G001

ABNORMALITIES OF THE CALCIUM PUMP IN ESSENTIAL HYPERTENSION. J. Sobrino*, A. de la Sierra*, L. Ribera, M.J. Adrian, M.T. Aguilera*, A. Coca*. *Hospital de l'Esperit Sant. Santa Coloma de Gramenet. Spain.*

Transmembrane Ca^{2+} fluxes are mediated, at least in part, by the Ca^{2+}-dependent ATPase. Thus, genetic or acquired abnormalities of this pump could explain the increase in free cytosolic Ca^{2+} content that has been observed in essential hypertensive patients. We carried out a kinetic study of the Ca^{2+} pump in intact erythrocytes from 49 essential hypertensive patients and 27 normotensive healthy. We used Sr^{2+} as a calcium analogue to measure Ca^{2+} fluxes dependent of the Ca^{2+} pump. The intracellular concentrations of Sr^{2+} and Ca^{2+} were modified using the A23187 ionophore in a Ringer isotonic solution. Hypertensive patients showed a significant increase of the maximal efflux rate for Sr^{2+} (V_{max}) with respect controls (6.6 ± 2.3 vs 5.2 ± 1.6 mmol/L.cels/h, p=0.006). Mean values of apparent dissociation constants for intracellular Ca^{2+} (K_{Ca}) were also increased in essential hypertensives (80.36 ± 53.46 vs 55.25 ± 15.13 µmol/L.cels, p=0.06). A significant correlation between V_{max} and age (r=0.342; p=0.016), and serum creatinine (r=0.446; p=0.001) was observed. The K_{Ca} only correlated with serum creatinine (r=0.402; p=0.004). Using the K_{Ca} confidence interval of 99% as the higher normal limit, patients were segregated into two subgroups depending on normal K_{Ca} values (33 patients, 67.3%) or increased K_{Ca} values (16 patients, 32.6%). Age (50.8 ± 13.5 vs 43 ± 10.2 years p=0.02), serum creatinine (1.13 ± 0.17 vs 0.95 ± 0.17 mg/dl, p=0.001), and serum uric acid (7.27 ± 3.32 vs 6.14 ± 1.49 mg/dl, p=0.04), were higher in patients with increased K_{Ca}. Finally, patients with increased K_{Ca} also showed increased values of V_{max} (9.13 ± 2.02 vs 5.38 ± 12.81 mmol/L.cels/h, p<0.0001). Essential hypertensive patients are heterogeneous regarding ion transport abnormalities, only affecting subgroups of hypertensive patients. We have observed abnormalities of the Ca^{2+}-dependent ATPase in 33% of essential hypertensive patients. These patients are older and tend to exhibit higher values of serum creatinine and uric acid.

Key Words: Essential hypertension, Ca^{2+}-dependent ATPase, Ca^{2+} pump.

G002

URINARY ALDOSTERONE AND SODIUM REABSORPTION IN THE DISTAL NEPHRON CORRELATE WITH THE ACTIVITY OF AMILORIDE-SENSITIVE SODIUM CHANNELS.

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In the distal nephron and colon, aldosterone-dependent Na^+ reabsorption is mediated by amiloride-sensitive epithelial Na^+ channels (ENaC). Aldosterone increases Na^- transport and thus increases transepithelial potential difference (PD). We have shown previously in mice that amiloride-sensitive rectal PD is related to urinary Na^+ excretion. However, whether it is related to sodium transport in the distal nephron is still unknown. Thus, we have assessed the relationship of amiloride-sensitive rectal PD to urinary aldosterone excretion and Na^+ reabsorption in the distal nephron. Amiloride-sensitive rectal PD, serum and urinary Na^+ and endogenous trace Li^+, and urinary aldosterone were measured in C57BL/6J mice on a normal, low and high sodium diet containing respectively 4, 1, and 16 mg Na^+/g food. Li^+ was measured by atomic absorption spectrophotometry and aldosterone was determined by radioimmunoassay after chromatography. Amiloride-sensitive rectal PD correlated with the urinary Na^+/K^+ ratio (r=0.86, n=39, $y=17x^{-0.6}$), linearly with the urinary aldosterone excretion rate (r=0.73, n=39, p<0.0001) and with the urinary Li^+/Na^+ ratio (r=0.81, n=36, p<0.0001). In addition, urinary aldosterone excretion also correlated with the urinary Na^+/K^+ ratio (r=0.82, n=39, $y=653x^{-0.4}$).

In conclusion, our results suggest that rectal ENaC activity correlates with aldosterone excretion and sodium reabsorption in the distal nephron.

Key Words: Aldosterone, ENaC, ions, amiloride-sensitive potential difference.

G003

ALTERED cAMP-RESPONSE IN T594M VARIANT OF AMILORIDE SENSITIVE SODIUM CHANNEL IN HUMAN TRANSFORMED LYMPHOCYTES. Y. Cui, Y.R. Su*, M. Layne, V. Carter*, A.G. Menon, M.C. Reif*, and R.Y.K. Pun. University of Cincinnati Medical Center, Cincinnati, OH.

Previously we reported the identification of a variant of T594M in the β-subunit of the amiloride-sensitive channel (ASSC) that occurs exclusively in the African American population (Su et al., 1996, J. Am. Soc. Nephrol., 7: 2543-2549). Liddle's syndrome is an autosomal hereditary hypertensive disease resulting from the truncation of the C-terminal of the β- or γ-subunit of ASSC. In Liddle'syndrome, basal sodium conductance of ASSC is elevated, while the T594M variant does not have a higher resting sodium conductance and shows an enhanced response to cAMP stimulation. To ascertain that the lack of changes in the basal response observed in the T594M variant is not related to the differences in the method used in the measurement of the sodium conductance, we compared the sodium conductance measured by step pulses (used in the evaluation of Liddle's lymphocytes) and by voltage ramp (used in our studies) on the same cell. Whole cell voltage clamp experiments were performed on both EBV-transformed lymphocytes harboring wild type and variant T594M channel. Slope conductance measured from the current-voltage relation (I-V) plot obtained with step pulses generated between −160 to −40 mV in 20 mV increment was compared to the slope conductance generated by voltage ramps between −150 to −50 mV (average of 4 ramps). The basal conductance was 0.44 ± 0.13 nS measured with steps and 0.42 ± 0.09 nS (mean ± s.d.; n=8) measured by ramp. In another population of cells, the effect of cAMP on sodium conductance was evaluated with cAMP included in the recording pipette and allowed to diffuse into the cell. In the presence of cAMP the mean value of the slope conductance obtained with steps was 1.52 ± 0.17 nS and the slope conductance obtained by ramp was 1.45 ± 0.21 nS. There was no significant difference between the values obtained between the two methods (paired t-test; p>0.05).

The lack of change in basal sodium conductance in the T594M variant indicates that this variant is fundamentally different from Liddle's mutation, although the mutations reside in the β-subunit.

Key Words: Amiloride-sensitive sodium channel, T594M, mutation, Liddle's syndrome

G004

DISTINCTION BETWEEN BUMETANIDE-SITES AT RAT Na-K-Cl COTRANSPORTERS BSC1 AND BSC2. D Pirot, M Alvarez-Guerra, C Nazaret, P Hannaert, RP Garay*. Laboratoire d'Informatique Biomédicale du CRAVEN, Crevant and INSERM U400, Faculté de Médecine de Créteil, France

Two bumetanide-sensitive Na-K-Cl cotransporters, products of two distinct genes, were recently identified : (i) BSC1, specifically localized at the apical border of the thick ascending limb of Henle's loop and (ii) BSC2, in almost all other cells. Here, we investigated BSC1/BSC2 selectivity for two loop diuretics, bumetanide and furosemide, in medullary thick ascending limb (BSC1), thymocytes and erythrocytes (BSC2) of the rat. Molecular modelling was used to identify structural differences between both molecules. Na-K-Cl cotransporter fluxes were equated to the bumetanide-sensitive rubidium uptake (measured by atomic absorption spectrophotometry). Bumetanide inhibited with similar potency BSC2 in thymocytes (pIC_{50} = 5.59 ± 0.05) and in erythrocytes (5.62 ± 0.18), and was 6-7 times more potent to inhibit Na-K-Cl cotransporter BSC1 (6.40 ± 0.09). Conversely, furosemide was : (i) 22-fold more potent to inhibit erythrocyte BSC2 (5.20 ± 0.02) than thymocyte BSC2 (3.86 ± 0.10) and (ii) of similar potency to inhibit BSC1 (4.86 ± 0.14) as erythrocyte BSC2 (5.20). Finally, erythrocyte and thymocyte BSC2 showed a similar regulatory response to cell shrinking. Molecular modelling showed that bumetanide possesses four potentially active groups, of which : (i) three are shared with furosemide at similar intergroup distances and should be recognized by the bumetanide binding site in BSC1 and erythrocyte BSC2, but only two should interact with thymocyte BSC2 and (ii) a fourth (phenoxy) group confers higher lipophilicity and should be recognized by the bumetanide binding site in BSC1 and thymocyte BSC2, but not in erythrocyte BSC2.

Key Words: Diuretics, Na-K-Cl cotransporters, cell membranes, ion transport