTA, which complexes Ca²⁺ and frees the medium

of the Ca ions (0.2-1 mM), 3-iso-butylmethylxanthine (IBM-X 0.4 μM).

Hematocrit of the erythrocyte suspension was set at 10% and measured with a cell counter (Sequoia Turner Cell Dyn 900). The osmolarity of the medium and of the suspension was checked by means of a Fiske Os Oximeter for a value of 290 mosm.

Cells were first pre-incubated in the medium with propranolol alone for 30 min, then Ca (0.2 mM) was added whose presence generally determines a flux of K⁺ and therefore an influx of Ca²⁺ of about 20%. The cell volume was kept constant throughout the test period. The kinetic examination was made by taking a quantity of 0.5 ml every 5 min for 90 min. The supernatant was rapidly separated to measure the flow of intracellular K⁺ with flame photometry (Eppendorf). The results were expressed as percentage values of the K⁺ outflux per cell compared to the baseline value time 0, according to the following equation:

$$A - (100 - B) \times C$$

) K cells ;

(1)

100

B

T₀

where A = total K⁺; B = hematocrit, C = K⁺ of the supernatant (at 0, 5, 15 ... 90 min).

The absolute value cannot be representative of the phenomenon, as the amount of cells in each different test can differ slightly for the number of the cells involved. The statistical analysis was carried out with the one way analysis of variance. Values of $p < 0.05$ were considered significant.

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Fig. 1. Percentage of K⁺ cell contents in the tested subjects. * $p < 0.01$.
Normotensive Hypertensive

subjects
ADPKD ADPKD
normotensive hypertensive

In healthy subjects, the K⁺ flow in the presence of 1 mM Ca²⁺ was 20% at 30 min the values persisting until 90 min. The pattern in normotensive ADPKD subjects was analogous (28%). In the subjects with primary hypertension and in ADPKD subjects with hypertension, the K⁺ flow in the presence of Ca was < 55% from 30 min to 90 min and at all stages it was greater than in the normotensive subjects ($p < 0.01$, fig. 1). The presence of IBM-X, a phosphodiesterase inhibitor, increased the K⁺ flow by 60% in healthy subjects and in normotensive subjects with ADPKD, while it did not affect the K⁺/Ca²⁺ flow induced in subjects with primary hypertension in the ADPKD subjects with hypertension.

Our results indicate that there is an anomaly in induced K⁺/Ca²⁺ transport in the erythrocytes of subjects with ADPKD and hypertension, which is identical to that found in subjects with primary hypertension [9] and might express a genetic membrane alteration. Although it has been suggested that renal cysts may give rise to hypertension independent of renal impairment [10], we believe that a particular genetic order that can compromise the function of many systems of ionic transport may participate in the genesis of hypertension in subjects with ADPKD.

References

- Grunfeld, JP, Bennet WM: Clinical aspects of autosomal dominant polycystic kidney disease. *Curr Opin Nephrol Hypertens* 1995;4:114-120.
- Zeir M, Ritz E, Geberth S, Gonzalo A: Genesis and significance of hypertension in autosomal dominant polycystic kidney disease. *Nephron* 1994;68:155-158.
- The European Polycystic Kidney Disease Consortium: The polycystic disease 1 gene encodes a 24 kb transcript and lies within a duplicated region on chromosome 16. *Cell* 1994;77:1-20.
- Romeo G, Devoto M, Costa G: A second genetic locus for autosomal dominant polycystic kidney disease. *Lancet* 1988;ii:8-10.
- Van Adelsberg J, Dale F: The PKD gene produces a developmentally regulated protein in mesenchyme and vasculature. *Nat Med* 1995; 1:359-364.
- Vareesangthip K, Thomas TH, Wilkinson R: Abnormal effect of thiol groups on erythrocyte Na-Li countertransport kinetics in adult polycystic kidney disease. *Nephrol Dial Transplant* 1995;10:2219-2223.
- Rutherford PA, Thomas TH, Wilkinson R: Erythrocyte sodium instructive or just phenomenology? *Clin Sci* 1992;82:341-352.
- Gardos G: The permeability of human erythrocyte to potassium. *Acta Physiol Acad Sci Hung* 1956;10:185-189.
- Buemi M, Allegra A, Marino D, Marino MT, D'Anna R, Palella S, Corica F, Frisina N: Effect of some growth factors on the potassium-calcium induced efflux in vitro in whole erythrocytes from hypertensive and pregnant subjects with gestation hypertension. *High Blood Pressure* 1995;4:204-208.
- Graham PC, Lindp GBM: The anatomy of the renin secreting cell in adult polycystic kidney disease. *Kidney Int* 1988;33:1084-1090.

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