P-109
IMPAIRED RESPONSE OF THE DENERVATED KIDNEY TO ENDOTHELIN RECEPTOR BLOCKADE IN NORMOTENSIVE WISTAR-KYOTO (WKY) AND SPONTANEOUSLY HYPERTENSIVE RATS (SHR)
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The exact role of endothelin (ET) acting through ETA receptors in renal sodium and water regulation and the potential implications of an interaction of the renal ET system with renal nerves in normotensive and spontaneously hypertensive rats is still unknown. Therefore, in the present study bilateral renal denervation (BRD) was performed in WKY rats and in SHR 7 days before the experiments. The ETA receptor blocker BQ-123 was infused i.v. at a rate of 16.4 nmol/kg/min. Renal papillary ET-1 content was 68% lower in SHR than in WKY. BRD decreased the ET-1 content in WKY by 74% but had no effect on the low ET-1 content in SHR. BQ-123 did not affect GFR or renal plasma flow. In WKY BQ-123 decreased urine flow and electrolyte excretion by approximately 50%. This effect was no more observed after BRD. In SHR BQ-123 only affected urine flow rate significantly but not electrolyte excretion. Again, this effect was no more observed after BRD. For comparison, the ETB receptor blocker BQ-788 had similar effects as BQ-123 to decrease urine flow and electrolyte excretion with no effects on excretion function in denervated WKY rats and SHR. In contrast to BQ-123, BQ-788 also had no effect on urine flow in intact SHR. We conclude that an interaction between the renal ET system and renal nerves is involved in the control of renal function where renal nerves participate in the regulation of ET-1 production. Decreased synthesis of ET-1 in the renal papilla of SHR may contribute to the development and/or maintenance of hypertension via modulation of renal excretox function.

Key Words: Endothelin, Renal Denervation, WKY and SHR

P-110
EFFECTS OF ERYTHROPOIETIN ON BLOOD PRESSURE AND HEMATOCRIT
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Erythropoietin (Epo) stimulates increased blood viscosity and raises blood pressure (BP). We examined effects of any circadian stage-dependence of Epo on BP and hematocrit (Hct) on 5 weeks old Sprague-Dawley rats kept on a daily light (04:00–18:00) cycle and alternating darkness with Purina chow and tap water freely available. To seek an effective treatment (Rx) time, rats were randomly assigned into control or Rx groups (n=5 per group) at 00, 04, 08, 12, 16 and 20 hours. Intraarterial BP and Hct were measured just before and immediately after the completion of a 4-week course of twice weekly Epo (50U/kg BW) or physiological saline injections. Before Rx, inter-group differences for BP, Hct and body weight (BW) were not significant. Epo Rx lowered BW and markedly, and increased Hct over all and at each of the 6 test times, all p<0.0001, and splenomegaly characterized each rat. Significantly increased BPs were detected at 12, 16, 20 and 00 hours, but not at 04 or 08 hours. Before Rx, two groups had similar circadian BPs. After Rx, circadian BP in Epo Rx group showed significantly higher than that of saline group, 135±2.6 vs. 116±1.7, p<0.0001. Furthermore, Epo Rx group had markedly increased amplitude than that of the saline group, 48.7 vs. 27.2, p<0.0001. The results conclude that the time of the Epo Rx may be important. Epo concentration in the clinical use should be reevaluated to reduce adverse effects, such as high BP and target organ damages. Key Words: Erythropoietin, angiogenesis, circadian blood pressure, splenomegaly

Key Words: Erythropoietin, Angiogenesis, Circadian Blood Pressure

P-111
EFFECTS OF PURE HUMAN ERYTHROPOIETIN-BINDING PROTEIN AND ITS ANTIBODY
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Biologically active human erythropoietin-binding protein (Epo-bp), MW=29 kDa, was purified from human Epo-receptor extracellular domain (EpoR) with a thrombin cleavage site (EpoRx-th). To produce human Epo-bp, recombinant vector JYL26 was constructed by inserting EpoR DNA PCR into a pGEX-2T plasmid vector, and transformed into the E. Coli strain JM109. EpoRx-th fusion protein, containing glutathione-S-transferase and Epo-bp, was extracted by affinity chromatography. After cleaving by thrombin, Epo-bp was purified by Epo-agarose chromatography, and verified by the SDS-gel electrophoresis and western blotting. Trypsin digestion of Epo-bp was performed to test a minimum sequence of EpoR required in ligand binding. The trypsin cleaved Epo-bp into ~20 kDa, however, completely eliminated the recognition of anti-Epo-bp antibody, as verified on western blotting. Effects of Epo-bp in ligand binding were verified using 125I-Epo ± unlabeled Epo. There was a strong binding affinity of Epo to the purified human Epo-bp in nanomolar concentrations, and unlabeled Epo eliminated the binding (p<0.0001). Thus, the binding of Epo to Epo-bp was specific, and requires an intact extracellular domain protein of EpoR. The binding sites of blood cell progenitors were elaborated using Epo-bp and its antibodies. Serum and plasma Epo and Epo-bp levels were similar: Epo 25.4±2.17; 24.2±2.35; and Epo-bp 24.2±1.84; 25.0±1.26 μM/ml, respectively. Serum anti-Epo and anti-Epo-bp antibody levels were similar but plasma Epo-bp antibody level was significantly lower than those of serum counterparts (p<0.025). The two methods are much simpler and more sensitive as compared to the conventional radioimmunoassay (Epo in serum 17.7±6.3 μM/ml). Biochemical values and cardiovascular parameters are compared in clinical samples.

Key Words: Erythropoiesis, Erythropoietin receptor, Epo-bp, Binding site, Cardiovascular parameters

P-112
AMINOGUANIDINE RESTORES REACTIVITY OF PULMONARY AND SYSTEMIC VESSELS IN RATS WITH MONOCROTALINE-INDUCED PULMONARY HYPERTENSION
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Objective. Monocrotaline (MCT)-induced pulmonary hypertension (PH) is associated with impaired endothelium-dependent relaxation and increased activity of inducible NO-synthase (iNOS). To examine a role of iNOS in MCT-induced PH, we used an iNOS inhibitor- amino guanidine (AG).

Design and Methods. PH was modeled by subcutaneous injection of 60 mg/kg MCT to Wistar rats; control rats were injected by saline. Then each group was separated to 2 sub-groups: 1-st was given drinking water (MCT-C and C-C groups) and the 2-nd was given AG in drinking water (15 mg/kg “day−1”) (MCT-AG and C-AG groups). Four weeks later right ventricular systolic pressure (RVP) to diagnostic PH was evaluated. Then perfusion pressure (PP) responses of isolated pulmonary (PPPA) and systemic (PPSA) arteries to acetylcholine (Ach) and activator of soluble guanylate cyclase (sGC), FPTO, were examined.

Results. PH has been developed: in MCT-C group RVP was increased [76±11 vs. 26±2 mm Hg in C-C group, p<0.05], but in MCT-AG group RVP was decreased to 47±7 mm Hg [p<0.05 vs. MCT-C group]. In MCT-C group decrease of relative PPA to 5±10−8 M Ach and 1±10−8 M FPTO was diminished [−24±5 and −41±13 % vs. −85±11 and −97±20 % in C-C group, respectively, p<0.05], decrease of relative