^{31}P NMR INVESTIGATION OF FREE INTRACELLULAR MAGNESIUM, pH AND ENERGY BALANCE IN STRIATED MUSCLE OF PATIENTS WITH KIDNEY DISEASE: RELATION TO INSULIN RESISTANCE

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^{31}P NMR VYŠETROVANIE VOĽNÉHO VNÚTROBUNKOVÉHO MAGNÉZIA, pH A ENERGETICKEJ ROVNOVÁHY V KOSTROVOM SVALE U PACIENTOV S OCHORENÍM OBLIČKY: VZŤAH K INZULÍNOVEJ REZISTENCII

Abstract

Sebekova K, Stefikova K, Krivosikova Z, Spustova V:
^{31}P NMR investigation of free intracellular magnesium, pH and energy balance in striated muscle of patients with kidney disease: relation to insulin resistance
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Background: **High prevalence (48 %) of insulin resistance (IR) in patients with mild to moderate kidney function reduction, and the potential pathogenetic role of magnesium (Mg) deficiency in IR prompted us to study skeletal muscle free Mg (fMg) concentration in patients with impaired kidney function.**
Methods**: fMg concentration, intracellular pH (pHi) and parameters of energy balance were determined employing ^{31}P NMR spectroscopy in the calf muscle of the dominant leg of 18 healthy controls (C) and 22 patients (P) with decreased kidney function. 10 patients suffered from insulin resistance (IR).**
Results: **No difference in fMg concentration in skeletal muscle was observed (C: 0.929±0.075; P: 0.948±0.062 mmol/l; x±SEM). In patients a slight shift of pHi towards acidic values was found (C: 7.036±0.0.004; P: 7.013±0.004; p<0.004), which was even more expressed in IR patients (7.008±0.005). Serum creatinine levels and creatinine clearance correlated with pHi in the patient's group. Adenosintriphosphate (ATP) to inorganic phosphate (Pi) ratio in skeletal muscle was lower, phosphocreatine (Pcr)/ATP ratio was higher, while that of Pcr/Pi showed only a trend towards an increase in the patient's group.**
Conclusion**: In patients with reduction of kidney function IR does not associate with a change in skeletal muscle free magnesium concentration, or deficiency in macroergic phospha-**

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Abstrakt

Šebeková K., Štefíková K., Krivošíková Z., Spustová V.:
^{31}P NMR vyšetrovanie voľného vnútrobunkového magnézia, pH a energetickej rovnováhy v kostrovom svale u pacientov s ochorením obličky: vzťah k inzulínovej rezistencii
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Pozadie problému: K štúdiu koncentrácií voľného magnézia (fMg) u pacientov s poruchou obličkových funkcií nás viedla vysoká prevalencia (48 %) inzulínovej rezistencie (IR) u pacientov s už mierne zníženými funkciami obličky a možná patogenetická úloha deficitu magnézia vo vzniku inzulínovej rezistencie.
Metódy: Koncentráciu fMg, intracelulárne pH (pHi) a ukazovatele energetického metabolizmu v lýtkovom svale dominantnej končatiny sme určovali metódou ^{31}P NMR spektroskopie u 18 zdravých dobrovoľníkov (C) a 22 pacientov (P) so zníženými funkciami obličiek, z ktorých 10 sme klasifikovali ako inzulínovorezistentných (IR).
Výsledky: Medzi skupinami sme nezaznamenali rozdiely v koncentrácii fMg v lýtkovom svale (C: 0,929±0,075; P: 0,948±0,062 mmol/l; x±SEM). U pacientov sme pozorovali mierny posun pHi k acidotickým hodnotám (C: 7,036±0,004; P: 7,013±0,004; p<0,004), ktorý bol najvýraznejší u IR pacientov (7,008±0,005). V skupine pacientov koncentrácia sérového kreatinínu a klírensu endogénneho kreatinínu korelovala s pHi. U pacientov bol pomer adenozíntrifosfátu (ATP) k anorganickému fosfátu (Pi) v kostrovom svale nižší, pomer fosfokreatínu (Pcr) k ATP bol vyšší, a v pomere Pcr/Pi sme pozorovali len trend k zvýšeným hodnotám v porovnaní so zdravými dobrovoľníkmi.
Záver: IR nie je u pacientov so zníženými funkciami obličiek spojená so zmenou koncentrácie voľného magnézia v kostrovom svale, alebo deficitom makroergických fosfátov. Na mechanizme IR u týchto pacientov sa môže zúčastňovať posun intracelulárneho pH

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te levels. Shift in intracellular pH towards acidic values may participate in IR. Decreased activity of Na^+/H^+ antiporter is suggested. ***(Fig. 5, Tab. 2, Ref. 22.)***
Key words: ^{31}P NMR, intracellular pH, insulin resistance, free intracellular magnesium, energy balance, kidney disease.

v kostrovom svale ku kyslým hodnotám. Autori predpokladajú možnú zníženú aktivitu Na^+/H^+ antiportera. *(Obr. 5, tab. 2, lit. 22.)*
Kľúčové slová: ^{31}P NMR, intracelulárne pH, inzulínová rezistencia, voľné intracelulárne magnézium, energetická rovnováha, ochorenia obličiek.

Both, hypertension and insulin resistance (IR) are risk factors of atherosclerosis (Pyorala, 1991; Connell and McLellan, 1991), its consequences such as coronary heart disease (Reaven, 1992), and obviously glomerulosclerosis related to atherosclerosis (Diamond, 1991). IR is almost a constant finding in advanced renal failure. Even in the early phases of kidney disease with only mild to moderate kidney function reduction prevalence of IR reaches 48 %, and it does not correlate with hypertension, plasma Mg concentration, anaemia, alteration in acid-base balance, or accumulation of endogenous inhibitors of glucose utilisation (Dzúrik et al., 1995).

About half of the patients with essential hypertension (EHT) suffer from IR without any correlation with plasma Mg (Štefíková et al., 1993). In EHT IR is associated with a decline in red blood cell intracellular free Mg concentration, due to an intracellular shift of Mg to its bound fraction (Resnick, 1992; Šebeková et al., 1993). However, in IR patients with kidney diseases no changes in free Mg concentration in erythrocytes were revealed. Relevance of this difference is not clear. However, IR is caused by impairment of glucose utilisation particularly in skeletal muscle, not red blood cells. To elucidate the role of Mg in IR of kidney disease patients, the skeletal muscle free Mg (fMg) concentration was determined in patients with mild to moderate reduction of kidney function.

Patients and methods

Patients and healthy volunteers: 22 normotensive patients with kidney disease (Table 1) and 18 healthy volunteers (9F, 9M, age = 28.2±1.6 years, body mass index = 21.6±0.45) with normal kidney function participated in the study. The study was approved by the Institutional Ethics Committee and was performed according to the Declaration of Helsinki. Written informed consent was obtained before participation.

^{31}P NMR spectroscopy: NMR spectra of the calf muscle of dominant leg positioned over 10 cm surface coil were recorded on Simens Magnetom SP 63 spectrometer operating at 1.5 T. Two 64 scan spectra were acquired using 80 sec pulses with 8 sec repetition time. fMg concentration was calculated from chemical shift differences between the mid point of the double peak of α-ATP and the central peak of ß-ATP (Gupta et al., 1978) (Fig. 1). Intracellular pH (pHi) was calculated using chemical shift difference between inorganic phosphate (Pi) and phosphocreatine (Pcr) peaks (Durozard et al., 1993), molar coefficients of phosphates (Šebeková et al., 1993) and the ratios of phosphate signals intensities were calculated.

Biochemical analysis: Insulin resistance was evaluated after standard oral glucose tolerance test (oGTT, oral glucose load of 75g in 300 ml water) with determination of glucose and insulin concentration before, and after 60 and 120 min. OGTT was classified according to WHO criteria. Patients with basal insulinaemia >20 μU/ml or >65 μU/ml 120 min after glucose load were classified as insulin resistant. In 4 patients the oGTT was not performed due to non-insulin dependent diabetes mellitus. Venous plasma was analysed for glucose, immunoreactive insulin,

Tab. 1. Characteristics of the patients.
Tab. 1. Charakteristika súboru pacientov.

	All Všetci pacienti (n=22)	Insulin sensitive Inzulín senzitívni (n=12)	Insulin resistant Inzulín rezistentní (n=10)
Gender [F/M] Pohlavie [Ž/M]	10/12	6/6	4/6
Age [years] Vek [roky]	54.8±2.7	55.7±4.1	53.7±3.5
S-Cr [μmol/l]	167.3±34.2	154.8±41.3	184.0±60.1
Cl_{Cr} [ml/s]	1.2±0.1	1.1±0.2	1.1±0.2
BMI [kg/m^2]	27.4±0.9 (n=18)	25.8±1.1 (n=12)	29.2±1.5* (n=6)
Glu t_o [mmol/l]	5.6±0.5	5.0±0.2	6.5±1.0*
Glu t_{60} [mmol/l]	9.0±0.9	7.3±0.6	11.3±1.7*
Glu t_{120} [mmol/l]	6.7±0.8	5.4±0.3	8.5±1.7*
IRI t_0 [μU/ml]	10.3±1.5	8.1±1.4	13.4±2.7*
IRI t_{60} [μU/ml]	79.1±8.2	64.3±8.9	100.1±11.7**
IRI t_{120} [μU/ml]	51.5±8.0	32.7±6.6	78.4±10.7**

F: female, M: male, S-Cr: serum creatinine concentration, Cl_{Cr}: creatinine clearance, BMI: body mass index, Glu: venous plasma glucose concentration, IRI: immunoreactive insulin, t: time interval in minutes after oral glucose load of 75 g, data are given as mean±SEM, *: $p<0.05$, **:$p<0.01$ insulin sensitive vs. insulin resistant patients with decreased renal function.

Ž: ženy, M: muži, S-Cr: koncentrácia kreatinínu v sére, Cl_{Cr}: klírens endogénneho kreatinínu, BMI: body mass index, Glu: koncentrácia glukózy vo venóznej plazme, IRI: immunoreaktívny inzulín, t: časový interval v minútach po orálnej záťaži glukózou v dávke 75 g, dáta sú prezentované ako priemer±SEM, *: $p<0,05$, **: $p<0,01$ inzulín senzitívni vs. inzulín rezistentní pacienti so zníženou funkciou obličiek.

Etiology of renal disease Etiológia ochorenia obličiek	(n=22)
Hypertension Hypertenzia	5
Glomerulonephritis Glomerulonefritída	4
Pyelonephritis Pyelonefritída	4
Diabetic nephrosclerosis Diabetická nefroskleróza	3
Interstitial nephritis Intersticiálna nefritída	1
Other Iné	5

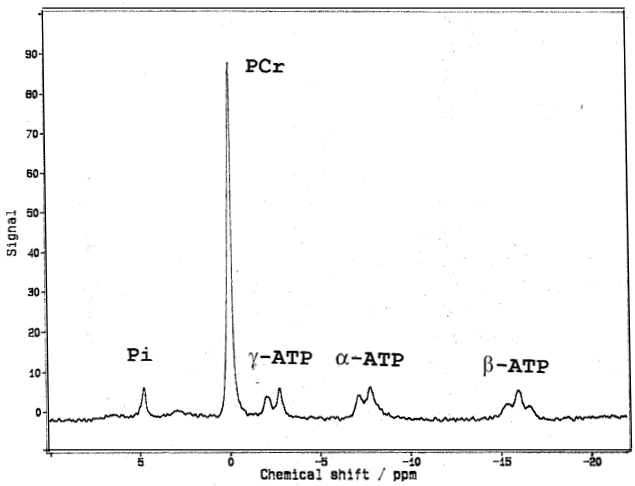

Fig. 1. ^{31}P NMR spectrum of resting calf muscle of a healthy volunteer. Pi: inorganic phosphate; PCr: phosphocreatine; α,ß,τ-ATP: phosphate peaks of adenosine triphosphate (ATP); intracellular Mg^{2+} binds to ATP within the cell and alters the chemical shift of α-ATP compared to ß-ATP, which is used to calculate fMg concentration.
Obr. 1. ^{31}P NMR spektrum lýtkového svalu zdravého dobrovoľníka v pokoji. Pi: anorganický fosfát; PCr: fosfokreatín; α,ß,τ-ATP: fosfátové píky adenozíntrifosfátu (ATP); intracelulárne Mg^{2+} sa v bunke komplexuje s ATP a mení chemický posun α-ATP v porovnaní s ß-ATP, čo sa využíva na výpočet koncentrácie voľného intracelulárneho Mg.

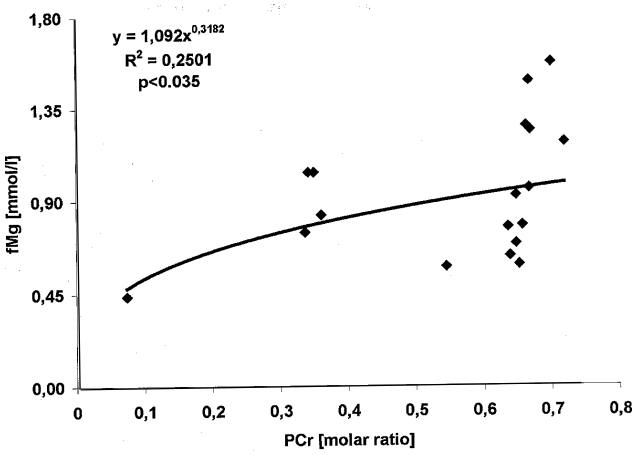

Fig. 3. Correlation of free magnesium concentration and the molar ratio of phosphocreatine in the resting calf muscle of the dominant leg in healthy volunteers. fMg: free magnesium concentration; PCr: phosphocreatine.
Obr. 3. Porovnanie koncentrácie voľného magnézia a molárneho zlomku fosfokreatínu v lýtkovom svale u zdravých dobrovoľníkov v pokoji. fMg: koncentrácia voľného magnézia; PCr: fosfokreatín.

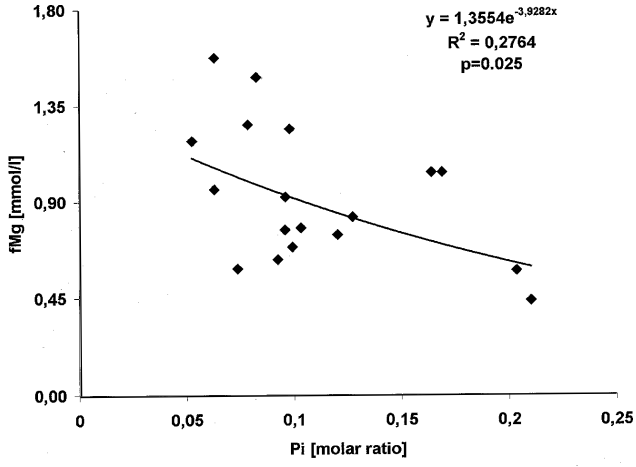

Fig. 2. Correlation of free magnesium concentration and the molar ratio of inorganic phosphate in the resting calf muscle of healthy volunteers. fMg: free magnesium concentration; Pi: inorganic phosphate.
Obr. 2. Porovnanie koncentrácie voľného magnézia a molárneho zlomku anorganického fosfátu v lýtkovom svale u zdravých dobrovoľníkov v pokoji. fMg: koncentrácia voľného magnézia; Pi: anorganický fosfát.

and creatinine. Creatinine clearance was estimated from 24 h collection of urine. In 15 patients acid base balance was determined (capillary blood).

Statistical analysis: For comparison (controls vs patients) Wilcoxon test was used. Differences among groups (controls vs IS and IR patients with kidney disease) were determined employing ANOVA and Newman—Keul test. Correlation and regression analyses were performed. Results are given as mean±SEM. $p>0.05$ was considered to be significant.

Results

Free Mg concentration in skeletal muscle: In healthy volunteers fMg concentration correlated with molar ratios of Pi (Fig. 2), Pcr (Fig. 3), and the ratios of Pcr/Pi ($r=0.549$, $p>0.018$) and Pcr/ATP ($r=0.462$, $p>0.045$).

Mean fMg concentration in resting skeletal muscle of the patients did not differ from that of the controls (Tab. 2). No correlation between fMg levels and parameters of muscle energy balance or intracellular pH was revealed in kidney diseased patients, regardless of IR.

Intracellular skeletal muscle pH: Patients with kidney diseases exhibited a slight but significant shift of pHi in striated muscle towards acidic values (Tab. 2). In 15/22 patients blood pH was determined, extracellular pH was within normal values (7.372±0.011), while intracellular acidosis was confirmed (7.013±0.006). No correlation was found between extracellular and intracellular pH. In the patient group serum creatinine concentrations ($r=-0.505$, $p>0.02$) as well as clearance of endogenous creatinine (Fig. 4) correlated with pHi, pointing to the association of the decreased kidney function with intracellular acidosis. Evaluating all studied subjects together, pHi values correlated inversely with BMI (Fig. 5), suggesting an association in alteration of metabolic regulation.

Energy balance: The relative amount of Pcr in the resting calf muscle was higher, while that of ATP was lower in patients with kidney disease if compared with controls (Tab. 2). However, the ATP/Pi ratio remained decreased in the patient group. Increase in

Tab. 2. Magnesium concentration, intracellular pH and parameters characterizing energy metabolism in the calf muscle of the dominant leg of the healthy controls and patients with kidney diseases at rest.
Tab. 2. Koncentrácia voľného magnézia, intracelulárne pH a ukazovatele charakterizujúce energetický metabolizmus v lýtkovom svale dominantnej nohy v súbore zdravých kontrol a inzulín-senzitívnych a inzulín-rezistentných pacientov so zníženou funkciou obličiek.

	Controls Kontroly (n=18)	All patients Všetci pacienti (n=22)	IS patients IS pacienti (n=12)	IR patients IR pacienti (n=10)
fMg_m [mmol/l]	0.929±0.075	0.948±0.062	0.940±0.097	0.957±0.081
pH_i	7.036±0.004	7.013±0.004**	7.018±0.006	7.008±0.005**
P_i [molar ratio] [molárny zlomok]	0.111±0.011	0.088±0.002*	0.090±0.003	0.085±0.004
Pcr [molar ratio] [molárny zlomok]	0.554±0.042	0.674±0.003**	0.670±0.007**	0.679±0.005**
ATP [molar ratio] [molárny zlomok]	0.335±0.034	0.237±0.004**	0.239±0.002**	0.235±0.004**
ATP/P_i	3.18±0.020	2.73±0.11*	2.72±0.18	2.73±0.12
Pcr/P_i	6.33±0.85	7.71±0.21	7.55±0.30	7.90±0.30
Pcr/ATP	2.04±0.23	2.84±0.06**	2.83±0.09**	2.85±0.07**

fMg_m: free intracellular magnesium concentration in calf muscle, pH_i: intracellular pH, P_i: inorganic phosphate, Pcr: phosphocreatine, ATP: adenosintriphosphate, data are given as mean SEM, *: p<0.05, **: p<0.01 vs. healthy controls.
fMg_m: koncentrácia voľného intracelulárneho magnézia v lýtkovom svale, pH_i: intracelulárne pH, P_i: anorganický fosfát, Pcr: fosfokreatín, ATP: adenosintrifosfát, dáta sú prezentované ako priemer±SEM, *: p<0,05, **: p<0,01 vs. zdravé kontroly.

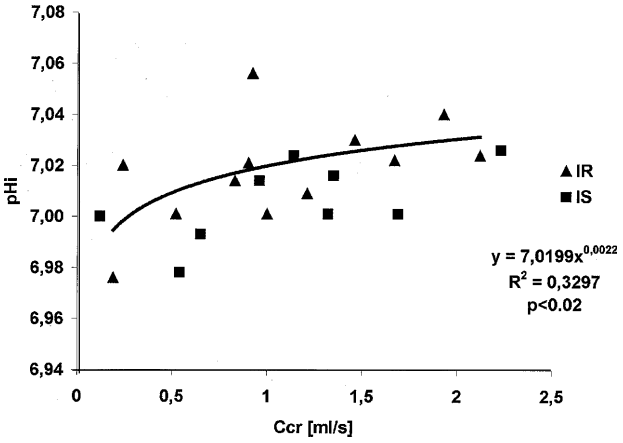

Fig. 4. Relationship of intracellular pH of skeletal muscle to creatinine clearance in patients with impaired kidney function. pH: intracellular pH; Ccr: clearance of endogenous creatinine.
Obr. 4. Vzťah medzi intracelulárnym pH v kostrovom svale a klírensom endogénneho kreatinínu u pacientov so zníženou funkciou obličiek. pH: intracelulárne pH; Ccr: klírens endogénneho kreatinínu.

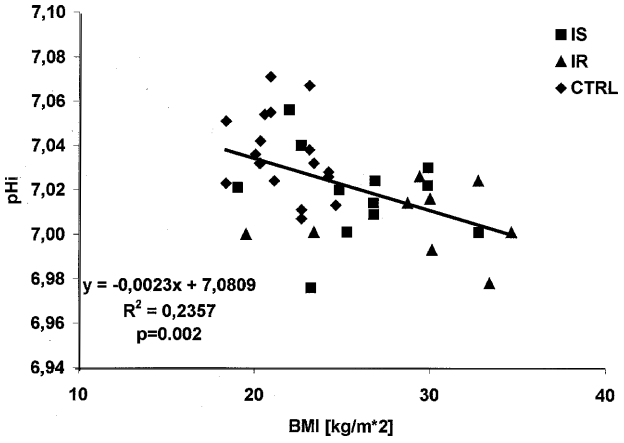

Fig. 5. Relationship of body mass index to intracellular pH of skeletal muscle in healthy controls and insulin sensitive and insulin resistant patients with kidney disease. BMI: body mass index; pHi: intracellular pH in the resting calf muscle; IS: insulin sensitive patients, IR: insulin resistant patients with kidney disease.
Obr. 5. Vzťah medzi BMI a intracelulárnym pH v kostrovom svale zdravých dobrovoľníkov a pacientov s ochorením obličiek. BMI: body mass index; pHi: intracelulárne pH v lýtkovom svale v pokoji; IS: inzulín-senzitívni pacienti, IR: inzulín-rezistentní pacienti s ochorením obličiek.

Pcr molar ratio resulted in an increase of Pcr/ATP ratio in our patients.

Insulin resistance: Comparison of the insulin sensitive and insulin resistant patients with the control group revealed differences in intracellular pH (IR patients) and Pcr/ATP ratio (Tab. 2). If fMg is responsible for IR in kidney diseased patients, the resting striated muscle fMg concentration should have been decreased. No correlation between fasting IRI levels or amount of insulin needed to metabolize a standard glucose load and intracellular pH or fMg concentration in skeletal muscle was found.

Discussion

Insulin resistance: Insulin resistance, almost constant in advanced renal failure, is a consequence of accumulation of various metabolic end-products, such as hippurate (Spustová and Gerykoová, 1989), pseudouridine (Dzúrik et al., 1993), 5-hydroxy-3-indoleactic acid (Šebeková et al., 1991, 1996), and with high probability also other accumulated inhibitors. However, IR is present even before the accumulation of unexcreted inhibitors and even in patients with mild kidney function reduction (Štefíková et al., 1993). Because of the acceleration of atherosclerosis, and obviously also

progression of kidney disease, IR is carefully studied by various nephrological groups (Mak and DeFronzo, 1992; DeFronzo et al., 1973).

IR in kidney diseased patients differs from that observed in essential hypertension: while patients with essential hypertension exhibit decreased intracellular free Mg concentration (fErMg) in red blood cells, in kidney diseased patients fErMg levels are within normal range. The presented, as well as other (Irish et al., 1997) results also exclude a selective decrease of fMg concentration in striated muscle, that is responsible for IR because of the decisive role of skeletal muscle in glucose utilisation. In essential hypertension IR is associated with a shift of red blood cell Mg from free to bound form. In our study the total Mg concentration in striated muscle was not evaluated, thus the intracellular shift could not be excluded. Moreover, subcellular distribution of ionised Mg in striated muscle should be taken into account. In vascular smooth muscle cells nuclear area contains a higher concentration of free ionised Mg than the peripheral area (Altura et al., 1993). The unchanged fMg concentration in skeletal muscle of our patients does not exclude the changes in free ionised Mg compartmentation, or shifts between its free and bound form. Thus, the pivotal role of intracellular fMg as a whole could be excluded, and intracellular or subcellular distribution of ionised free Mg should be investigated, or, more probably, another mechanism responsible for IR in kidney diseased patients should be sought.

Energy balance: IR could have been caused by the energetic deficiency with changes in intracellular concentrations of macroergic compounds, and by opening the ATP-dependent K^+ channel (Fosset et al., 1988), increase in transmembrane potential, closure of the voltage dependent Ca channel and decreased glucose utilisation (opposite to mechanism involved in stimulation of glucose utilisation by sulfonylurea antidiabetics). This mechanism participates in IR caused by various uremic inhibitors, such as hippurate (Spustová and Dzúrik, 1991), pseudouridine (Dzúrik et al., 1993), or 5-hydroxy-3-indoleacetic acid (Šebeková et al., 1996). This alternative might be excluded, since in macroergic phosphates no deficiency was observed.

Intracellular muscle pH: The unexpected result of the present study was the finding of a slight but significant intracellular acidosis without extracellular metabolic acidosis or hypokaliemic alkalosis accompanying intracellular acidosis in patients with even mild decline in kidney function.

Normotensive non-insulin dependent diabetics exhibited the shift in intracellular pH in red blood cells towards alkalosis, while in patients with essential hypertension, and surprisingly also in patients with NIDDM and high blood pressure pH values in red blood cells were significantly decreased (Resnick, 1992). Hemodialyzed patients with chronic renal insufficiency exhibited lower, or comparable values of intracellular pH in skeletal muscle at rest than healthy volunteers (Durozard et al., 1993; Moore et al., 1993; Táborský et al., 1993; Irish et al., 1997). Thompson et al. (1994) reported a non-significant decrease of intracellular pH in uremic muscle of patients without any form of renal replacement therapy.

In case of normal extracellular acid-base balance, intracellular pH depends significantly on the activity of Na^+/H^+ antiporter (Aviv, 1992). Augmented activity of Na^+/H^+ antiport was suggested to cause IR in patients with essential hypertension (Aviv, 1992). Indeed, in spite the decreased skeletal muscle pH found in patients with essential hypertension, in the subgroup of IR patients fasting IRI levels as well as the summed insulin response to glucose load correlated positively with intracellular pH. On the other hand no correlation between the severity of IR and intracellular pH or fMg concentration was found in the present study. This findings support the view, that IR in patients with renal disease might be of different aetiology from that found in essential hypertension. Intracellular acidosis, which participates on development of IR directly or indirectly, must be therefore caused by the decreased activity of Na^+/H^+ antiporter. The effect of undefined inhibitor is anticipated. In any case its accumulation is not a consequence of decreased renal function, since half of the patients even with mild kidney function reduction suffers from IR.

In conclusion, IR in kidney disease patients does not seem to be caused by the decreased free Mg concentration (as found in essential hypertension), or by change in macroergic phosphate levels (found to be caused by several uremic inhibitors of glucose utilization). Intracellular striated muscle acidosis is an early sign of renal disease, and could participate in IR. However, its mechanism remains to be elucidated. The decreased activity of Na^+/H^+ antiporter is suggested.

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