C001
DIFFERENTIAL EXPRESSION AND REGULATION OF
DOPAMINE-1(D1) AND DOPAMINE-5D-5
RECEPTOR FUNCTION IN HUMAN KIDNEY
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The renal dopaminergic system plays an important role in the regulation of blood pressure, sodium balance, and kidney function. Two D1-like receptors (D1 and D5) are expressed in mammalian kidney, however, their distribution and cAMP linkage in human kidney have not been studied.

With immunohistochemistry, we found that the D1 receptor is expressed in luminal and basolateral membrane of proximal tubules (PT), distal convoluted tubules (DCT), loop of Henle (LH), cortical collecting duct (CCD), juxtaglomerular apparatus (JGA), and intima and smooth muscles of renal arterioles. The D5 receptor, like the D1 receptors, is found in proximal tubules but only in luminal membrane. The D5 receptor, unlike the D1 receptor, is not found in the other nephron segments or arterioles. Neither the D1 nor D5 receptors to D1-like agonist stimulation of cAMP production, in proximal tubules in culture. We studied the effect of dopamine and fenoldopam, a D1-like agonist, in the presence or absence of sense or antisense oligonucleotides, since no ligand can distinguish the D1 from the D5 receptor. Basal cAMP production was 1107 ± 32 fmol/mg protein/min (n = 8). Dopamine and D1-like agonist, fenoldopam (10−6 to 10−4) dose-dependently increased cAMP production, Emax = 32 ± 3% and 67 ± 1% respectively, D1 antisense oligonucleotides decreased the ability of fenoldopam (10−6 M) to increase cAMP production (−76 ± 5%, p < 0.05, n = 6). In contrast, D5 antisense oligonucleotides had little effect (−15 ± 5%, n = 4). Sense or scrambled oligonucleotides D1 and D5 had negligible effect of cAMP production. We conclude that D1 and D5 receptors are differentially expressed in the human kidney. In renal proximal tubules where both D1-like receptors are expressed, D1 receptor predominates over D5 receptors action on cAMP production.

Key Words: Dopamine; dopamine receptor; cAMP

C002
EFFECT OF LEAD ACETATE ON VASCULAR
RESPONSIVENESS IN VITRO
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A prevailing mystery in lead toxicology, for which this study seeks an explanation, is why low levels of lead cause hypertension in man and the experimental animal whereas high levels have no effect. In previous studies we have shown that blood vessels removed from rats made hypertensive by feeding 100 ppm lead acetate have no alterations in contractile response to norepinephrine or relaxation response to acetylcholine (Purdy RE et al, Am J Hypertens 1997;10:997–1003). In this study, we examined the in vitro response of thoracic aorta removed from normal Sprague-Dawley rats to graded doses of lead acetate from (10−9 to 10−3 M). Experiments were done in intact and endothelium-denuded blood vessels, examining responses to norepinephrine (10−8 to 10−6 M), acetylcholine or methylfurmethide (10−8 to 10−6 M), potassium chloride (6–80 mM) and phorbol ester (phorbol-12-myristate-13-acetate) (2–4 μM). Lead acetate had no effect on contraction in either intact or denuded blood vessels induced by norepinephrine, potassium or phorbol ester in doses ranging from 10−9 to 10−6 M. At 10−6 M, lead acetate had equivocal effect on contraction induced by potassium and norepinephrine whereas at 10−5 M the effects were definite. Likewise lead reduced the relaxation effect of acetylcholine at 10−5 M lead acetate. In contrast, lead acetate at 10−5 M had no effect on the calcium-independent contraction caused by phorbol ester in a calcium-free medium. In view of the coordination of blood pressure and reactive oxygen species measurements in low lead-treated animals given various scavengers of reactive oxygen species we speculate that the reactive oxygen species are responsible for the low lead-induced-hypertension, whereas a direct inhibitory effect of high lead on calcium channels counteracts this effect.

Key Words: Lead; vascular reactivity; norepinephrine; acetylcholine; potassium; phorbol ester

C003
ENTERAL NACl ABSORPTION IN NORMOTENSIVE
SUBJECTS
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Although amiloride-sensitive Na channels are present in most epithelial cells, any information is available in regard to Amiloride (AI) effects on enteral NaCl absorption. To assess this effect we measured plasma (Na, K, Cl, Ca++, ionized Osmol) RBC (Nai, Ki) and 12-h night urine (Na, K, Cl, Ca++, Osmol, pH) electrolytes, in a double-blind placebo-controlled study, in two age-sex matched groups: Group A (n = 7): 30 ± 1 yr., BMI 25 ± 2 Kg/m2², BP 122 ± 12/77 ± 8 mm Hg vs. Group B (n = 6): 31 ± 2 yr., BMI 23 ± 2 Kg/m²², BP 116 ± 8/77 ± 5 mm Hg. All on normal diet with large NaCl intake (uNa ≥ 145 mmol/l, Cl ≥ 123 mmol/l, Osmol ≥ 635 mosmol), starting 5 mg AI (Group A) or placebo (Group B) for 1 week. BP, HR, BMI and plasma (Na, K, Cl, Ca++, ionized, Osmol) RBC (Nai, Ki) and 12-h night urine (Na, K, Cl, Ca++, Osmol, pH) electrolytes were obtained before drug (0 day) followed by 12-h urine collection (days 1–7) and all studies repeated after 1 week washout period (day 14). AI rapidly decrease uNa 78 ± 19 mmol/l, Cl 86 ± 26 mmol/l and Osmol 375 ± 109 mosmol (p < .0001) at 3rd day and uNa 84 ± 26, Cl 79 ± 21, Osmol 47 ± 167 at 7th day (p < .0001) with decrease Nai (5 ± 0.2 mmol/l/c, p = 0.003) and normal plasma Na. Basal uK 38 ± 12 was decreased 21 ± 13 mmol/l at 3rd day (p = 0.002) and 17 ± 4
at 7th day (p<0.001) without changes in Ki and plasma K. At the 14th day, all plasma and urine electrolytes returned to previous basal levels. In Group B, all studies were unchanged during placebo trial. We present the first evidence of an enteral effect of AI, which explain some of its pharmacological action.

Key Words: Amiloride; NaCl enteral absorption; sodium; potassium

C004  
IN VITRO EFFECTS OF CATHECOLAMINES ON INTRACELLULAR FREE MAGNESIUM IN HUMAN LYMPHOCYTES  
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A catecholamine-induced Mg$^{2+}$ efflux has been described, nevertheless no changes of intracellular free magnesium (Mgi) following β-agonist addition have been reported in human cell types. We in vitro measured Mgi in human lymphocytes incubated with either norepinephrine (NE) or isoproterenol (IS). Peripheral blood lymphocytes from healthy donors were isolated and Mgi was measured using a method based on the dye furaptra. Lymphocytes incubated with NE (10⁻⁶ mol/l) showed significantly reduced Mgi values as compared to control cells (Mgi: 538 ± 6 mol/l; NE: 172 ± 13 mol/l, P < 0.0001). The NE-induced Mgi decrease was prevented by incubating the cells with propranolol (PR), 2⁻⁶ mol/l solution (Mgi: 308 ± 9 mol/l; NE: 238 ± 29 mol/l, P = 0.0001). The NE-induced Mgi decrease was abolished by incubating the cells in a Na⁺-free solution (M ≥ SD, n = 6, Mgi, control: 238 ± 32 mol/l; NE and PR: Mgi, 239 ± 30 mol/l, N.S.). The catecholamine-induced Mgi decrease is abolished by incubating the cells in a Na⁺-free solution (M ≥ SD, n = 6, Mgi, Na⁺ solution: 233 ± 172 mol/l; Na⁺ solution and NE: 172 ± 42 mol/l, p = 0.01; Na⁺-free solution: 538 ± 89 mol/l; Na⁺-free solution and NE: 530 ± 70 mol/l, N.S.). By artificially increasing intralymphocyte AMPc, we found a Mgi decrease similar to the catecholamine-induced effect (M ≥ SD, n = 3, control: 265 ± 21 mol/l; AMPc cells: 199 ± 13 mol/l, P < 0.01). These data are in agreement with data collected in non-human cell types and are in favour of the hypothesis that catecholamines may regulate Mgi by means of binding to membrane β-adrenergic receptors, AMP cyclic stimulation and activation of membrane Na⁺-Mg$^{2+}$ exchanger.

Key Words: Magnesium; hypertension; lymphocytes

C005  
PRESSURE RESPONSE TO ISOMETRIC EXERCISE IN YOUNG NORMOTENSIVE SUBJECTS: INFLUENCE OF THE INTRACELLULAR POTASSIUM  
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Isometric Exercise (IE), as a physical Stress, may reveal cardiovascular abnormalities not present in at rest. Several studies have reported that young normotensive offspring of hypertensive patients presents abnormal hemodynamic responses during IE. Intracellular Potassium (Ki) plays an important role in the pathophysiology of Arterial Hypertension (AH) and its has been proposed as an Intermediate Phenotype. The aim of the study was to evaluate the effect of Ki (mEq/l) in the pressure response during IE. 39 subjects participated in the study (Female n = 20, Male n = 19), age 21.66 ± 2.64 yr., BMI 23.91 ± 2.85 Kg/m². Screening for Family History for AH (FH) and physical examination was performed. Blood sample for red blood cell Ki was taken at basal conditions. Pulse and Blood pressure (SBP, DBP, MBP) were obtained at basal and during IE. Later patients and data were grouped according to FH (Kim < 88/FH+) and FH (Kim ≥ 88/FH-). Statistical difference was observed in basal conditions, a progressive increase of Ki, from group (1) to (4) was observed. ANOVA test showed statistical difference in Ki (F = 17.870, p < 0.0001) and MBP (F = 14.63, p < 0.0001) within the 4 groups; not quite statistical significance in DBP (F = 2.645, p = 0.069). Statistical difference was obtained in DBP (p = 0.046) and MBP (p = 0.042) only when (1) vs (4) groups were compared. Besides, a tendency of decrease of blood pressure and pulse with progressive increase of Ki, was also observed. During IE, ANOVA showed statistical difference in DBP (F = 3.693, p = 0.020) and MBP (F = 3.341, p = 0.030). Statistical difference was observed in DBP and MBP when compare (1) vs (4): (DBP p = 0.020 y MBP p = 0.035) y (K3) vs (4): (DBP p = 0.006 y MBP p = 0.0419). When comparing data at basal and during IE, statistical difference was found in groups (1) and (2). Pulse shows no difference in group (3). No statistical difference was observed in group (4). Ki, together with FH, influence the pressure response at IE in young normotensive subjects.

Key Words: Potassium; isometric exercise; physical stress; intracellular potassium