Resumos
Setor 04
Farmacologia Cardiovascular e Renal

04.001

PROGRAMMING THE ENDOTHELIUM DYSFUNCTION *IN UTERO*: ROLE OF SUPEROXIDE ANION, NITRIC OXIDE AND ANGIOTENSIN II

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Aim: It is well known that intrauterine undernutrition (IU) contributes to the development of cardiovascular disease in adulthood. In addition, the vascular diseases that have been linked to IU are characterized by endothelial dysfunction. The aim of this study was to explore the mechanisms involved in programming of endothelium dysfunction *in utero*. **Methods:** Female pregnant Wistar rats were fed either normal or 50% of the normal intake diets, during the whole gestational period. In male offspring, arteriolar diameter was measured *in vivo* by intravital microscopy before and after application of bradykinin (BK) or acetylcholine (ACh) in the absence or presence of SOD mimetic, tetrahydrobiopterin (BH_4) (NOS cofactor) or apocynin (NADPH-oxidase inhibitor). Superoxide anion generation (hydroethidine method) was studied in the absence or presence of apocynin, BH_4 or losartan. Arterial blood pressure, NOS and SOD activities, NO production (DAF-2), angiotensin II (ANGII) concentration (HPLC) and AT_1, $p22^{phox}$, $gp91^{phox}$ and eNOS gene expression (RT-PCR) were determined. **Results:** IU induced hypertension, decreased vasodilation to ACh (4.53± 0.4*vs.*10.3± 0.4%) and BK (5.87± 0.6*vs.* 10.9± 0.9%). Topical application of SOD mimetic, apocynin and BH_4 significantly improved the altered arteriolar responses to ACh and BK. Decreased SOD and NOS activities, reduction in NO production (2.1 ± 0.2*vs.*2.9 ± 0.2), increased superoxide concentration (25.84 ± 2.60 *vs.*10.83 ± 1.72%), enhanced local ANGII concentration, attenuation of oxidative stress by BH_4, apocynin and losartan and improvement of NO production after treatment with BH_4 were observed. IU did not alter the gene expression for eNOS, AT_1, $p22^{phox}$ and $gp91^{phox}$. **Conclusion:** This study shows that IU programmed endothelium dysfunction by: 1) enhancing oxidative stress, which is associated with decreased SOD activity and increased activation of NADPH oxidase via ANGII-mediated mechanism; 2) decreasing NO production by impairment of BH_4 pathways. **Supported by:** FAPESP

04.002

ENDOTHELIN-1 (ET-1) CONTRIBUTES TO THE SEXUAL DIFFERENCES IN STRUCTURAL RENAL INJURIES IN DOCA-SALT RATS.

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We investigated whether ET-1 is involved in the modulation by the ovarian hormones of renal damage in DOCA-salt hypertension. Male and female control and DOCA-salt rats were treated with the ET_A antagonist BMS182874 (40 $mg.Kg^{-1}.day^{-1}$) or vehicle. Females were also ovariectomized (OVX) and treated with estrogen (0.5 mg/pellet), progesterone (50 mg/pellet) or estrogen+ progesterone. Histological analysis of renal sections was performed to evaluate the presence of renal damage. Arbritary units (AU= 0, no change; AU= 5, presence of severe renal damage) were used. Renal injuries were more severe in male (DOCA= 3.4 vs control= 0) than female DOCA-salt rats (DOCA= 1.5 vs control= 0). BMS treatment ameliorated renal damage in male (AU= 1.7) and female (AU= 0.6) DOCA rats. OVX exacerbated renal injuries in female DOCA-salt (AU= 2.5), but did not induce renal damage in control rats (AU= 0). Treatment with estrogen+progesterone (AU= 1.6) and progesterone (1.9), but not estrogen alone (AU= 3.0), attenuated renal damage in OVX DOCA-salt rats. BMS treatment greatly ameliorated renal damage in OVX DOCA rats (AU= 1.0). Extension of renal damage in male DOCA rats was partially related to blood pressure (BP) values since treatment with hydralazine partially attenuated both BP (154±10 mmHg vs 189±5 mmHg DOCA-salt) and renal damage (AU= 2.9). Pre-pro-ET-1 mRNA expression was increased in male DOCA (vs control) and OVX DOCA-salt (vs. female DOCA) rats. In conclusion, the ovarian hormones display a protective role in the renal structural alterations in female DOCA rats and ET-1, via ET_A receptors, contributes to the more severe renal damage in male and OVX female DOCA rats. **Supported by:** CNPq, FAPESP, PRONEX,

04.003

EFEITOS DE TRATAMENTO CRÔNICO COM ATENOLOL E CAPTOPRIL SOBRE A DENSIDADE CAPILAR MUSCULAR E CUTÂNEA DE RATOS ESPONTANEMENTE HIPERTENSOS (SHR).

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Introdução: A rarefação dos vasos da microcirculação é uma alteração característica da hipertensão arterial primária. Investigamos os efeitos de um tratamento crônico com o beta-bloqueador atenolol (ATE) e com o inibidor da enzima conversora de angiotensina captopril (CAP) sobre a densidade capilar cutânea e no músculo esquelético (grácil) de SHR. **Métodos:** Ratos SHR machos com 12-14 semanas, receberam tratamento oral por gavagem com ATE (50 mg/kg/dia), CAP (25 mg/kg/dia) ou veículo (grupo controle) durante 4 semanas. Após o término do tratamento os animais foram anestesiados e a densidade capilar avaliada através de microscopia intravital por epi-iluminação com fluorescência. **Resultados:** O tratamento com os anti-hipertensivos normalizou a pressão arterial sistólica dos ratos SHR [176 ± 11 mmHg para 125 ± 11 mmHg ($P< 0,05$, n=8) e de 170 ± 5 mmHg para 122 ± 7 mmHg ($P< 0,05$, n=8), com ATE ou CAP, respectivamente]. Foi observado aumento significativo da densidade capilar muscular esquelética de ratos SHR tratados com ATE ou CAP (405 ± 62 e 464 ± 69 capilares/mm^2, respectivamente) quando comparados com o grupo controle (305 ± 40 capilares/mm^2, $P<0,05$). Também foi observado aumento da densidade capilar cutânea (372 ± 43 e 359 ± 30 capilares/mm^2, para tratamento com ATE e CAP, respectivamente). **Discussão:** O presente estudo demonstrou que o tratamento crônico com os anti-hipertensivos ATE ou CAP induz aumento da densidade capilar cutânea e muscular esquelética em ratos SHR. **Apoio Financeiro:** CNPq/FAPERJ

04.004
VASCULAR ADAPTIVE RESPONSE TO CHRONIC STRESS IN PREGNANT RATS
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Introduction: maternal homeostasis can be altered by stress exposure. Previous data from our laboratory have shown that the vascular adaptive response to stress is characterized by a hyporreactivity to noradrenaline (NA) in both male and female rats. The goal was to determine the vascular adaptive response to chronic stress (CS) in aorta from pregnant (PRG) rats. **Methods:** curves to NA were obtained in aorta, with (+E) and without (-E) endothelium, of PRG and non-pregnant (NPRG) female rats submitted or not (NR) to stress (1-h immobilization/7 days during PRG days 12-19). The rats were killed on the PRG day 20. **Results:** Pregnancy did not alter the aorta reactivity to NA [maximum response (g of tension): +E NPRG NR 4.4±0.3 PRG NR 5.1±0.2 E NPRG NR 6.4±0.2 PRG NR 6.6±0.2, n=7-10, P>0.05]. Similar to that in NPRG rats, stress determined a hyporreactivity to NA in aorta +E of PRG rats [maximum response (g of tension): NPRG CS 3.2±0.3* PRG CS 2.8±0.2*, n=11-12, *P<0.05 related to respective NR]. The reactivity of aorta (-E) was not altered by stress. **Discussion:** Pregnancy did not alter the vascular adaptive response to stress. **Supported by:** FAPESP

04.005
AORTA ADAPTIVE RESPONSE TO STRESS IN SHR RATS: ROLE OF NITRIC OXIDE.
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Introduction: Stress induces a decrease in the reactivity of aorta with endothelium (+E) to noradrenaline (NA), which is a consequence of an endothelial nitric oxide (NO) system hyperactivity (Cordellini & Vassilieff *Gen. Pharmacol.*, 30:79, 1998). It was investigate the aorta adaptive response to stress in SHR with emphasis on NO system. **Methods**: Curves to NA in the absence or presence of L-NAME were obtained in aorta with and without (-E) endothelium from Wistar and spontaneously hypertensive (SHR) rats, 6 weeks of life, submitted (SR) or not (NR) to stress (20-min swimming and 1-h immobilization 25 min apart). **Results:** At the age of 6 weeks, SHR are normotensive [Blood pressure (mmHg): Wistar 123.3±2.1, SHR 123.3±3.4, n=6, P>0.05]. Subsensitivity to NA was observed in both aortas +E and E from SHR related to Wistar [maximum response (g tension) +E: Wistar NR 3.7±0.2 SHR NR 2.5±0.2*, -E Wistar NR 5.9±0.3 SHR NR 4.3±0.4*, n=4-6, *P<0.05 related to Wistar]. Similar to Wistar, stress determined a hyporreactivity to NA in aorta +E from SHR [maximum response (g tension): Wistar SR 2.3±0.2^{+}, SHR SR 1.5±0.2$^{\#+}$ n=5-7, $^{\#}$P<0.05 related to Wistar SR, $^{+}$P<0.05 related to respective NR]. L-NAME abolished the stress-induced adaptive response [maximum response (g tension): Wistar NR 5.9±0.2 SR 6.5±0.6, SHR NR 4.0±0.4* SR 3.7±0.4*, n=4-6, *P<0.05 related to respective Wistar]. The reactivity of aorta (-E) was not altered by the different procedures (P>0.05). **Discussion:** The vascular adaptive response to stress involved an increase in the endothelial NO system activity in both strains. **Supported by:** CAPES

04.006
MECHANISMS UNDERLYING VASOPROTECTION IN AORTIC RINGS FROM ETHANOL CRONICALLY TREATED FEMALE RATS
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Introduction: Gender influences the effects of chronic ethanol intake on vasoconstrictive responsiveness. It has been described that the effect of chronic ethanol consumption is more pronounced in males than females. This study was designed to determine the mechanisms underlying this proposed cardioprotective effect in female rats. **Methods**: Female and Male Wistar rats were divided into 3 groups: Control (C; received water), Ethanol (E, received a solution of ethanol 20%) and Isocaloric (I, received isocaloric amounts of sucrose instead ethanol). The experiments were performed at the end of 4 weeks. Concentration-response curves for phenyleprhine (Phe) were obtained in isolated rat aorta. **Results:** Blood ethanol levels in the treated rats averaged (mg/mL) 1.38 ± 0.09 in the female and 1.87 ± 0.21 in male. There was an enhancement in the maximal effect (Emax: grams of developed tension) induced by Phe in in intact rings from male ethanol treated rats (C: 1.64 ± 0.14; I: 1.72 ± 0.67; E: 2.17 ± 0.09*) and in endothelium-denuded rings (C: 2.25 ± 0.11; I: 2.29 ± 0.16; E: 2.70 ± 0.11*). In endothelium-intact rings from female rats the Emax values for Phe was not altered by ethanol intake (C: 1.51 ± 0.13; I: 1.52 ± 0.17; E: 1.51 ± 0.12) although in endothelium-denuded rings Emax values for Phe was greater in ethanol treated female rats (C: 1.87 ± 0.14; I: 1.70 ± 0.23; E: 2.53 ± 0.19*). The ovariectomy did not alter the effect of chronic ethanol consumption on female rats. The treatment with ethanol did not alter sodium nitroprusside-induced relaxation. The L-NAME-induced contraction on Phe-pre-contracted rings were greater in rings isolated from ethanol-treated rats. In endothelium-intact female aorta, the incubation with L-NAME, aminoguanidine and L-NNA but not 7-NI enhanced the Phe-induced maximum tension in ethanol treated rats. *Different from groups C and I (ANOVA). **Conclusions:** The effect of chronic ethanol intake on vasoconstrictive responsiveness is gender dependent. The vasoprotective effects observed in female is endothelium-dependent and involves an increasement of nitric oxide production by activation of iNOS and eNOS. **Supported by:** Fapesp

04.007
MECHANISMS UNDERLYING THE ENDOTHELIUM-INDEPENDENT RELAXATION INDUCED BY ANGIOTENSIN II IN RAT AORTA
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Introduction: Some studies have provided evidences that angiotensin II (Ang II) induces vascular relaxation but little is known about the cellular basis of this effect on rat aortic rings. This work aimed to investigate the mechanisms underlying angiotensin II-induced vasorelaxation in isolated rat aortic rings. **Methods:** Rat aortic rings were isolated and placed in an organ chamber. The rings were pre-

contracted with phenylephrine and when the contraction reached a plateau angiotensin II (1 μM - 30 μM) was added cumulatively. The Emax values (% of relaxation) was compared. **Results:** Angiotensin II produced concentration-dependent vasorelaxation (95.54± 3.57%, n=5), which was not changed by removal of endothelium (75.48± 11.17%, n=7) or incubation with EDTA (0.75 mM), a non-selective inhibitor of methaloproteases (83.04±7,68%, n=4). PD123,319 (0.05 nM 100 nM) inhibited the Ang II-induced relaxation (n=5-6) but losartan (100 nM) did not (73.12± 10.17%, n=6). HOE-140 (100 nM), the selective antagonist of the bradykinin B_2 receptor and amiloride, abolished the Ang II-induced vasorelaxation (n=6). Administration of exogenous bradykinin (0.30 μM 10 μM) on pre-contracted tissues produced concentration-dependent relaxation (49.62±5.89%, n=7), which was also inhibited by HOE (12.64±5.15%, n=7). Indomethacin (10 μM), abolished the vasorelaxation induced by Ang II (n=5). L-NAME (100 μM) inhibitors of NO-synthase enzyme and ODQ (1 μM) cGMP synthesis inhibitor also abolished the vasorelaxation (n=5). Tetra-ethylammonium chloride (1 mM), a non-selective blocker of K^+ channels, and 4-aminopyridine (1 mM), a selective blocker of voltage-dependent K^+ channels, inhibited the vasorelaxation (n=4-5). Glibenclamide (3 μM), the selective blocker of K_{ATP} channels and apamin (1 μM), the selective blocker of the small condutance K_{Ca} channels, did not produce any significant effect (58.32±12.15 and 51.94±9.08% respectively, n=5). **Conclusions:** The present study shows functionally that stimulation of angiotensin II AT_2 receptor causes a significant vasorelaxation in endothelium-denuded rat aorta artery via stimulation of B_2 receptors by bradykinin. Stimulation of B_2 receptors results in activation of the relaxant prostaglandins-NO-cGMP pathway leading to opening of $I_{K(V)}$ and vasorelaxation. **Supported by:** FAPESP

04.008

THE ABILITY TO COPE WITH STRESS IS IMPAIRED IN SHR

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Objective: In Wistar rats (WR), the acute stress (AS) induced a hyporreactivity of aorta to noradrenaline (NA) that was potentiated by previous chronic stress (CS) (Cordellini & Vassilief *Gen Pharmacol*, 30:79,1998). It was studied the vascular adaptive response to AS and CS in WR and SHR, before and after established hypertension. **Methods:** Curves to NA were obtained in aorta with (+E) and without (-E) endothelium from 6 (prehypertensive) and 14 (hypertensive)-week-old WR and SHR submitted or not to AS (20-min swimming and 1-h immobilization 25 min apart), preceded or not to CS (2 sessions 2 days apart of a daily immobilization, 1hour/day for 5 consecutive days). **Results:** Reactivity [maximum response (g of tension)] to NA was decreased in aortas +E and E from 6-week-old but not 14-week-old SHR related to age-matched WR [6-week-old +E: WR 3.2±0.1, SHR 1.7±0.1*; -E: WR 4.2±0.2, SHR 2.8±0.1*, *P<0.05 relate to WR; 14-week-old +E: WR 3.8±0.1, SHR 4.0±0.1; -E: WR 5.5±0.3$^{\#}$, SHR 5.0±0.2$^{\#}$, $^{\#}$P<0.05 relate to respective aorta +E; n=6-18]. Stress did not alter the reactivity of aorta E. In aorta +E, AS induced hyporreactivity to NA similar in both strains, CS potentiated this adaptive response in 6 and 14-week-old WR but not in 6-week-old SHR, and aorta reactivity of 14-week-old SHR exposed to CS was similar to that of non-stressed SHR at same age (6-week-old AS: WR 1.9±0.1, SHR 0.8±0.1*; CS: WR 1.3±0.2*, SHR 0.8±0.5*; 14-week-old AS: WR 2.7±0.1, SHR 2.2±0.1; CS: WR 1.9±0.2*, SHR 4.1±0.2*$^{\#}$; *P<0.05 related to WR/AS; $^{\#}$P<0.05 related to SHR/AS; n= 6-18). **Conclusions:** The ability to cope with stress is different between strains and impaired in SHR. **Supported by:** CAPES

04.009

CHARACTERIZATION OF POTASSIUM CHANNEL ON NITRIC OXIDE ANTIPROLIFERATIVE EFFECT IN VSMC STILL IS POORLY UNDERSTOOD

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Introduction: We previously showed that nitric oxide (NO) inhibits rat aorta smooth muscle cell line A7r5 proliferation probably via soluble guanylate cyclase (sGC) and some sub-types of potassium channels (KC). Here, we sought to characterize the importance of KC on NO antiproliferative effect in primary culture of rat aorta VSMC. **Methods and Results**: Cells were isolated from enzymatic digestion of smooth muscle media of Wistar rat (20-day old) aortas and used until passage 6. Cell proliferation and viability were simultaneously evaluated after 6 days by counting cells in Neubauer chambers with Trypan blue. NO donor SNAP (S-nitroso-acetyl-DL-penicillamine; 1-100 μM) inhibited proliferation dose-dependently (CE_{50} ~ 10 μM), without cell death. Tetraethylammonium (TEA; a non-selective blocker of KC; 300 μM); clotrimazole (CLT; a blocker of intermediate-conductance calcium-activated KC; 10 nM) and 4-aminopyridine (4-AP; a blocker of voltage-activated KC; 10 μM) reversed NO inhibitory effect (control 52 ± 1; SNAP 11.5±0.2; SNAP + TEA 38±1.5; SNAP + CLT 41.5±5.5; SNAP + 4-AP 32.5±5 thousand cells/mm^3; n=3). KC blockers alone failed to affect cell proliferation, at least in the concentrations used. **Conclusions**: NO anti-proliferative effect is strictly dependent on KC in primary smooth muscle cells. Therefore, derangement in KC working may be of importance in atherosclerosis as well as their pharmacological manipulation may be of interest in the treatment of cardiovascular diseases. **Supported by:** CNPq, PRONEX and CAPES

04.010

ATORVASTATIN (A) ENHANCES SILDENAFIL (S) - INDUCED VASODILATION THROUGH NITRIC OXIDE (NO)-MEDIATED MECHANISMS

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Introduction: Statins increase the expression of endothelial nitric oxide synthase and activate the NO-cGMP pathway. We investigated whether pretreatment with A for two weeks affects the vascular reactivity to S, a phosphodiesterase 5 inhibitor that increases cGMP levels. **Methods:** Wistar rats received tap water (controls) or A (30 mg/kg/day; p.o.) for two weeks. Vasodilatory responses to S (1 pM - 100 mM) were studied in isolated aortic rings pre-contracted with 1 μM phenylephrine after incubation with L-NAME 10 μM (or saline). Nitrite and nitrate plasma levels were determined by chemiluminescence. **Results**: Pretreatment with A increased the potency (pD_2 = log of effective dose producing 50% of maximum effect) of sildenafil-induced vasodilatory responses (pD_2 increased from 6.61 ± 0.47 to 8.28±0.04; N=5; P<0.01). L-NAME blunted A-induced increase in the potency of sildenafil-induced vasodilatory responses (pD_2 decreased from 8.28±0.04 to 5.72±0.38; N=5; P<0.01). Non significant changes were observed in the maximum vasoralaxing effects of S. Pre-treatment with A increased plasma nitrite/nitrate concentrations (from 25±5 to 84±23 μM; N=6; P<0.05). **Discussion:** Our results suggest that A can increase the bioavailability of NO and enhance the sensitivity of S-induced vasorelaxation. The clinical significance of this drug interaction should be evaluated. **Supported by:** FAPESP, CAPES, CNPq

04.011

ENDOTOXINA DE *E. coli* REDUZ A REATIVIDADE VASCULAR DA ADRENALINA EM RATOS

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Introdução: A "sepsis" é caracterizada por sinais e sintomas de hipotensão e redução da reatividade vascular às substâncias vasoconstritoras e aumento desta reatividade às substâncias vasodilatadoras. Esta redução é determinada por intensa liberação de fatores essencialmente endoteliais consequente da ação de substâncias liberadas da parede bacteriana, que podem levar à morte. A redução da reatividade à adrenalina, nestas condições é observada no homem. Neste estudo avaliamos a mudança da reatividade vascular à adrenalina (Adr) após administração de endotoxina de *E. coli* (Etx) e após tratamento com NωNLA em ratos. **Material e Métodos:** Ratos Wistar pesando 250-320 g foram anestesiados com Nembutal (40 mg/kg, i.p), tendo canulados: traquéia (para livre ventilação pulmonar), veia jugular direita (para injeção de drogas) e artéria carótida esquerda (para conecção a transdutor de tensão isométrica em polígrafo, para registro da pressão arterial média [PAM]). Após 20 min. de registro da PAM foram injetados Adr (1, 2, 4 μg/kg, i.v.) com 5 min. de intervalo entre as doses [protocolo I] e a PAM foi registrada. Após 20 min. os animais animais foram tratados com Etx (3 mg/kg, i.v.) e após 1 h o [protocolo I] foi repetido e a PAM registrada. Após 30 min. os animais foram tratados com NωNLA (5 mg/kg i.v.) e após 1 h o [protocol I] foi repetido e a PAM registrada. Os resultados mostrados representam a média ± erro padrão da média (M ± EPM) e a análise estatística dos resultados foi feita pela ANOVA e para p<0,05 os resultados foram considerados significativos. **Resultados:** A PAM nos animais controle foi de 125 ±7 mmHg e após Adr (1, 2, 4 μg/kg) foi de 143±5; 159±7 e 176 ± 8 mmHg, respectivamente. Após 1 h de incubação com Etx (3 mg/kg), o [protocolo I] foi repetido e a PAM foi 102 ±4 ; 121±6 e 143±5 respectivamente. Após o NωNLA (5 mg/kg), o [protocolo I] foi repetido e a PAM foi 141±6; 156±4 e 172±6 mmHg, respectivamente. **Discussão:** Nossos resultados mostram que houve uma redução da potência da Adr após administração da Etx, totalmente revertida pelo tratamento dos animais, com o inibidor seletivo da NO sintase endotelial constitutiva (NωNLA). Esta observação sugere que a via L-arginina-NO entre outros prováveis fatores, também está envolvida tanto na hipotensão como nesta hiporreatividade vascular Etx-induzida semelhante aquela observada no choque séptico no homem. **Apoio Financeiro:** FCF-Unesp/Araraquara

04.012

VASCULAR EFFECTS OF ANGIOTENSIN II ON THE PORTAL VEIN OF NORMOTENSIVE AND HYPERTENSIVE RATS.

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Objective: To investigate Ang II-induced venoconstriction in Wistar and Spontaneously Hypertensive Rats (SHR). **Methods:** Rings of portal veins (n=6-8) were mounted in organ baths and concentration-response curves (CRC) to Ang II were generated. Veins were incubated with losartan (0.1mM), PD 123,319 (0.1mM), indomethacin (10mM) or L-NAME (10mM) for 30 min before CRC. SHR veins were studied after treatment of animals with enalapril (10mg/kg/8d, *p.o.*). Levels of mRNA for AT1 receptors were determined in portal veins through Real Time PCR and tissue ACE activity was analyzed by fluorimetric assay. **Results:** Ang II contracted Wistar and SHR veins. CRCs were shifted to the right by losartan but not by PD. Indomethacin and L-NAME had no effect in Wistar veins. SHR preparations exhibited reduced maximal response to Ang II (0.51±0.1*) in comparison to Wistar rings (0.92±0.1). This decreased response was reversed after enalapril treatment (0.89±0.1*) and by indomethacin (1.33±0.2*), but not by L-NAME. (*P<0.01). No differences between groups were observed in mRNA levels for AT1 receptors and in ACE activity. **Conclusions:** In SHR, Ang II venoconstriction may be counterbalanced by vasodilator prostaglandins. ACE blockade restores the responses to Ang II in SHR, suggesting an important role of the Renin-Angiotensin System in

modulating the venous reactivity. **Supported by:** FAPESP

04.013

RENAL ISCHEMIA AND REPERFUSION DECREASE VASCULAR RESPONSE TO BRADYKININ IN THE ISOLATED RAT KIDNEY

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Introduction: Vascular changes in ischemia/reperfusion (I/R) injury are associated with microvascular dysfunction. The present study investigates the effect of renal ischemia and reperfusion on renal vascular reactivity to bradykinin in the perfused rat kidney. **Methods:** Wistar rats (200-280g) were anaesthetized with thiopental (40mg/kg, i.p.) and the left kidney was excised through a flank incision. Renal ischemia was performed in the contralateral kidney by total interruption of renal artery flow for 45 min. After 45 min renal reperfusion was reestablished, and at the end of 4 hours, the right kidney was canulated, isolated and transferred to a closed-circuit system to analyze the renal vascular resistance (RVR). After the bradykinin addition, perfusate samples were collected to evaluate renal bradykinin degradation by bioassayed using an isolated guinea pig ileum preparation. **Results:** Basal perfusion pressure and perfusion flow were not significantly affected by ischemia followed by 4 hours of reperfusion (113±2 versus 113±8 mmHg and 21±3 versus 25±3 ml/min, in I/R and control kidneys, respectively). In control kidneys, bradykinin (BK, 0.5 mm) produced a transient decrease in RVR by 27±4%. However, in the I/R kidneys, the reduction in the RVR induced by BK was one third of that observed in control kidneys (9±1%). In addition, BK inactivation rate was greatly faster in I/R than in control kidneys (46± 6%/min in I/R versus 9±2%/min in control). **Conclusion:** Our data indicate that the decrease in the renal vascular reactivity to bradykinin may be due to an increase in the activity of one or more renal kininases after ischemia and reperfusion. **Supported by:** CAPES, FAPEMIG

04.014

ALTERAÇÕES RENAIS DA SER-THR-LYS-GUANILINA EM PERFUSÃO DE RIM ISOLADO DE RATO

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Introdução: A guanilina é um peptídeo endógeno, que ativa a guanilato ciclase, modificando a absorção de fluidos e eletrólitos nos rins e intestino. No presente trabalho foram analisadas as alterações renais provocadas pela ser-thr-lys-guanilina, com o objetivo de verificar se essa substância sinteticamente modificada causa os mesmos efeitos dos derivados endógenos da guanilina, bem como do seu análogo lysine-1 guanilina. **Métodos:** Foram utilizados rins de ratos Wistar (250-300 g) segundo a técnica descrita por Fonteles (1983). O grupo controle (C), onde os rins foram perfundidos com solução de Krebs-Henseleit modificada (6% de albumina bovina), foi comparado com o grupo tratado (T) com ser-lys-guanilina (0,1 µg/ml), adicionada aos 30 minutos de perfusão. Os dados foram analisados por teste t de Student *p<0,05. **Resultados:** O grupo tratado mostrou um aumento significativo no fluxo urinário (C_{120}=0,16±0,01; T_{120}=0,22± 0,02) e uma diminuição no transporte tubular total de sódio (C_{120}=85,03± 0,28; T_{120}=79,03±2,59) e potássio (C_{90}=65,12±3,93; T_{90}=42,59±2,59), assim como transporte proximal de ambos. Não houve alteração significativa nos demais parâmetros avaliados, como pressão de perfusão, resistência vascular renal, transporte de cloreto e ritmo de filtração glomerular. **Discussão:** Os resultados mostram que a ser-lys-guanilina apresenta efeitos diurético, natriurético e caliurético similares aos da guanilina e uroguanilina endógenas, e aos da lysine-1 guanilina sintética. **Apoio Financeiro:** CNPq

04.015

MECHANISMS UNDERLYING PROPRANOLOL-INDUCED RELAXATIONS OF RAT MESENTERIC ARTERY

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Goal: High concentrations of propranolol (PROP), a β-adrenoceptor (βAR) antagonist, are thought to relax vascular preparations independent on βAR blockade. This work aimed to investigate the mechanisms by which PROP relaxes rat mesenteric artery (MA). **Method:** MA rings were mounted in 5-ml myographs and isometric force was recorded using a PowerLab® data acquisition system. Concentration-response curves (CRC) to DL-, R (+)- and S (-)-PROP (10-100 µM, each) were constructed in absence or presence of L-NAME (100 µM; NO synthase inhibitor) or ODQ (1 µM; guanylyl cyclase inhibitor). CRC for exogenous calcium chloride ($CaCl_2$; 0.1-10 mM, in free Ca^{2+} Krebs medium) were made in absence or presence of PROP. **Results:** DL-, R (+)- and S (-)-PROP evoked a maximal relaxation (E_{max}) of 100 ± 1%, which was unaffected by either L-NAME or ODQ. However, L-NAME and ODQ significantly reduced relaxations elicited by 30 µM of DL-(43 and 35% inhibition, respectively), R (+)- (39 and 34% inhibition, respectively) and S (-)-PROP (30 and 26% inhibition, respectively). The E_{max} for $CaCl_2$, in presence of 100 µM of DL-, R (+)- and S (-)-PROP was reduced by 32, 35 and 40%, respectively. Nifedipine (1 µM) reduced by 39% the CRC to $CaCl_2$. **Conclusions:** The PROP-induced MA relaxations are independent of βAR blockade, and involve the stimulation of NO-cGMP pathway as well as inhibition of calcium mobilization in the smooth muscle. **Supported by:** FAPESP

04.016

TETRAHYDROBIOPTERIN CORRECTS NITRIC OXIDE/REACTIVE OXYGEN SPECIES IMBALANCE IN FEMALE DIABETIC RATS

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Introduction: Tetrahydrobiopterin (BH_4) is an essential cofactor for nitric oxide synthase (NOS). With reduced levels of BH_4, NOS generates superoxide anions instead of NO. Endothelial dysfunction in male diabetic animals has been demonstrated to involve NO/reactive oxygen species (ROS) imbalance, but this aspect is not well understood in females. **Methods:** To investigate the role of ROS production in the endothelial dysfunction and the effect of BH_4 in the NO/ROS production in alloxan-diabetic female rats, the arteriolar responses to acetylcholine (ACh) and bradykinin (BK) were studied by intravital microscopy before and after administration of BH_4 and of MnTMPyP, a superoxide dismutase (SOD) mimetic. The expression (by RT-PCR) and activity of SOD and eNOS, the NO production (by diaminofluorescein method), and the generation of ROS (by hydroethidine) were evaluated. **Results:** The reduction of 36.8% in the arteriolar response to ACh and 39.7% to BK in female diabetic rats were corrected by both MnTMPyP and BH_4. Enzymatic activity of NOS (38.2%), but not of SOD, was impaired. The expression of the enzymes, however, was not modified. Arterioles of female diabetic rats exhibited lower basal (30.6 %) and stimulated (with ACh 51.8% and BK 49.6%) NO production and increased ROS production (110.6%) that were also corrected by BH_4. **Conclusions:** Our data suggest that endothelial dysfunction in female diabetic rats involves impaired NOS activity and increased oxidative breakdown of NO due to enhanced formation of ROS, which is possibly caused by a deficiency of BH_4. **Supported by:** FAPESP/PRONEX

04.017

L-NOARG AND ODQ ANTAGONIZE THE REDUCTION ON ARTERIAL BLOOD PRESSURE INDUCED BY STEVIOSIDE

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Introduction: Activation or blockage of the L-arginine (L-Arg)- nitric oxide (NO) synthase (NOS)- NO-Guanylyl cyclase (GC)- cGMP pathway modifies arterial blood pressure. It has been shown that stevioside, a glycoside obtained from the leaves of the plant *Stevia rebaudiana* Bertoni, reduces the arterial blood pressure blocking Ca^{++} channels in preparations of isolated aortic rings of rats. Nevertheless, the effects of stevioside have not been investigated when *in vivo* preparations of rats were treated with agents that reduce the activity of NOS (N^G Nitro-L arginina, L-NOARG) or of GC (1H-[1,2,4]oxadiol[4,3-a]quinoxalin-1-one, ODQ). **Objective**: To verify effects of stevioside on arterial blood pressure of rats treated with L-NOARG or ODQ. **Methods:** Carotid artery and jugular vein of male Wistar rats (250-280g) were catheterized to verify the arterial blood pressure and to permit the injections of drugs, respectively. Data were submitted to ANOVA, followed by the Bonferroni ($P<0.05$). **Results**: L-NOARG (1mg/kg) or ODQ (2.5 µg/kg) alone did not change the arterial blood pressure, but antagonized (L-NOARG= 5.3±0.5 %, n= 4, ODQ= 3.8±0,8 %, n= 4) the reduction on arterial blood pressure (25.3±3.6 %, n= 4; 50.2±7.4%, n= 4) induced by stevioside (16 to 64 mg/kg). **Conclusion**: It is possible to admit that the reduction on arterial blood pressure induced by stevioside is dependent on the L-Arg-NOS-NO-GC-cGMP pathway. **Supported by:** CNPq (400875/2002)

04.018

EFFECTS OF L-ARGININE (L-ARG) ON ACUTE PULMONARY EMBOLISM (APE)-INDUCED PULMONARY HYPERTENSION

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Introduction: APE is a common cause of death. The pulmonary vasodilator nitric oxide (NO) is produced from L-Arg, which has been used in primary pulmonary hypertension (PH).We examined the effects of L-Arg pulmonary vasodilation in a model of APE. **Methods:** an isolated lung perfusion (9 ml/min) rat model of APE was used to examine the vasodilator effects of L-Arg (0.1, 0.5, 3 and 10 mM) added to the lung perfusate solution 5 min before induction of APE (injection 6.6 g/kg of Sephadex microspheres into the pulmonary artery). Mean pulmonary artery pressure (MPAP) was measured throughout the study. L-NAME 4mM or Methylene Blue (MB) 30uM were also given before APE, with and without L-Arg 3 mM. Nitrate+nitrite (NOx) concentrations in lung perfusate were measured. **Results:** While L-Arg (0.5, 3 or 10 mM) attenuated ($P<0.05$) APE-induced increases in MPAP, no statistically significant effects were observed with L-Arg 0.1 mM. L-NAME completely reversed L-arg 3mM pulmonary vasodilator effects. MB partially reversed L-arg 3mM pulmonary vasodilator effects. No significant changes were observed in NOx concentrations. **Discussion:** L-Arg attenuates APE-induced PH. Although NOx concentrations did not increase with L-Arg, the reversal of L-Arg effects by L-NAME suggests that the pulmonary vasodilator effects of L-Arg are mediated by NO. In addition, the partial reversion of L-Arg effects by MB suggests that L-Arg dilates pulmonary vessels through mechanisms that are only partially dependent on NO-stimulated production of cGMP. **Supported by:** FAPESP, CAPES, CNPq

04.019

ENDOTHELIUM-DERIVED NITRIC OXIDE CONTRIBUITES TO THE VASORELAXANT RESPONSE INDUCED BY CMMTT (A MESOIONIC COMPOUND) IN RATS

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Introduction: The present study was designed to determine whether 2-(4-chlorophenyl)-3-methyl-4-(4-methoxyphenyl) 1;3-tiazol-5-thiolate (CMMTT), a mesoionic compound synthesized in our laboratory, may have a pharmacological effect on superior

mesenteric artery rings isolated from male Wistar rats, and if so, to investigate the mechanism by which CMMTT may exerts its pharmacological effect. **Methods and Results:** Superior mesenteric artery rings from adult male Wistar normotensive rats were suspended in organ baths containing Tyrode solution (37°C). In endothelium-intact rings, CMMTT (10^{-14}3x10^{-6} M) inhibited in a concentration-dependent manner the contractions induced by phenylephrine 10 µM (IC_{50} =5,5±2,7 x 10^{-9}M). The effect of CMMTT on phenylephrine-induced contractions was significantly ($p<0.001$) attenuated after removal of the vascular endothelium. Similar results were obtained after pre-treatment of the rings with L-NAME 100 mM (a nitric oxide synthase inhibitor), hydroxocobalamin 30 µM (a nitric oxide scavenger) or ODQ 10 mM (an inhibitor of soluble guanylate cyclase). L-arginine (1 mM) completely restored the inhibitory effect of L-NAME (100 µM) (IC_{50}=5,5±3,5 x 10^{-9}M) on CMMTT induced vasorelaxations. In contrast, the effect of CMMTT on phenylephrine-induced contractions was not affected by indomethacin 1mM (IC_{50}=2±1,9 x 10^{-9}M) or atropine 1 nM (IC_{50}=7,5±4,7 x 10^{-9}M). **Discussion**: These findings suggest that NO contributes significantly to the vasorelaxant responses induced by CMMTT in rat superior mesenteric arteries. **Supported by:** PRONEX/CNPq/Brazil

04.020

CHARACTERIZATION OF CELLULAR PROCESS INVOLVED IN VASCULAR SMOOTH MUSCLE RELAXATION INDUCED BY A NEW NITRIC OXIDE DELIVERER COMPLEX

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Aim: investigate the cellular mechanisms involved in relaxation induced by trans-[RuCl([15]aneN$_4$)NO]$^{2+}$ (15-ANE) in rat aortas. **Methods**: in denuded rings, contractile responses were induced by norepinephrine (NE 0.1uM) and when they reached the plateau, cumulative concentrations of 15-ANE (0.1nM to 300uM) were added to the bath. Similar protocols were performed for 15-ANE after incubation with the guanylyl-cyclase inhibitor (10uM ODQ), non-selective K^+ channels blocker (1mM TEA), guanylyl-cyclase inhibitor and non-selective K^+ channels blocker and selective K^+ channels blockers (3uM glibenclamide, 1mM 4-aminopyridine (4-AP), 1uM apamin and 0.1uM iberotoxin) prior to the addition of NE. **Results**: the relaxation induced by 15-ANE was concentration-dependent in denuded aortas (maximum effect (ME):99.09±1.41% and pD_2:5.03±0.15, n=6). pD_2 and ME for 15-ANE were reduced by ODQ (ME:53.19±8.24% and pD_2:4.24±0.42, n=4) and TEA (ME:39.40±2.01% and pD_2:4.51±0.13, n=6). In presence of ODQ and TEA the relaxation was abolished. All selective K^+ channels blockers reduced ME and pD_2 values for 15-ANE (glibenclamide: ME 79.07±2.58% and pD_2 4.18±0.09 (n=4), 4-AP: ME 87.14± 2.14% and pD_2 4.32±0.07 (n=4), apamin: ME 76.92± 3.24% and pD_2 4.20±0.07 (n=4) and iberotoxin: ME 86.22±6.98% and pD_2 4.27±0.05 (n=4)). **Conclusion:** the relaxation induced by 15-ANE involves guanylyl cyclase and K^+ channels activation. Furthermore, the K^+ channels (K_{ATP}, voltage-dependent K^+ channel and Ca^{2+}-dependent K^+ channel) are involved in this relaxation. **Supported by:** FAPESP

04.021

INVOLVEMENT OF SOLUBLE GUANYLATE CYCLASE IN NITRIC OXIDE-MEDIATED HYPORESPONSIVENESS TO PHENYLEPHRINE FOLLOWING ENDOTOXIN CHALLENGE

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Introduction: We investigated the role of soluble guanylate cyclase (sGC) in endotoxin (LPS)-induced hyporesponsiveness to phenylephine. **Methods and Results:** The effects of phenylephrine (Phe) or glyceril trinitrate (GTN) on female Wistar rats blood pressure were evaluated at 2, 8 and 24 h after injection of LPS (12.5 mg/kg, i.p.). Vasoconstrictive response to Phe was reduced by 40-50% in all time periods. Methylene blue (MB, a sGC inhibitor, 15 µmol/kg, i.v.) restored the reactivity to Phe only 2 and 24 h after LPS injection. The vasodilatatory response to GTN was reduced (20%) at 8 h, but not 2 or 24 h after LPS injection. Lungs were harvested, minced and incubated in vitro with sodium nitroprusside (SNP; 100 µM) or saline, and processed for cGMP determination. SNP increased lung cGMP levels (4-fold) in control animals, as well as in lungs of LPS-treated animals 24 h before. However, SNP failed in increasing lung cGMP levels of rats treated with LPS 8 h before. **Conclusion:** In animals injected 8 h before with LPS i) MB failed in restoring the responses to Phe; ii) SNP failed in increasing lung cGMP levels and iii) GTN yielded a smaller vasodilatory effect of rats treated 8 h before with LPS, whereas all these effects were observed in animals injected 2 or 24 h before with LPS (similar to control animals). It is concluded that there is a temporal window of 8 h after LPS injection in which sGC is not functional. Thus, the putative use of inhibitors of sGC in sepsis may be beneficial only at early periods. **Supported by:** CAPES, CNPq and PRONEX

04.022

HYPERHOMOCYSTEINEMIA IMPAIRS THE PHENYLEPHRINE-INDUCED RELAXATION OF RAT CAROTID ARTERY VIA ACTIVATION OF α_{1D}-RECEPTORS

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Introduction: One of the mechanisms by which hyperhomocysteinemia (HHcy) has been described to interfere on the vascular reactivity is decreasing the biodisponibility of NO. **AIM:** We aimed to investigate the consequence of HHcy on the relaxation induced by phenylephrine (Phe) via activation of α_{1D}-receptors, on rat carotid artery and the mechanisms involved in this response. **Methods:** Male Wistar rats were divided into Control (C: received water) and HHcy (received a solution of DL-Hcy thiolactone 1g/kg/day) groups. The rats were sacrificed at 15 days after the treatment. The carotid artery was removed and placed in an organ chamber. Concentration-response curves for Phe (10^{-13}-10^{-10}M) were obtained, in endothelium intact or denuded rings pre-contracted with

$PGF_{2\alpha}$. **Results:** The treatment enhanced the plasmatic levels of homocysteine (uM) from 5.31±0.82 to 63.23±6.89*. Phe-induced relaxation was observed only in intact endothelium rings. The Emax (% Relaxation) induced by Phe was reduced in the arteries from HHcy group (C: 38.22±1.28; HHcy 20.50±3.21*). Prazosin, a selective α_1-adrenoceptor antagonist, reduced Phe-induced relaxation in both C (6.19±0.49*) and HHcy (4.32±0.22*) rings, when compared to the curves obtained in the absence of the antagonist. Similarly, BMY 7378, a selective α_{1D}-adrenoceptor antagonist inhibited the relaxation, in both, C (2.32±0.25*) and HHcy (1.96±0.19*) groups. L-NAME inhibited the relaxation responses in C: 4.10±0.90* and HHcy: 1.73±0.28*. The curves obtained with the purpose to investigate the inhibition of Phe-induced relaxation, showed that this response was, in part, of endothelial NOS activation, since L-NNA (specific inhibitor of eNOS) inhibited the relaxation in C (85.88±5.44) and HHcy (54.32±6.41*) rings. However, HHcy induced an activation of inducible NOS since 1400W (specific inhibitor of iNOS) produced a more accentuated inhibition of Phe-induced relaxation (C: 11.48±5.42; HHcy: 34.90±6.76*). *Means significant difference from control group (ANOVA, Dunnett´s post test). **Conclusions:** The relaxation induced by Phe in carotid arteries occurs via endothelial activation of α_{1D}-adrenoceptor, which involves the releasing of NO. Hyperhomocysteinemia impairs this relaxation via reduction of biodisponibility of NO. These observed effect are independent of the relaxation hability of the muscle, because the curves for sodium nitroprusside (NPS), an NO donor, induced relaxation concentration-dependent. **Supported by:** FAPESP

04.023

ALTERATIONS ON BLOOD PRESSURE INDUCED BY HYPERHOMOCYSTEINEMIA IN MALE ADULT RATS

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Introduction: Hyperhomocysteinemia (HHcy) has been described to interfere on the vascular reactivity in part of by decreasing the biodisponibility of NO. In this way, this reduction on the vasodilator factor could interfere on blood pressure. **AIM:** We aimed to investigate the consequence of HHcy on the arterial blood pressure on male adult rats. **Methods:** Male Wistar rats were divided into Control (C: received water), HHcy (received a solution of DL-Hcy thiolactone 1g/kg/day) and HHcy-W (received a solution of DL-Hcy thiolactone 1g/kg/day for 15 days and after this period received water for 15 days). The rats were sacrificed at 15 days after the periods. The mean arterial pressure (MAP) and the heart rate (HR) were acquired through a catheter implanted on right femoral artery. **Results:** The treatment with homocysteine for a period of 15 days on HHcy group had an increase on the MAP (116,8 ± 2 mmHg, *F= 8,4) and HR (363,4 ± 3,8 bpm, *F= 10,5), when compared to control group (MAP 104 ± 1,8 mmHg e HR= 339 ± 3,2 bpm). However, the animals from HHcy-W had no alterations on MAP (118, 6 ± 3,2, 6 mmHg, P>0,05) e HR (348,7 ± 4,1 bpm, P>0,05), when compared to control. *Means significant differences (ANOVA followed by Dunnett). **Conclusion:** Hyperhomocysteinemia induces an increase on arterial blood pressure, however, when the intake turns to physiological (water), the blood pressure turns to values similar to control group. **Supported by:** FAPESP

04.024

CHRONIC ETHANOL CONSUMPTION REDUCES ET_B-MEDIATED RELAXATION IN RAT CAROTID ARTERIES.

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Introduction: Conflicting reports describe increases, decreases and no change in vascular reactivity to constrictor and relaxing agents in ethanol treated rats. We aimed to investigate the effects of chronic ethanol consumption on endothelin-1(ET-1)-induced contraction. **Methods:** Male Wistar rats were divided into 3 groups: Control (C; water), Ethanol (E; solution of ethanol 20%) and Isocaloric (I; sucrose instead of ethanol). The rats were sacrificed at the end of the 2nd, 6th and 10th week (wk) and the carotid artery was removed for organ bath studies. **Results:** Blood ethanol levels in the treated rats averaged (mg/mL) 2.16 ± 0.17 in the 2nd wk, 2.15 ± 0.21 at wk 6 and 1.48 ± 0.19 at wk 10. No differences were found in seric glucose levels among the groups. There was an enhancement in the maximal effect (E_{max}: grams) for ET-1 after 2 wks (C:0.44± 0.04; I:0.48± 0.03; E:0.58± 0.04*), 6 wks (C:0.43± 0.04; I:0.46± 0.04; E:0.56± 0.03*) and 10 wks (C:0.42± 0.05; I:0.42± 0.03; E:0.55± 0.04*) only in endothelium-intact rings. The treatment reduced the pA_2 values for BQ123. IRL1620-induced constriction was abolished by BQ788, not affected by BQ123 and augmented after endothelium removal. The treatment with ethanol enhanced IRL1620-induced contraction in endothelium-intact rings. ET-1-induced relaxation was augmented in the presence of BQ123, reduced in the presence of BQ788 and abolished after endothelium removal. IRL1620 induced relaxation that was abolished by BQ788 and endothelium removal. The relaxation response induced by both agonists was reduced by the treatment. Pre-incubation of intact rings with L-NAME, ODQ, indomethacin or tetraethylammonium reduced IRL1620-induced relaxation. 4-amynopiridine but not apamin, glibenclamide or charybdotoxin

reduced IRL1620-induced relaxation. None of these compounds had effect in the rings from ethanol treated rats. The mRNA expression for ET_A and ET_B receptors, is similar for the 3 groups. *ANOVA. **Conclusions:** Chronic ethanol consumption reduces ET_B-mediated relaxation by acting on the cellular pathways involved in this response. FAPESP

04.025

MECHANISMS UNDERLYING CHRONIC ETHANOL CONSUMPTION ENHANCEMENT OF PHENYLEPHRINE-INDUCED CONTRACTION IN RAT AORTA.

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Introduction: Reports in the literature describe increases on α adrenergic-induced contraction after chronic ethanol consumption but the mechanisms underlying this response are poorly understood. This work aimed to: 1) analyzes the temporal effects of ethanol consumption on phenylephrine (Phe)-induced contraction; 2) investigate the mechanism (s) underlying these effects. **Methods:** Male Wistar rats were divided into 3 groups: Control (C; received water), Ethanol (E; received a solution of ethanol 20%) and Isocaloric (I; received isocaloric amounts of sucrose instead ethanol). The rats were sacrificed at the end of the 2nd, 6th and 10th week (wk). Concentration-response curves for Phe were obtained in isolated rat aorta. **Results:** Blood ethanol levels in the treated rats averaged (mg/mL) 2.19 ± 0.15 in the 2nd wk, 2.10±0.18 at wk 6 and 1.49±0.10 at wk 10. No differences were found in seric glucose levels among the groups. In intact rings, there was an enhancement in the maximal effect (E_{max}: grams of developed tension) induced by Phe after treatment for 2 wks (C:1.61±0.08; I:1.77±0.11; E:2.25± 0.14*), 6 wks (C:1.71±0.11; I:1.71± 0.16; E:2.26±0.19*) and 10 wks (C:1.51±0.11; I:1.51±0.12; E:2.28± 0.19*). Similarly, the E_{max} values in denuded rings were also altered after treatment for 2 wks (C:2.16±0.11; I:2.23±0.11; E:2.78±0.16*), 6 wks (C:2.16±0.15; I:2.02±0.12; E:2.68± 0.19*) and 10 wks (C:1.96±0.09; I:1.99± 0.14; E:2.84±0.20*). Indomethacin and SQ29548, but not AH6809 abolished the enhancement in the E_{max} values for Phe. $CaCl_2$-induced contraction in Ca^{2+}-free medium increased after treatment being this response abolished by SQ29548. The treatment with ethanol did not alter Phe-induced contraction in Ca^{2+}-free medium as well as acetylcholine or sodium nitroprusside-induced relaxation. L-NAME-induced contraction on Phe-pre-contracted rings did not differ among the groups. *Different from groups C and I (ANOVA). **Conclusions:** The increased Phe-induced contraction after treatment with ethanol is endothelium-independent and involves the release of TXA_2 or PGE_2, which in turns promote an enhancement in extracellular Ca^{2+} influx. **Supported by:** FAPESP

04.026

SILDENAFIL SELECTIVELY INHIBITS ACUTE PULMONARY EMBOLISM (APE)-INDUCED PULMONARY HYPERTENSION (PH)

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Introduction: Selective pulmonary vasodilators attenuate APE-induced PH. We examined the effects of inhibition of phosphodiesterase type 5 (PDE5) with sildenafil on the hemodynamic and respiratory changes caused by APE in anesthetized dogs. **Methods** APE was induced by intravenous (i.v.) injections of 300 µm microspheres to increase mean pulmonary artery pressures (MPAP) by 20 mmHg. Hemodynamic evaluation was performed at baseline, 15, and 30 min after APE was induced, and then 15, 30, and 45 min after the sildenafil infusion (1mg/kg followed by 0.3 mg/kg/h) started in the Sildenafil group (n=7), or saline infusion started in the Control group (n=8). **Results:** APE induced sustained PH with a 325% increase in pulmonary vascular resistance index (PVRI) without other significant hemodynamic changes. Control dogs showed no further changes in MPAP and PVRI. A significant decrease in MPAP and PVRI (-25% and -45%, respectively; P<0.05 both) was observed with sildenafil. No changes were observed in the mean arterial pressure in both experimental groups. APE produced marked and sustained decreases in arterial oxygen pressure (PaO_2), which were mildly attenuated by sildenafil (P<0.05). **Discussion**: At this dose, sildenafil (i.v.) selectively dilates the pulmonary circulation without producing systemic hemodynamic effects. These effects may lead to an increased survival rate after APE. **Support:** FAPESP, CNPq and CAPES

04.027

CLONIDINA INDUZ VASODILATAÇÃO DEPENDENTE DE ÓXIDO NÍTRICO E EDHF EM LEITO MESENTÉRICO DE RATO

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Introdução: O efeito vasodilatador da clonidina é parcialmente reduzido, mas não completamente abolido pelo tratamento com L-NAME em leito arterial mesentérico de rato (LAM) (Figueroa *et al*, *Br. J. Pharmacol.*, 134:957, 2001), sugerindo que outro (s) mecanismo (s) possam participar deste efeito. Portanto, no presente estudo, avaliamos a participação das prostaglandinas e do fator hiperpolarizante derivado do endotélio (EDHF) na vasodilatação induzida pela clonidina. **Método e Resultados**: Ratos *Wistar* machos (250-350 g) foram sacrificados em câmara de CO_2 para o isolamento do LAM. A preparação foi perfundida segundo McGregor (*J. Physiol.*, 21:177, 1965) e contraída com norepinefrina (6-30 mM). A clonidina (10300 pmol) induziu um efeito vasodilatador dose-dependente que foi significativamente (P<0.05) reduzido pela remoção do endotélio com ácido deoxicólico (2.5 mM). O efeito vasodilatador da clonidina (300 pmol) também foi reduzido (P<0.05) pelo L-NAME (0.3 mM, 49±4 vs 30±5%) e pelo KCl (45 mM; 61±2 vs 43±4%). A combinação de L-NAME com K^+ elevado inibiu completamente a resposta vasodilatadora (52±6 vs 1±1%). Entretanto, o tratamento do LAM com indometacina (3 mM), glibenclamida (1 mM), 4-aminopiridina (1 mM) não modificou a resposta vasodilatadora. **Conclusão**: Os resultados demonstram que a clonidina induz um efeito vasodilatador dependente do endotélio,

envolvendo provavelmente a liberação de NO e EDHF. Este efeito é independente de prostaglandinas ou da ativação de canais de K^+ dependentes de voltagem e ATP. **Apoio Financeiro:** CNPq

04.028

EFEITO DA HIPERTENSÃO E DA IDADE SOBRE A RESPOSTA DA ANGIOTENSINA II EM LEITO ARTERIAL MESENTÉRICO DE RATO

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Introdução: Estudos de nosso laboratório mostram que a [Ileu^5]-Ang II (Ang II) induz um efeito vasodilatador em leito arterial mesentérico (LAM) de ratos (Soares de Moura *et al.*, *Br. J. Pharmacol*, 141:860, 2004). O objetivo do presente estudo é avaliar o efeito da Ang II em LAM de ratos normotensos e espontaneamente hipertensos (SHR), jovens (150 g) e idosos (400 g). **Métodos e Resultados**: O LAM de ratos Wistar machos normotensos jovens (JN) e idosos (IN) ou SHR jovens (JH) e idosos (IH) foi perfundido segundo McGregor (J. Physiol., 1965) e o efeito da Ang II (3-300 nmol) foi estudado na presença de norepinefrina (6-30 mM). A Ang II produziu vasodilatação nos grupos de ratos JN, IN e IH. Apenas em preparações de ratos JH, a Ang II produziu um efeito bifásico, vasodilatação seguida de vasoconstrição (300 nmol-31±8%). O efeito vasodilatador da Ang II (300 nmol) foi significativamente maior em ratos jovens comparados com ratos idosos (JN, 68,8±4%; IN, 27,5±3%, *P*<0,05; JH, 62,5±3%; IH, 25,6±3%, *P*<0,05) e a hipertensão não modificou as respostas. O efeito vasodilatador da acetilcolina (ACh, 10 pmol) foi reduzido apenas em preparações de ratos IN comparado ao de ratos JN (48,8±5% vs 64,1±3%, *P*<0,05), não havendo diferença entre as respostas de ratos JH e IH (76±4% vs 64,6±2%). **Conclusão**: A Ang II produz um efeito vasodilatador maior em LAM de ratos jovens comparado ao de ratos idosos, que não é modificado pela hipertensão. Uma possível disfunção endotelial e não a hipertensão poderia justificar a menor resposta em animais idosos. **Apoio Financeiro:** CNPq

04.029

VASCULAR REACTIVITY AND BLOOD PRESSURE OF ADULT RATS, EXPOSED TO LEAD DURING PREGNANCY AND POSTNATAL PERIOD.

Fresneda da Silva, A.; Cordellini, S. UNESP - Farmacologia

Introduction: Several diseases developing during adulthood were probably determined during early stages of life, under the effect of exposure or preferential mother diet during pregnancy. It was investigated the vascular reactivity and blood pressure of rats exposed to lead (Pb) during pregnancy and postnatal days (PN) 1-70. **Methods:** Wistar rat dams received 300 ppm of Pb (acetate) or 400 ppm of sodium acetate in the drinking water during pregnancy and lactation. Pups received similar doses of Pb and sodium acetate in the drinking water on PN22-70. Male pups were killed on postnatal day 70. Curves to noradrenaline (NA) were obtained in aorta, with (+E) and without (-E) endothelium, from pups exposed or not to Pb. Beginning on PN22, systolic blood pressure was weekly determined in consious rats. **Results:** Increased reactivity to NA was observed in aorta +E from rats exposed to Pb related to age-matched Wistar. Pb did not alter the reactivity of aorta E. Maximum response (g tension) +E: Pb 3.7±0.1*, Wistar 3.1±0.2; -E: Pb 4.2±0.3, Wistar 4.9±0.2 n= 13-20, *P>0.05 relate to respective Wistar. Blood pressure was significantly increased in rats exposed to Pb (mmHg PN22: Pb 120.6±2.9*, Wistar 100.8±1.9, PN70: Pb 129.9±3.2, Wistar 121.0±2.6, n= 19-38, *P>0.05 related to respective Wistar). **Discussion:** Exposure to Pb during pregnancy and postnatal period determined hypertension associated with aorta-increased reactivity to NA in rats. **Supported by:** CAPES

04.030

VASCULAR REACTIVITY AND BLOOD PRESSURE OF ADULT RATS, EXPOSED TO LEAD DURING PREGNANCY AND LACTATION.

Fresneda da Silva, A.; Cordellini, S. UNESP - Farmacologia

Introduction: Several diseases developing during adulthood were probably determined during early stages of life, under the effect of exposure or preferential mother diet during pregnancy. It was investigated the vascular reactivity and blood pressure of rats exposed to lead (Pb) during pregnancy and lactation. **Methods:** Wistar rat dams received 300 ppm of Pb (acetate) or 400 ppm of sodium acetate in the drinking water during pregnancy and lactation. Male pups were killed on postnatal day 70 (PN70). Curves to noradrenaline (NA) were obtained in aorta, with (+E) and without (-E) endothelium, from pups exposed or not to Pb. Beginning on postnatal day 22 (PN22), systolic blood pressure was weekly determined in consious rats. **Results:** No reactivity alteration to NA was observed in aortas +E and -E from rats exposed to Pb related to age-matched Wistar [maximum response (g tension) +E: Pb 3.2±0.1, Wistar 3.1±0.2; -E: Pb 4.5± 0.3, Wistar 4.9±0.2 n= 13-19, P>0.05 related to respective control]. Blood pressure was significantly increased by Pb exposure [blood pressure (mmHg) PN22: Pb 120.6±2.9*, Wistar 100.8± 1.9, PN70: Pb 139.1±3.2*, Wistar 125.6± 1.9, n=18-35, *P<0.05 related to age-matched Wistar]. **Discussion:** Despite determines hypertension in rats, perinatal lead exposure did not alter the rat aorta reactivity to NA. **Supported by:** CAPES

04.031

MECANISMO DO EFEITO VASODILATADOR DO EXTRATO HIDROALCOÓLICO DE CASCAS DE UVAS VITIS LABRUSCA (GSE) NO LEITO VASCULAR MESENTÉRICO DE RATO

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Objetivos: O GSE apresenta atividade vasodilatadora dependente do endotélio e via NO (Soares de Moura *et al.*, *J Pharm Pharmacol*, 54:11, 2002). Neste estudo investigamos o possível envolvimento do GMP_c e EDHF, além dos receptores muscarínicos, B_2, H_1, e α_2-adrenérgicos no efeito vasodilatador do GSE. **Métodos e resultados:** O leito vascular mesentérico (LVM) de ratos Wistar machos (250-350g) foi isolado e perfundido com Krebs segundo McGregor (McGregor, *J Physiol*, 177:21, 1965). O efeito vasodilatador

do GSE (10600 μg), acetilcolina (ACh), nitroglicerina (NG), histamina, bradicinina (BK) e clonidina foram avaliados em LVM de rato pré-contraído com noradrenalina (NA; 0,3μM), antes e após tratamento com ODQ, atropina, HOE 140, caribdotoxina (Chtx), apamina, L-NAME, pirilamina, 4-aminopiridina (4-AP) e ioimbina. O GSE apresentou efeito vasodilatador com IC_{50} de 37±8 μg (n=6). A resposta vasodilatadora do GSE (30 μg) foi reduzida de 44±17% para 17±4% pelo ODQ (10 μM; n=6) e de 36±8% para 4±3% pelo tratamento com Chtx 0,1 μM + apamina 0,1 μM (n=6). A resposta vasodilatadora do GSE foi totalmente abolido pelo tratamento com Chtx + apamina + L-NAME (1 mM; n=6). Entretanto, o efeito vasodilatador do GSE permaneceu inalterado após tratamento com atropina (0,03 μM), HOE 140 (0,03 μM), pirilamina (1 μM), 4-AP (1 mM) e ioimbina (3 μM) em LVM de rato (n=6, para cada grupo). **Conclusão:** Nossos resultados demonstram que há a participação da via NO-GMP_c e do EDHF no efeito vasodilatador do GSE, e descarta o envolvimento dos receptores muscarínicos, B_2, H_1, e α_2-adrenérgicos. **Apoio Financeiro:** CNPq, FAPERJ, CAPES.

04.032

EFEITO VASODILATADOR E ANTIHIPERTENSIVO DO VINHO TINTO BRASILEIRO (BRW)

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Introdução: O efeito vasodilatador do vinho tinto é controverso, pois nem todos apresentam as mesmas ações farmacodinâmicas. Dessa forma, estudamos o efeito do BRW em leito arterial mesentérico de rato (LAM) e um possível efeito anti-hipertensivo em ratos com hipertensão induzida pelo L-NAME. **Métodos e Resultados**: O resíduo liofilizado do BRW (C. Sauvignon-Miolo, 10600 mg) induziu em LAM precontraído com norepinefrina (6-30 mM), um efeito vasodilatador dose-dependente. O efeito vasodilatador do BRW (100 mg) foi reduzido (P<0.05)com o ácido deoxicólico (2,5 mM, 55±5 vs 5±3%), L-NAME (0.3 mM, 38±1 vs 20±3%), ODQ (1 mM, 38±1 vs 20±5%) e caribdotoxina (ChTx, 0.1 mM) mais apamina (0.1 mM, 34±5 vs 11±4%). A combinação de L-NAME com ChTx mais apamina inibiu completamente a resposta vasodilatadora. Entretanto, o tratamento com atropina (0.03 mM), pirilamina (1 mM), ioimbina (3 mM), HOE 140 (0.01 mM), glibenclamida (1 mM) e indometacina (0.1 mM), não alterou a resposta. As pressões sistólica, média e diastólica foram registradas na cauda do rato antes (146±3; 97±4; 76±2 mmHg) e após 12 dias de tratamento com L-NAME (50 mg/kg/dia; 206±10; 142±7; 110±4). Estes valores foram reduzidos (P<0.05) com a administração do BRW (100 mg/kg/dia) por 10 dias (160±6; 113±3; 90±3). **Conclusão**: Os resultados demonstram que o BRW induz um efeito vasodilatador dependente do endotélio que é provavelmente devido a liberação de NO-GMPc e EDHF. O efeito antihipertensivo do BRW, deve exercer um papel significativo na ação cardioprotetora do consumo crônico de vinho tinto. **Apoio Financeiro:**CNPq

04.033

MATRIX METALLOPROTEINASE ACTIVITIES IN RATS TREATED CHRONICALLY WITH N^{ω}-NITRO-L-ARGININE METHYL ESTER

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Introduction: Matrix metalloproteinases (MMPs) are proteolytic enzymes involved in extracellular matrix turnover. MMP activity and expression can be modulated by oxide (NO). In this work, we examined the activities of MMP-2 and MMP-9 in cardiac and renal tissue of rats treated with N^{w}-nitro-L-arginine methyl ester (L-NAME).**Methods:** Male Wistar rats (>150 g) received L-NAME (20 mg/rat/day) in the drinking water for 2, 4 and 8 weeks. Tissues (heart and kidney) were processed for gelatin zymography. Enzyme activity (AUF/min/mg of protein) was assayed using the substrate Ac-pro-Leu-Gly-(2-mercapto-4-metilpentanoil)-Leu-Gly-OEt for MMP2/9. **Results:** Zymography showed that, in control and L-NAME-treated rats, cardiac tissue contained predominantly MMP-2 (72 kDa) whereas renal tissue contained predominantly MMP-9 (92 kDa). Renal tissue also had gelatinolytic bands >100 kDa, possibly multimers of MMP-9. The gelatinolytic activity of both tissues was inhibited by EDTA (10 mM) and phenanthroline (10 mM). Densitometry revealed no significant differences between the intensities of the bands seen in control and L-NAME-treated rats. Enzyme activity in cardiac tissue was 0.12±0.04, 0.08±0.02 and 0.11±0.02, and in renal tissue 0.04±0.00, 0.03±0.00, and 0.03±0.01 (mean±S.D., n=7) after 2, 4 and 8 weeks, respectively. These values were unaltered by L-NAME.**Conclusions:** Treatment with L-NAME did not affect MMP-2 and MMP-9 activities in rat cardiac and renal tissue. This lack of effect may reflect adaptation to the chronic absence of NO. **Supported by:** CAPES, FAPESP

04.034

RELAXAMENTO INDUZIDO POR ACETILCOLINA (ACh) EM AORTA DE RATOS TRATADOS CRONICAMENTE COM SALBUTAMOL.

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Introdução: Em cultura de células mesangiais e endoteliais, o acúmulo de AMPc estimula a expressão de óxido nítrico sintase (NOS). Em ratos com hipertiroidismo induzido, situação que envolve aumento da estimulação β-adrenérgica, ocorre um aumento da expressão de NOS. Assim, objetivamos investigar se o estímulo prolongado de adrenoceptores β_2, com conseqüente acúmulo de AMPc, promove aumento do relaxamento da aorta induzido pela ACh via NOS. **Métodos:** Ratos Wistar machos (n=8) foram divididos em 2 grupos: controle e tratado com salbutamol durante 5 semanas (6mg/100 ml de água dos bebedouros). Curvas concentração-resposta (contração isométrica) para ACh foram obtidas em anéis de aorta torácica pré-contraídas com fenilefrina (10^{-6}M), em presença de salina, indometacina (10^{-5}M) e indometacina (10^{-5}M) + L-NMMA. (3×10^{-4}M). Os parâmetros analisados foram o pD_2 (-log CE_{50}) e o Rmax (relaxamento máximo). **Resultados:** O tratamento dos animais com salbutamol não modificou

significativamente (P>0,05; ANOVA) as respostas de relaxamento induzida pela ACh nas preparações estudadas.

	Controle		Tratado Salbutamol	
Incubação	pD_2	Rmax	pD_2	Rmax
Salina	8,38±1,02	88,93±3,24	7,75±0,24	88,95±4,14
INDO	7,04±0,99	79,55±5,33	6,06±0,93	78,84±5,26
INDO + L-NMMA	--	13,77±3,84	--	17,42±3,05

Conclusão: O tratamento com salbutamol não modificou o relaxamento NOS-dependente, induzido pela Ach.

04.035

EFEITO DA SERTRALINA I.C. SOBRE O REFLEXO DE BEZOLD-JARISCH EM RATOS MACHOS E FÊMEAS Pires, J. G. P.; Castro, M. E. C.; Modenesi, C. A.; Futuro Neto, H. A. UFES - Fisiologia

Introdução: 5HT participa da modulação central dos reflexos cardiovasculares. Inibidores seletivos da recaptação de 5-HT (SSRI) são úteis no tratamento clínico da síncope vasovagal. Investigou-se aqui os efeitos da administração intracisternal (i.c.) do SSRI sertralina (ST) sobre o reflexo de Bezold-Jarisch (BJ) elicitado por fenilbiguanida (PBG). **Métodos**: Ratos Wistar (250-320 g) foram anestesiados (uretana) e instrumentados para registros de pressão arterial e freqüência cardíaca (FC). A veia jugular E foi canulada para administração de drogas. Os animais foram colocados no estereotáxico e a membrana atlanto-occipital foi exposta, para permitir a injeção i.c. (20 mL). Os ratos receberam atenolol (1 mg/kg i.v.), para permitir mensurar o componente vagal da bradicardia reflexa. Após 20 min, o reflexo de BJ era elicitado com PBG (15 mg/kg i.v.) a intervalos de 15 min, até que 3 bradicardias similares fossem obtidas; 10 min após a última bradicardia controle, uma injeção i.c. era feita com ST (0,2 ou 0,5 mg/kg) ou salina. As administrações subseqüentes de PBG foram feitas aos 5, 20, 35 e 50 min após a injeção i.c.. Os dados são expressos como média±EPM (N=6/grupo). Testes paramétricos apropriados foram usados para comparar as médias. O nível de significância foi arbitrado em 5%. **Resultados**: A ST causou uma atenuação dose-dependente do reflexo de BJ, em particular da bradicardia vagal, não tendo sido detectadas diferenças significativas entre machos e fêmeas [0,2 mg/kg, -DFC (bpm): (a) machos: pré-ST = 196±15; 5-min: 120±19 (*P*<5%); 20-min: 153±40; 35-min: 155±38; (b) fêmeas: pré-ST = 234±25; 5-min: 159±23 (*P*<5%); 20-min: 249±22; 35-min: 272±11]. A atenuação máxima da bradicardia foi, respectivamente, de 37% e 52%, nas doses menor e maior de ST. Salina i.c. não alterou o BJ. **Discussão**: Estes resultados sugerem que a administração i.c. de SSRIs reduz a excitabilidade dos motoneurônios vagais cardíacos.

04.036

cGMP REDUCES Ca^{2+} INFLUX IN NORMOTENSIVE (2K) AND IN RENAL HYPERTENSIVE (2K-1C) AORTAS Molin, J. C.; Bendhack, L. M. USP - FCFRP

Activation of protein kinase-G by cGMP can induce vascular smooth muscle relaxation by several mechanisms including Ca^{2+} influx reduction via voltage-operated Ca^{2+} channels (VOCs) activated by membrane depolarization induced by high extracellular K^+ concentrations. The aim of this study was to investigate the effect of cGMP on the contraction induced by Ca^{2+} influx through VOCs in 2K and in 2K-1C rat aortas. After intracellular Ca^{2+} depletion with phenylephrine, concentration-effect curves for Ca^{2+} stimulated with 30 mM KCl were constructed in 2K and 2K-1C aortas in the absence (control) and presence of 100 mM cGMP, incubated for 20 min. The control curves were also performed in intact endothelium aortas and the curves realized after incubation with cGMP were done only in denuded aortas. The maximum effect (ME) and the pD_2 values were analyzed. Endothelium removal increased the contraction in 2K (ME:1.75±0.19g (n=7) vs 2.46±0.18g (n=8) and pD_2:3.05±0.03 vs 3.14±0.06 (p<0.01), but the response was not changed in 2K-1C (ME:1.61±0.12g (n=8) vs 1.72±0.13g (n=8) and pD_2:3.04±0.05 vs 3.05±0.05). The response to extracellular Ca^{2+} was similar in 2K and 2K-1C with endothelium. However, in denuded arteries the response was greater (p<0.01) in 2K than in 2K-1C aortas. cGMP decreased the contraction (p<0.01) in 2K (ME:0.83±0.21g; pD_2:2.60±0.02) and 2K-1C (ME:0.71±0.18g; pD_2:2.54±0.02). These results indicate:1) that endothelium reduces Ca^{2+} influx only in 2K aortas; 2) in 2K denuded aortas Ca^{2+} influx is higher than in 2K-1C; 3) cGMP decreases Ca^{2+} influx in 2K and 2K-1C denuded aortas. **Supported by:** FAPESP, CNPq

04.037

EFEITO DE DERIVADOS TIENILHIDRAZONAS NA FUNÇÃO CONTRÁTIL DE CORPO CAVERNOSO DE COBAIO

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Introdução: Dentre 7 derivados do agente inotrópico positivo, LASSBio 294 (L294) testados, três (L785, L786, L788) apresentaram efeito relaxante vascular. Neste trabalho foi investigada a hipótese, destes derivados também serem ativos em relaxar a musculatura lisa de corpos cavernosos, devido a possível interação com a fosfodiesterase tipo 5 (PDE5). **Métodos:** Corpos cavernosos de cobaio (250-300 g) foram montados em cubas verticais de 15 ml de volume interno contendo solução Tyrode oxigenada (95% O_2/5% CO_2) à 37°C e preparados para registro de tensão isométrica. Após uma hora de equilíbrio, a preparação foi estimulada com 30 μM de fenilefrina e, ao atingir a contratura máxima foram adicionadas concentrações crescentes e cumulativas dos derivados em teste (10 a 200 μM). **Resultados:** Os análogos L785 (n=6) e L786 (n=6) não reverteram significativamente a contratura induzida pela fenilefrina. Entretanto, o L788 (n=4) provocou relaxamento significativo a partir da concentração de 100 μM, atingindo o efeito máximo com 200 μM, o que correspondeu a 46,5±8,8% (P<0,05) da contratura controle. **Conclusões:** Os derivados L785 e L786 não provocaram relaxamento da musculatura lisa do corpo cavernoso. Porém, o efeito do L788 pode estar relacionado à inibição da PDE5 para o efeito de relaxamento do músculo liso. **Apoio Financeiro:** CNPq, PRONEX, FAPERJ, FUJB

04.038

ILIDENOS E CARBAMATOS PROLONGAM O EFEITO HIPNÓTICO DO PENTOBARBITAL

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Introdução: Com a intenção de se desenvolver novos compostos que atuassem no sistema nervoso central, foram sintetizadas várias substâncias (ilidenos e carbamatos) resultantes da introdução de diferentes radicais no nitrogênio da estrutura da isatina. Este trabalho descreve a interferência de 9 substâncias na duração da hipnose induzida por barbitúricos como pentobarbital. **Métodos:** Pentobarbital sódico foi administrado na dose de 25 mg/kg por via venosa na cauda de camundongos suíços machos (18-25 g) para se determinar a duração da hipnose induzida pelo barbitúrico. Dois grupos experimentais foram formados com 15 camundongos cada, para injeção intraperitoneal prévia (30 min) do veículo (DMSO) ou dos diferentes derivados. A duração de hipnose induzida pelo pentobarbital foi comparada entre os dois grupos. **Resultados:** Todas as substâncias testadas prolongaram o tempo de hipnose induzida pelo pentobarbital. Dois ilidenos (MB29, MB108) e dois carbamatos (MB68, MB79) foram os mais potentes em prolongar o efeito hipnótico. A potência destes derivados em ordem decrescente foi MB68>MB29>MB79>MB108. Para MB68, o efeito farmacológico máximo é observado na dose de 500 µg/kg, onde a duração de hipnose aumentou de 19,3±0,3 para 71,2±3,2 min. **Conclusões:** A modificação da estrutura da isatina proporcionou a formação de ilidenos e carbamatos com potente efeito em prolongar a hipnose induzida por barbitúrico. **Apoio Financeiro:** FAPERJ, FUJB

04.039

ESTEREOSELETIVIDADE DA AÇÃO VASODILATADORA DOS ISÔMEROS DO TRAMADOL

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Introdução: Tramadol é um analgésico de ação central comercializado como mistura racêmica dos isômeros S (+) e R (-)tramadol. RS (±)tramadol produz vasodilatação dependente parcialmente da função endotelial. Este trabalho investiga se a vasodilatação provocada pelo tramadol está relacionada a um ou aos dois isômeros. **Métodos:** Anéis de aorta torácica de ratos Wistar (200-300 g) foram preparados para registro de tensão isométrica. Os anéis foram posicionados em cubas preenchidas com solução nutridora oxigenada a 37±0,5 °C. A preparação foi contraída com 10 µM de fenilefrina e exposta a concentrações crescentes e cumulativas de RS (±)tramadol e isômeros. A ação vascular do tramadol foi avaliada na presença e ausência do endotélio íntegro, L-NAME (200 µM), inibidor da enzima óxido nítrico-sintase, e naloxona (100 µM), antagonista de receptor opióide. **Resultados:** RS (±) e R (-)tramadol produziram relaxamento de forma dose-dependente dos anéis de aorta pré-contraídos com fenilefrina. Mesmo efeito não foi observado com S (+)tramadol. O relaxamento vascular foi parcialmente revertido com a remoção do endotélio vascular e após o pré-tratamento com naloxona. A concentração de R (-)tramadol para inibir 50% da contratura máxima induzida por fenilefrina foi 0,4±0,06 e 1,2±0,12 mM (P<0,05) na ausência e presença de naloxona, respectivamente. A vasodilatação produzida pelo R (-)tramadol também foi revertida pelo L-NAME. **Discussão:** R (-)tramadol promove vasodilatação mediada tanto por mecanismos dependente como independente do endotélio vascular. **Apoio Financeiro:** Cristália, FAPERJ e FUJB

04.040

FORSKOLIN REDUCES THE CONTRACTION INDUCED BY PHENYLEPHRINE IN NORMOTENSIVE (2K) AND IN RENAL HYPERTENSIVE (2K-1C) RAT AORTA

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Introduction: The relaxation of vascular smooth muscle cells can be due to activation of adenylyl-cyclase (AC), formation of cAMP and increase in the Ca^{+2} storage into the sarcoplasmic reticulum (SR). Forskolin (FSK) directly activates the enzyme AC. **Objective:** investigate the effect of FSK on the contraction of phenylephrine (Phe) and to compare the effect of FSK between 2K and 2K-1C rat aorta. **Methods:** Aortic rings were isolated from 2K-1C that presented systolic arterial pressure above 160 mmHg and 2K for isometric tension measurement. **Results:** In denuded arteries pre-contracted with 100 nM Phe, concentration-effect curves were constructed for FSK (0.1 nM to 1 µM). The effect of FSK (EC_{50}) on the contraction to Phe was compared in normal Ca^{+2} solution and in Ca^{+2} free solution. In normal Ca^{+2} solution (PSS), the contractile response to Phe was reduced ($p<0.01$) by FSK in 2K (from 2.18±0.25 g; n=6 to 0.98±0.10 g; n=6) and in 2K-1C (from 2.33±0.24 g; n=6 to 1.14±0.05 g; n=6). In Ca^{+2} free PSS the contraction to Phe was also decreased by FSK in both 2K (from 0.66±0.14 g; n=6 to 0.45±0.06 g; n=6) and in 2K-1C (from 0.76±0.11 g; n=6 to 0.52±0.03 g; n=6). Either in 2K and 2K-1C denuded aortas, the contraction induced by Phe was lower in Ca^{+2} free PSS than in normal Ca^{+2} PSS. **Conclusion:** FSK reduced the contraction induced by Phe in 2K and in 2K-1C aortas, in presence and in absence of extracellular Ca^{+2}. **Supported by:** FAPESP

04.041

EFFECT OF A NEW NITRIC OXIDE DONOR IN RENAL HYPERTENSIVE 2 KIDNEY-1CLIP RATS (2K-1C)

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Introduction: Nitric oxide (NO) is involved in several regulatory events as the control of arterial pressure. However, the NO has a half-life of about 5 seconds in biological systems. Since it

is difficult to study the effects of NO itself, it was of interest to synthesize new chemical compounds that can release NO in a controlled manner. **Object:** The effect of the compound *trans*-[RuCl ([15]aneN$_4$)NO]$^{2+}$ (15-ane), a ruthenium complex that is able to release NO^0, was investigated in the blood pressure of conscious hypertensive (2K-1C) and normotensive (2K) rats. **Method:** Male Wistar rats (180 g) were anesthetized and after a midline laparatomy, a silver clip (0.2 mm ID) was placed around the left renal artery. The 2K rats were submitted to laparatomy only. Six weeks after surgery, the systolic arterial pressure was measured by the tail-cuff method. Rats were considered to be hypertensive (2K-1C) when systolic blood pressure was higher than 160 mmHg. **Results:** In hypertensive rats, the 15-ane reduced the blood pressure in ± 30 mmHg (14%) of basal mean arterial pressure. This effect was observed one hour after administration of the compound and the hypotensive effect lasted for 6 hours. On the other hand, 15-ane failed in inducing hypotensive effect in 2K rats. In contrast, SNP reduced the blood pressure in both normotensive and hypertensive rats. However, the effect was shorter (2 ± 0.3 min, n=3) either in 2K and 2K-1C rats than that of 15-ane. **Conclusion:** These results are consistent with the slow rate release of NO^0 from this compound[1] (5000 s). [1]Oliveira et al., Inorg Chem Comms, v.7, p.160, 2004. **Supported by:** Fapesp

04.042

PAPEL DA ENDOTELINA E DO SISTEMA RENINA-ANGIOTENSINA SOBRE AS ALTERAÇÕES DE RESPOSTA CONSTRITORA À FENILEFRINA EM AORTAS DE RATOS TRATADOS COM OUABAÍNA.

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Objetivos: Em nível central, a endotelina-1 (ET-1) e o sistema renina angiotensina (SRA) participam da gênese e/ou manutenção da hipertensão induzida pelo tratamento crônico com ouabaína. Em aorta de ratos esse tratamento reduz, de maneira dependente do endotélio, a resposta constritora à fenilefrina (FE). Investigamos o papel da ET-1 e do SRA sobre as alterações da resposta contrátil à FE em aorta de ratos tratados cronicamente com ouabaína. **Métodos e Resultados:** A resposta contrátil à FE, expressa em % da contração ao KCl, foi estudada em aortas de ratos Ouabaína (OUA, » 8 µg/dia por 5 semanas, *s.c.*, n=9) e Veículo (VEH, n=13), tratados ou não com BMS182874 (40 mg/Kg/dia, *v.o.*, n=10) ou Losartan (Los, 15 mg/kg/dia, *v.o.*, n=16). Foram usadas aortas com e sem endotélio ou na presença de L-NAME (LN, 100 µM). A expressão do RNAm para ET-1 e seus receptores (ET_A e ET_B) foi analisada por RT-PCR. O grupo OUA apresentou pressão sistólica (PAS) elevada comparada ao grupo VEH (OUA: 156 ± 5 *vs.* VEH: 122 ± 1 mmHg, P<0,05). O tratamento com BMS bloqueou a elevação da PAS no grupo OUA (OUA BMS: 124 ± 2 *vs.* VEH BMS: 123 ± 2 mmHg). O tratamento com Los reduziu a PAS em ambos grupos, bloqueando a elevação da PAS no grupo OUA (OUA Los: 100 ± 2 *vs.* VEH Los: 102 ± 3 mmHg). A resposta à FE em aortas de ratos OUA apresentou-se reduzida comparada ao VEH (E_{max}, OUA: 62,3 ± 9,8 *vs.* VEH: 93,0 ± 4,7 %, P<0,05; pD_2, OUA: 6,65 ± 0,09 *vs.* VEH: 7,17 ± 0,09, P<0,05) e a remoção do endotélio ou o LN aumentaram essa resposta em maior magnitude no grupo OUA, igualando-a ao grupo VEH. O tratamento com Los não alterou esse padrão de resposta no grupo OUA e VEH porém o tratamento com BMS restaurou a resposta à FE do grupo OUA ao nível do grupo VEH (E_{max}, OUA BMS: 110±7 *vs.* VEH BMS: 111 ± 7 %; pD_2, OUA BMS: 6,82 ± 0,12 *vs.* Vehicle BMS: 6,82± 0,05), reduzindo também a maior modulação do endotélio e do óxido nítrico. O tratamento com ouabaína induziu um aumento da expressão do RNAm para a ET-1 e ET_A mas não para o ET_B. **Conclusão**: Esses resultados sugerem a participação da ET-1, mas não do SRA, sobre as alterações de reatividade vascular à FE em aortas de ratos hipertensos pelo tratamento crônico com ouabaína. **Apoio Financeiro:** CAPES, CNPq e FAPESP - Brasil.

04.043

EFFECTS OF VASOPEPTIDASE INHIBITORS ON MICROVASCULAR REACTIVITY: OBSERVATION IN THE HAMSTER CHEEK POUCH

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Introduction: Vasopeptidase inhibitors (VI) block simultaneously the angiotensin converting enzyme (ACE) and the neutral endopeptidase (NEP) and increase the biodisponibility of bradykinin (BK) thus augmenting the vasodilation. OBJECTIVES: To evaluate the effects of these substances upon mean arteriolar diameter (µm). **Material and Methods:** Male anesthetized hamsters *(Mesocricetus auratus)* were divided into 7 groups (6 animals in each) and the cheek pouch prepared for intravital microscopy observations, coupled with a closed circuit TV system. All vasoactive substances were applied topically and diameters were measured during control conditions, addition of BK and its combination with VI, CPA (carboxipeptidase inhibitor) and PDI59790 (endothelin inhibitor). Internal diameter measurements were performed using an image shearing device. RESULTS: Addition of (l) MDL100240 and omapatrilat (VI) increased the vasodilation elicited by BK 35.4% and 20.0%, respectively; (2) CPA increased BK mediated vasodilation on 8.4% and (3) PDI59790 increased BK vasodilation on 30.4%. **Conclusion:** Our results have shown that VI and PDI59790 potentiate the vasodilation elicited by bradykinin, further supporting the idea of increased biodisponibility. **Supported by:** Astra Zeneca

04.044

EFFECTS OF VASOPEPTIDASE INHIBITORS ON MACRO-MOLECULAR PERMEABILITY INCREASE INDUCED BY BRADYKININ IN THE HAMSTER CHEEK POUCH (HCP)

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Introduction: Vasopeptidase inhibitors (VI) block simultaneously the

angiotensin converting enzyme (ACE) and the neutral endopeptidase (NEP) (Weber MA, Lancet, 2001). These drugs could be more effective in the treatment of many cardiovascular diseases than either ACE or NEP inhibitors. However, the incidence of angioedema due to VI seems higher probably due to a potentiation of bradykinin (BK) effects. **Objectives:** To evaluate the effects of VI, ACE and NEP inhibitors on macromolecular permeability increase induced by BK. **Material and Methods:** Hamsters were anesthetized with a-chloralose and the cheek pouch prepared for intravital microscopy. The observation of plasma leakage (leaks) at postcapillary venules was performed using FITC-dextran as a tracer and fluorescent light. Topical applications of BK (100 nM) by itself, BK + drug 10-8 M, BK + drug 10-7 M and BK + drug 10-6 M were performed. **Results:** (maximum number of *leaks/cm*^{2} ± SD): control group (only BK): 1st BK 313±25 *leaks/cm*^{2}, 2nd BK 285±11 leaks/cm^2, 3rd BK 285±23 leaks/cm^2 and 4th BK 279±23 leaks/cm^2. Addition of (1) VI (omapatrilat or MDL) doubled the BK response and (2) NEP inhibitor (candoxatrilat) or ACE inhibitor (enalapril) increased the BK response by 50%. **Conclusion:** These results further support that VI have greater effects on bradykinin induced macromolecular permeability than either ACE or NEP inhibitors separately. **Supported by:** Astra Zeneca

04.045

TRANSPORTE DE L-ARGININA EM UM MODELO ANIMAL DE UREMIA

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Uremia é uma síndrome complexa associada com uma disfunção da via L-arginina-óxido nítrico (NO). Nosso grupo demonstrou previamente que o transporte de L-arginina, precursor da sintese de NO, está ativado em células sanguínes de pacientes urêmicos em hemodiálise, correlacionando-se significativamente com os níveis plasmáticos reduzidos de L-arginina. O presente estudo investigou o transporte de L-arginina em eritrócitos e concentrações plasmáticas de aminoácidos em um modelo animal de insuficência renal crônica. **Métodos:** Doze ratos Wistar machos foram submetidos a nefrectomia parcial (5/6). Após 3 meses, foram anestesiados e o sangue coletado por punção cardíaca. Os eritrócitos foram isolados e incubados a 37° em presenca de concentrações crescentes de L-arginina tritiada (5-500 µM). O transporte de L-arginina foi isolado em sistemas y^+ e y^+L através da inibição irreversível por N-etilmaleimida (1 mM). A análise dos níveis plasmáticos de aminoácidos foi realizada através de cromatografia de alta performance reversa. **Resultados:** O influxo de L-arginina (µmol/l céls/h) pelo sistema y^+ em eritrócitos de ratos urêmicos (V_{max} = 512 ±68) foi similar aos controles (V_{max} = 407±138). Também não houve diferença significativa no transporte de L-arginina em eritrócitos de controles (V_{max} = 38±12) e de ratos urêmicos (V_{max} = 48±4). Os níveis plasmáticos de L-citrulina estavam elevados em ratos urêmicos (135±7 µM) comparados com controles (62±3 µM) enquanto que a concentração de L-arginina foi similar nos dois grupos (102±13 vs 76±11 µM). **Conclusão:** Em contraste com nossos prévios resultados em pacientes urêmicos, este modelo animal de uremia não apresentou uma ativação do transporte de L-arginina nem uma redução dos níveis plasmáticos de L-arginina. É possível que estas alterações só ocorram em graus avançados de insuficiência renal crônica de modo a influenciar as características moleculares dos eritrócitos urêmicos. **Apoio Financeiro:** Wellcome Trust

04.046

L-ARGININE BLUNTS THE INCREASE IN OXIDATIVE STRESS CAUSED BY ACUTE PULMONARY EMBOLISM (APE)

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Introduction: Nitric oxide is a vasodilator formed from L-arginine (L-ARG). Supplementation with L-ARG is used to treat primary pulmonary hypertension. Although L-ARG attenuates pulmonary hypertension, it is possible that L-ARG may also attenuate the increase in oxidative stress observed during APE. We examined this hypothesis here. **Methods:** APE was induced in isolated Wistar rat lung perfusions by injecting 6.6 g/kg of Sephadex microspheres into the pulmonary artery. L-ARG (0, 0.1, 0.5, 3 and 10 mM) was added to the lung perfusate solution 5 min before induction of APE. In some experiments L-ARG (0, 3 and 10 mM) was added to the lung perfusate and saline was injected into the pulmonary artery instead of Sephadex microspheres (non embolized lungs). Lung perfusate samples were collected and thiobarbituric acid reactive substances (TBARS) were measured in plasma to evaluate oxidative stress. **Results:** APE increased TBARS by 11.2±2.0 nmol/L malondialdehyde (MDA). L-ARG significantly attenuated the increases in TBARS, which were 1.0±2.0, 2.6±3.2, 4.4±1.4, and 2.8±1.7 nmol/L MDA when L-ARG 0.1, 0.5, 3 and 10 mM, respectively, was added to lung perfusate before APE (all $P<0.05$). No significant increases in TBARS were observed when L-ARG (0, 3 and 10 mM) was added to the lung perfusate and no APE was induced. **Discussion:** The results of the present study suggest that L-ARG blunts the increase in oxidative stress caused by APE and may help to explain the beneficial effects of L-ARG. **Supported by:** FAPESP, CAPES, CNPq

04.047

HEMODYNAMIC EFFECTS OF DIETHYLENETRIAMINE NONOATE (DETA/NO) IN A DOG MODEL OF ACUTE PULMONARY EMBOLISM

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Introduction: Inhaled nitric oxide (NO) has been used in experimental acute pulmonary embolism (APE) as a selective pulmonary vasodilator. We examined the effects of a slow NO-releasing drug (DETA/NO) on the hemodynamic and respiratory changes caused by APE in anesthetized dogs. **Methods:** Sham operated animals received only saline infusions. APE was induced by stepwise intravenous injections of 300 µm microspheres in amounts adjusted to increase mean pulmonary artery pressures (MPAP) by 20 mmHg. Hemodynamic evaluation and arterial blood gas analysis were performed at baseline, 15, and 30 min after APE, and then 15, 30, and 45 min after the DETA/NO infusion (1

μMol/kg; i.v.) in the DETA group, or saline infusion started in the Control group. **Results:** results are presented as mean±SD. No significant hemodynamic changes were observed in sham animals (n=3). APE increased MPAP from 10±7 to 34±12 mmHg and pulmonary vascular resistance index (PVRI) from 225±156 to 934±398 din.s.cm^{-5}.m^{-2}(both P<0.05). No further changes were observed in the control group (n=8). Treatment with DETA (n=9) decreased MPAP from 34±12 to 30±14 mmHg and PVRI from 934±398 to 703±323 din.s.cm^{-5}.m^{-2} (both P<0.05), without significant changes in the other parameters. No significant changes were observed in mean arterial pressure and systemic vascular resistance. **Conclusion:** This dose of intravenous DETA/NO attenuates APE-induced pulmonary hypertension without significant systemic effects. **Supported by:** FAPESP, CNPq and CAPES

04.048

SILDENAFIL ATTENUATES ACUTE PULMONARY EMBOLISM (APE)-INDUCED OXIDATIVE STRESS

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Introduction: APE is a major cause of secondary hypertension, which can be attenuated with intravenous sildenafil, a selective inhibitor of phospho-diesterase-5. Although it is well known that sildenafil increases tissue concentrations of cGMP, it possible that sildenafil attenuates the increased oxidative stress observed during APE. We examined this hypothesis in this study. **Methods:** APE was induced in two experimental settings: 1) In isolated Wistar rat lung perfusions in which APE was induced by injecting 6.6 g/kg of Sephadex microspheres into the pulmonary artery. 2) In anesthetized mongrel dogs in which APE was induced by intravenous injections of microspheres to increase mean pulmonary artery pressures by 20 mmHg. Sildenafil (or saline) was administered before APE in both experimental settings (1 mg/kg). Blood samples were collected and thiobarbituric acid reactive substances (TBARS) were measured in plasma to evaluate oxidative stress. **Results:** Sildenafil significantly attenuated the increase in TBARS after APE in isolated lung perfusion (saline: 11.2±2.0 nmol/L MDA; sildenafil 1.1±1.5; P<0.05). In addition, sildenafil produced similar effects in dogs. Sildenafil attenuated the increase in TBARS after APE in anesthetized dogs (saline: 16.9±4.4 nmol/L MDA; sildenafil 4.5±3.5; P<0.05). **Discussion:** The results of the present study show in two different experimental settings that sildenafil attenuates the increase in oxidative stress caused by APE. **Supported by:** FAPESP-CAPES-CNPq

04.049

GENDER DIFFERENCE IN THE HYPERHOMOCYSTEINEMIA MODULATING THE VASCULAR REACTIVITY OF RAT CAROTID ARTERY

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Hyperhomocysteinemia (HHcy) has been described to interfere on the vascular reactivity. Additionally, the gender difference appears to play a role in the vascular responses. The present study aimed to investigate the consequence of HHcy on reactivity of rat carotid artery to Phe in male and ovarietomized female Wistar rats. Male and female rats were each divided into control (received water) and HHcy (received a solution of DL-Hcy thiolactone 1g/kg/day). The experiments were performed 15 days after the treatment beginning. The carotid artery was removed and placed in an organ chamber for reactivity study. Concentration-response curves for Phe (10^{-10}-10^{-5}M) were obtained, in endothelium intact or denuded rings. The treatment enhanced the plasmatic levels of homocysteine (uM) from 5.31±0.82 to 63.23±6.89* in male and from 4.34±0.55 to 207.17±16.79* in female. Body weight was reduced in male from 337.92±5.73 to 311.93±5.15* and in female from 377.47±8.57 to 305.31±5.96*. There were no alterations on blood pressure, food and liquid intake. In male rats, the Emax (g/mg of tissue) of Phe in intact ring were greater in HHcy group (0.95±0.08*) than in control (0.77±0.05). In female, the Emax values to Phe in HHcy group (0.58±0.05) and Control (0.70±0.07) were similar. In denuded rings, the Emax value induced by Phe on HHcy group (1.08±0.08) were not different from control (0.92±0.08) in male rats, however, in female the Emax was increased in HHcy group (1.2±0.08*) when compared to Control (0.72±0.05).*Significant different from control groups (Student t test , P<0.05). Data obtained in our study indicate that HHcy affect differently the reactivity of carotid arteries in male, in which the effects of HHcy are endothelium-dependent. On the other hand, the effect of HHcy in female is endothelium independent. **Supported by:** FAPESP

04.050

TEMPORAL CONSEQUENCE OF HYPERHOMOCYSTEINEMIA ON THE VASCULAR REACTIVITY IN YOUNG RATS

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Introduction: Hyperhomocysteinemia (HHcy) has been described to interfere on the vascular reactivity. Additionally, the vascular responses to injury are different in adult when compared to young. **AIM:** We aimed to investigate the consequence of HHcy on reactivity of carotid artery to phenylephrine (Phe) in young rats. **Methods:** Young male Wistar rats (21 days) were divided into Control (C: received water) and HHcy (received a solution of DL-Hcy thiolactone 1g/kg/day) groups. The rats were sacrificed at 7, 14, 28 and 56 days after the treatment. The carotid artery was removed and placed in an organ chamber. Concentration-response curves for Phe (10^{-10}-10^{-6}M) were obtained. **Results:** The treatment enhanced the plasmatic levels of homocysteine (uM) at 7 (C: 1,42±0,14; HHcy: 98,67±9,22*), 14 (C: 2,19±0,27; HHcy: 122,42±12,25*), 28 (C: 2,77±0,39; HHcy: 135,97±22,23*) and 56 days (C: 4,16±0,41; HHcy: 200,02±49,59*). Phe induced contraction concentration-dependent. The Emax (g/mg dry tissue) induced by Phe in rings with endothelium intact

was not altered at 7 and 14 days after treatment between C and HHcy groups, however, it was enhanced in rings from HHcy group at 28 (C: 0.64±0.08; HHcy 0.88±0.07*) and 56 days (C: 0.38±0.05; HHcy 0.72±0.06*). The Phe-Emax was not altered in arteries in the absence of endothelium between C and HHcy groups. *Means significant difference from control groups (Student t-test, unpaired). **Conclusion:** Data obtained indicate that the HHcy cause an increase on the reactivity of Phe on carotid arteries in young male rats. We have also shown that this effect is dependent of endothelium. **Supported by:** CNPq

04.051

EFFECTS OF LERCANIDIPINE (LER) ON PLASMA METALLOPROTEINASE (MMP)-2 AND MMP-9 ACTIVITY IN HYPERTENSIVE (H) AND HYPERTENSIVE DIABETIC (HD) PATIENTS

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Introduction: Increased activity of MMPs has been described in many cardiovascular disorders and is of major significance in vascular remodelling. Inhibition of MMPs may be a pharmacologic target. We examined the effects of LER on plasma MMP-2 and MMP-9 activities in H and HD patients. **Methods:** LER 20 mg (or placebo) was given to H (n=7) and DH (n=7) patients for 30 days. 24 hour ambulatory blood pressure monitoring (ABPM) was used to evaluate the effects of LER on mean arterial pressure (MAP). Venous blood samples were drawn after LER (or placebo) treatment and gelatin zymography of MMP-2 and MMP-9 from plasma was performed. Samples were subjected to electrophoresis on 12% SDS-PAGE co-polymerized with gelatin (0.1%). Gels were washed Triton X-100 and incubated at 37°C for 16 h in $TrisCaCl_2$ buffer, and stained with Coomassie Brilliant Blue. Enzyme activity was assayed by densitometry. **Results:** While LER produced no effects on MAP in HD patients, a significant reduction was observed in H patients (from 106±4 to 100±3 mmHg; P=0.03). Moreover, LER decreased active MMP-9 by 29% and 100%, respectively, in plasma from H and HD patients (both $P<0.05$). LER decreased active MMP-2 in plasma from HD patients by 12% ($P<0.05$). **Discussion:** Our results show that LER decreases MMP-9 and MMP-2 activities in HD patients and MMP-9 activity in H patients. Therefore LER may delay the development of atherosclerosis in such patients. **Supported by:** FAPESP-CAPES-CNPq

04.052

MECANISMO DA AÇÃO VASODILATADORA DA EUXANTONA EM VASOS DE RESISTÊNCIA

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Introdução: No presente estudo avaliamos o mecanismo da ação vasodilatadora da euxantona, isolada de *Vismia latifolia*, em pequenas artérias mesentéricas de resistência. **Métodos e Resultados:** Os anéis (1,6-2,0 mm) de artérias mesentéricas de rato Wistar foram montadas como descrito por Côrtes *et al.*, *Br. J. Pharmacol.*, 133, 849, 2001. A euxantona induziu vasodilatação em vasos contraídos com fenilefrina (3μM) e com KCl (50 mM), com CI_{50} = 3,6±1,9 μM e 9,5±2,7 μM, respectivamente. L NAME (100 μM) e indometacina (10 μM) não alteraram o efeito vasodilatador da euxantona (CI_{50} = 3,1 ± 0,6 μM e 1,7±1,0 μM, respectivamente) em vasos pré-contraídos com fenilefrina. H-89 (1 μM) e Rp-8-pCPT-cGMPS (3 μM), respectivos inibidores de proteína quinase A e G, também não modificaram o efeito da euxantona (CI_{50} = 3,8±1,6 μM e 5,2±0,3 μM, respectivamente). A euxantona (10 μM) inibiu em 93,3±3,8 % as contrações induzidas por fenilefrina (3 μM) dependentes da liberação de Ca^{2+} dos estoques intracelulares. As contrações dependentes do influxo de cálcio induzidas por KCl (80 mM) também foram inibidas por euxantona (10 μM) em 32,3±7,9%. Da mesma forma, as contrações induzidas pelo influxo capacitativo de Ca^{2+} também foram inibidas. As contrações induzidas por éster de forbol (PMA; 10 μM) foram inibidas por euxantona (10 μM), tanto em meio extracelular contendo ou livre de Ca^{2+} (93,0±2,9% e 71,7±8,7%, respectivamente). O mesmo efeito foi observado na presença de staurosporina (3 μM). **Conclusão:** Podemos concluir que a euxantona produz um efeito vasodilatador independente da participação de fatores endoteliais, assim como da ativação das proteínas quinases A ou G. Estes resultados sugerem que a euxantona exerce o seu efeito via inibição do influxo de cálcio e da inibição de proteína quinase C. **Apoio Financeiro:** CNPq

04.053

RAPID RECOVERY OF NITRIC OXIDE-MEDIATED HYPORESPONSIVENESS TO PHENYLEPHRINE IN THE ISOLATED AND PERFUSED RAT KIDNEY

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Introduction: Nitric Oxide (NO) production during septic shock is the major cause of the vascular hyporesponsiveness to vasoconstrictors. This study evaluated the vasoconstrictor effect to phenylephrine (PHE) in isolated kidney perfused after rat exposure of exogenous NO. **Methods and Results:** The right kidney was obtained from Wistar rats, the urether was ligated and the organ was perfused with oxygenated Krebs-Henseleit solution at 4 ml/min. Sodium nitroprusside (SNP; 4 mg/kg) was injected either 4 or 7 h before the surgery and perfusion. After equilibration, the initial tone (~80 mm Hg) was raised to 110-120 mm Hg with PHE (0.05-2.5 μg/ml Krebs). After a stable baseline has been reached, increasing dosis of PHE were injected. In rats treated with SNP 4 h before, the vasoconstriction was inhibited by more than 90%, but in animals injected with SNP 7 h before, PHE response was normal (data shown in table).

PHE	Control	4 h after SNP	7 h after SNP
nmol	? mmHg SEM N	? mmHg SEM N	? mmHg SEM N
0.5	9.5 1.5 4	1.0 0.3 4	16.5 8.4 4
1.0	22.0 4.0 4	1.1 0.1 4	30.6 3.0 4
2.0	40.5 7.3 4	3.5 1.6 4	52.9 3.6 4
3.0	61.3 1.9 4	4.0 1.3 4	72.7 5.3 4

Conclusion: In the rat kidney, NO-induced decrease in vasoconstrictor response is short-lasting, which is in sharp contrast to the long-lasting effect of NO donor on vascular responsiveness seen in blood pressure or aorta rings. This rapid recovery in the response to vasoconstrictors may help to explain why the kidney exhibits a constrictive response in situations of high production of NO, such as septic shock. **Supported by:** CAPES, CNPq and PRONEX

04.054

EFFECTS OF L-ARGININE (L-ARG) ON ACUTE PULMONARY EMBOLISM (APE)-INDUCED INCREASES IN LUNG METALLOPROTEINASE-2 ACTIVITY

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Introduction: Matrix metalloproteinases (MMP) break down the extracellular matrix. Moreover, MMP-2 regulates the vascular reactivity by cleaving big endothelin-1, a vasoconstrictor released during APE. We examined whether MMP-2 and MMP-9 are activated during APE and whether L-ARG, a precursor of nitric oxide, affects MMPs activation. **Methods:** APE was induced in isolated Wistar rat lung perfusions by injecting 6.6 mg/kg of Sephadex microspheres into the pulmonary artery. L-ARG 0.5 mM (or saline) was added to the lung perfusate solution 5 min before induction of APE. Gelatin zymography of MMP-2 and MMP-9 from lung and plasma samples were performed. Samples were subjected to electrophoresis on 12% SDS-PAGE co-polymerized with gelatin (0.1%) as the substrate. Then gels were washed Triton X-100 and incubated at 37°C for 16 h in $TrisCaCl_2$ buffer, and stained with Coomassie Brilliant Blue. Enzyme activity was assayed by densitometry. **Results:** APE did not change plasma MMP-2 and MMP-9 activities, and lung MMP-9 activity. However, APE increased lung MMP-2 activity from 389±129 (controls; N=4) to 1203±491 arbitrary units (N=8; P<0.05). When L-ARG 0.5 mM was added to the lung perfusate, MMP-2 increased significantly less (up to 687±245 arbitrary units; P<0.05) after APE. **Discussion:** The results of the present study suggest that L-ARG attenuates the increase in MMP-2 activity caused by APE and may help to explain the beneficial effects of L-ARG. **Supported by:** FAPESP, CAPES, CNPq

04.055

ATORVASTATIN ATTENUATES ACUTE PULMONARY EMBOLISM (APE)-INDUCED INCREASES IN LUNG METALLOPROTEINASE-9 ACTIVITY

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Introduction: Matrix metalloproteinases (MMPs) may be activated during APE. Atorvastatin may increase nitric oxide production and downregulate MMPs expression. We examined whether MMP-2 and MMP-9 are activated during APE and whether atorvastatin affects MMPs activation. **Methods:** Wistar rats were treated with water or atorvastatin 30mg/kg/day p.o. for 14 days. APE was induced by injecting 9 mg/kg of microspheres (or saline) into the caudal vein. One day after APE, rats were killed and gelatin zymography of MMP-2/MMP-9 from lung was performed. Samples were subjected to electrophoresis on 12% SDS-PAGE co-polymerized with gelatin (0.1%). Gels were washed with Triton X-100 and incubated at 37°C for 16 h in $TrisCaCl_2$ buffer, and stained with Coomassie Brilliant Blue. Enzyme activity was assayed by densitometry. **Results:** MMPs´activities are reported as mean±SD arbitrary units (N=12/group). Higher lung active MMP-9 and pro-MMP-9 activities were observed in embolized lungs vs. controls (3.2±0.4 vs. 1.1±0.2 and 0.6±0.2 vs. 0.1±0.0, respectively; both P<0.05). Atorvastatin attenuated (P<0.05) the increases of lung active MMP-9 (activity=2.3±0.3) without significant effects on lung pro-MMP-9 and MMP-2 activities. Moreover, atorvastatin increased 24 hour survival rate from 35% to 52% (P<0.05). **Discussion:** Our results show that atovastatin attenuated the increases in lung active MMP-9 and significantly reduced APE-mortality rate. **Supported by:** FAPESP, CAPES, CNPq

04.056

INHIBITION OF METALLOPROTEINASES WITH DOXYCYCLINE ATTENUATES ACUTE PULMONARY EMBOLISM (APE)-INDUCED SYSTEMIC HYPOTENSION

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Introduction: Matrix metalloproteinases (MMPs) are zinc dependent proteinases involved in the degradation of extracellular matrix. Recent studies have shown that MMP-2 regulates the vascular reactivity by cleaving big endothelin-1, a pulmonary vasoconstrictor released during APE. In this study we examined whether MMPs inhibition with doxycycline (a non-specific MMPs inhibitor) attenuates the hemodynamic responses to APE. **Methods:** Wistar rats were anesthetized with urethane (1 g/kg, i.p.) and the trachea was cannulated. Rat lungs were mechanically ventilated. The right carotid artery and left femoral vein were cannulated for the measurement of mean arterial blood pressure (MAP) and drug administration, respectively. The arterial catheter was connected to a pressure transducer and MAP was monitored throughout the experiments, which were initiated after at least 20 min of stabilization. Doxycycline (40 mg/kg) or saline was injected intravenously 10 minute before APE was induced by injecting 9 mg/kg of Sephadex microspheres (or saline) intravenously. **Results:** MAP decreased by 34±3 mmHg 10 minutes after APE induction with microspheres in rats pre-treated with saline. When rats received doxycycline, significantly lower decreases in MAP were observed (8±3 mmHg; P<0.05). **Discussion:** Our results show that the non-specific MMPs inhibitor doxycycline attenuates APE-induced hypotension in rats. This finding suggests that MMPs have a role in the hemodynamic changes caused by APE. **Supported by:** FAPESP, CAPES, CNPq

04.057

INTERETHNIC DIFFERENCES IN THE DISTRIBUTION OF THE CLINICALLY RELEVANT T^{-786}C ENDOTHELIAL NITRIC OXIDE SYNTHASE (eNOS) GENE VARIANTS IN BRAZILIANS

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Introduction: Nitric oxide (NO) plays a major role in the maintenance of vascular homeostasis. It is produced by eNOS, which exhibits genetic polymorphisms. The clinically relevant T^{-786}C eNOS polymorphism in the promoter region of eNOS gene is associated with a 50% reduction in eNOS expression and increased risk of developing cardiovascular diseases. We compared the distribution of the T^{-786}C polymorphism in two brazilian ethnic groups (blacks and whites). **Methods**: We studied 154 white subjects and 55 black subjects. Genomic DNA was extracted from the cellular component of 1 mL of whole blood by a salting-out method. Genotypes for the T-786C polymorphism were determined by polymerase chain reaction (PCR) and fragment restriction digestion with MspI. **Results**: The frequency of the genotypes TT, TC, and CC were significantly different (P=0.02, by Chi-squared test) in white subjects (31%, 54%, and 15% respectively) compared with black subjects (40%, 56%, and 4%, respectively). **Discussion**: We found that the distribution of the T^{-786}C gene variants in white subjects differs from that in black subjects. Similar interethnic differences were previously shown in the American population. Taken together, these findings suggest that such interethnic differences are consistent in different populations and may be related to ethnic disparities in cardiovascular risk and response to drugs. **Supported by:** FAPESP, CNPq, CAPES.

04.058

REDUÇÃO DA CARDIOTOXICIDADE DO HALOFANTRINO VEICULADA EM NANOCÁPSULAS

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A cardiotoxicidade induzida por drogas é uma preocupação importante na terapêutica da malária. O halofantrino (Hf) é capaz de prolongar o intervalo QT do eletrocardiograma (ECG), predispondo à arritmia cardíaca severa. Formulações de Hf encapsulado em nanocápsulas (NC-Hf) administradas *in vivo* pela via intravenosa reduzem a sua toxicidade aguda [Mosqueira *et al., 2001*]. **Objetivo:** Determinar a dose letal (DL_{50}) de Hf livre e NC-Hf e avaliar os efeitos das formulações sobre os parâmetros do ECG de ratos. **Métodos e Resultados:** Ratos Wistar machos foram anestesiados pelo tiopental, tiveram catéteres implantados para injeção de drogas e registro de PA, e eletrodos introduzidos no tecido subcutâneo para registro do ECG na derivação DII. A diferentes grupos de animais foram administrados: Hf livre em DMA/PEG/glicose (100 e 150mg/kg); Hf base encapsulado (100 e 150mg/kg) e soluções controle. A DL_{50} para Hf e NC-Hf calculada foi de 158 e 237mg/kg, respectivamente. Os intervalos QT (27% e 55%) e PR (37% e 87%) apresentaram aumentos dose-dependentes significativos após administração das doses de 100 e 150mg/kg de Hf livre, respectivamente. No entanto, a administração NC-Hf induziu a aumentos dos intervalos QT (9% e 8%) e PR (23% e 21%) de maneira independente da dose, e esses aumentos foram significativamente menores que os induzidos pelo Hf livre. **Conclusão**: Os resultados sugerem que a encapsulação foi capaz de modificar a distribuição do fármaco no organismo reduzindo a concentração no tecido cardíaco, evitando uma liberação rápida de altas doses capazes de provocar alterações eletrofisiológicas cardíacas. **Apoio Financeiro:** UNDP/World Nbank/WHO/TDR: ID A00790; CNPq; UFOP; FAPEMIG

04.059

PEPTIDASE ACTIVITIES AND EXPRESSION IN SPONTANEOUSLY HYPERTENSIVE RATS

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Introduction: Peptidases can modulate cardiovascular functions. In this work, we examined the activities of some peptidases in spontaneously hypertensive rats (SHR). **Methods:** Tissues (aorta, brain, heart, kidney, liver, lung) from adult male SHR and Wistar-Kyoto (WK) rats were processed for activity assays (as nmol of product formed or arbitrary fluorescence units/min/mg of protein) and immunoblotting (as arbitrary densitometric units) of aminopeptidase M (APM), dipeptidyl peptidase IV (DPPIV), metalloendopeptidase 24.15 (MEP 24.15) and neutral endopeptidase 24.11 (NEP 24.11) (Linardi *et al.*, *Biochem. Pharmacol.*, 2004, in press). **Results:** APM activity was unaltered in SHR vs. WK rats. DPPIV activity increased in brain (WK: 0.17$\pm$0.03 vs. SHR: 0.3$\pm$0.05; mean$\pm$S.D., n=5; *p<0.05; Student´s *t*-test). and decreased in aorta (WK: 1.3$\pm$0.4 vs. SHR: 0.2$\pm$0.2*); immunoblotting confirmed these changes (WK: 8439$\pm$1111 vs. SHR: 6690$\pm$1505* for aorta) and (WK: 7844$\pm$1228 vs. SHR: 10534$\pm$588* for brain). MEP 24.15 activity decreased in aorta (WK: 23.4$\pm$6.9 vs. SHR: 11.3$\pm$2.7*) but increased in lung (WK: 524.5$\pm$87.0 vs. SHR: 865.5$\pm$199.5*); expression was reduced in aorta (WK: 7884$\pm$1533 vs. SHR: 5914$\pm$1507*). NEP 24.11 activity increased in kidney (WK: 158.2$\pm$12.8 vs. SHR: 231.9$\pm$46.7*) and lung (WK: 6.0$\pm$0.9 vs. SHR: 9.8$\pm$1.8*), with no changes in protein expression. **Conclusion:** Peptidase activities are altered in SHR and this may contribute to cardiovascular alterations in this model. **Supported by:** FAPESP

04.060

BENEFICIAL EFFECTS OF EXERCISE TRAINING IN ERECTILE RESPONSES OF NITRIC OXIDE-DEFICIENT RATS

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Goals: The beneficial effects of regular exercise training in preventing cardiovascular diseases are well established, but prevention of male sexual dysfunction by exercise is poorly studied. We tested here if regular exercise improves the relaxations of rat corpus cavernosum (CC) in response to electrical field stimulation (EFS; 232 Hz), BAY41-2272 (NO-independent activator of soluble guanylate cyclase; 0.0110 μM) and sildenafil (PDE-5 inhibitor; 0.0110 μM) in two different conditions: untreated and L-NAME-treated rats. **Methods:** Wistar rats were divided into 4 groups: Sedentary-Untreated (SU), Trained-Untreated (TU), Sedentary-L-NAME (SN) and Trained-L-NAME (TN). Exercise training was performed for 5 days/week in sessions that lasted 60 min each during 8 weeks. L-NAME was given daily in the drinking water (2 mg/rat/day) for the last 5 weeks. **Results:** Electrical-field stimulation induced frequency-dependent CC relaxations in all groups. In SN group, EFS (16 Hz)-induced relaxations were significantly lower (38±4%) in compared with either SU (53±6%) or TU (73±5%). However, exercise training significantly restored the CC relaxations in L-NAME-treated rats to control values (63±4.5%; $P<0.05$). On the other hand, neither the potency nor the maximal responses to sildenafil or BAY41-2272 were altered in all groups. **Conclusions:** Dynamic exercise improves EFS- (but not sildenafil- or BAY41-2272)-induced CC relaxations, suggesting that physical training acts to increase NO release from nitrergic fibers or endothelium **Supported by:** FAPESP

04.061

CONTRACTILE RESPONSE TO CARBACHOL IN DETRUSOR SMOOTH MUSCLE FROM HYPERTENSIVE RATS SUBMITTED TO EXERCISE TRAINING

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Introduction: It is well established the beneficial effects of the physical exercise on the arterial hypertension, but no studies have been addressed the influence of dynamic exercise on the urinary bladder. Thus, the aim of this study was to evaluate the potency and maximal response to carbachol, in detrusor smooth muscle from rats treated with L-NAME. **Methods:** Animals were divided into four groups: sedentary (SD), L-NAME (LN), trained (TR), and L-NAME/trained (LN/TR). Training program was performed in a treadmill and L-NAME treatment was carried out according to Ribeiro et al. (1992) for 4 weeks. After the training program, the animals were sacrificed and the detrusor smooth muscle strips was isolated. Concentration-response curves were constructed to carbachol and potency and maximal responses (Emax) were calculated. **Results:** Chronic L-NAME administration significant increase the BP (160±2 mmHg) as compared to SD and ET groups (129±1 and 132±1 mmHg). The increase in BP induced by L-NAME treatment was prevented by TR (136±1 mmHg). The potencies for carbachol were not modified by either dynamic exercise or L-NAME tretament (SD: 5.98±0.05; LN: 5.57±0.19; TR: 6.01±0.05; LN/TR: 5.80±0.03). Similarly, the Emax for carbachol was unaltered in all studied groups (SD:101±0.29; TR:102±3.9; LN:99±2.5; LN/TR:103±1.7 mN). **Conclusion:** Our findings show that dynamic exercise for 8 weeks did not affect the sensitivity of detrusor smooth muscle to carbachol in L-NAME-treated rats.

04.062

RELAXING EFFECTS OF SILDENAFIL ANALOGUES IN THE RABBIT ISOLATED AORTA

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Goal: Sildenafil (SILD), a selective phosphodiesterase type 5 (PDE5) inhibitor, has been shown to enhance NO-induced responses by preventing cGMP breakdown. We have developed 2 analogues of SILD (namely SILD-1 and SILD-3) and aimed to investigate their effects in rabbit aorta (RbA). Method: Thoracic aortic rings (AR) were mounted in organ baths, and force was recorded using isometric transducers connected to a PowerLab® data acquisition system. Concentration-response curves (CRC) to SILD, SILD-1 and SILD-3 (0.001-10 μM) were constructed in absence or in presence of intact endothelium, L-NAME (100μM; NO synthase inhibitor) or ODQ (1 μM; guanylyl cyclase inhibitor). **Results:** SILD, SILD-1 and SILD-3 concentration-dependently relaxed the AR (pEC_{50} values: 7.24±0.05, 7.10± 0.19 and 7.21±0.13, respectively). The maximal responses (E_{max}) to SILD-1 (63 ± 8%) and SILD-3 (59 ± 3%) were similar to SILD (76±8%). Removal of AR endothelium caused significant rightward shifts in CRC (pEC_{50}) to SILD, SILD-1 and SILD-3 (6.41±0.08, 6.54±0.18 and 6.73±0.14, respectively). Endothelium removal reduced E_{max} values to SILD, SILD-1 and SILD-3 by approximately 32-54%. Incubation of AR with either L-NAME or ODQ reduced pEC_{50} to all three agents (L-NAME: 6.52±0.05, 6.33±0.21 and 6.47±0.11; ODQ: 6.49±0.11, 6.53±0.15 and 6.28±0.10, respectively, for SILD, SILD-1 and SILD-3). **Conclusions**: The vasorelaxant responses induced by SILD-1 and SILD-3 are similar to SILD, and partly involve the endothelium integrity. **Supported by:** FAPESP

04.063

RATOS NORMOTENSOS E GENETICAMENTE HIPERTENSOS DEFICIENTES EM ÓXIDO NÍTRICO TÊM ALTERAÇÕES QUANTITATIVAS DA PAREDE DA AORTA E ARTÉRIAS DE RESISTÊNCIA

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Objetivo: Avaliar a remodelagem da parede aórtica e de artérias de distribuição em ratos Wistar (W) e ratos espontaneamente hipertensos (SHR), sob inibição crônica da síntese do óxido nítrico. **Métodos e Resultados:** Foram estudados 4 grupos de ratos adultos machos (n=5): controle (W e SHR) e tratados com L-NAME (LN) (W e SHR). LN (30 mg/kg/dia) foi administrado durante 22 dias. Após a eutanásia, a artéria aorta (Ao) e o músculo glúteo (MG) foram extraídos para análise através de microscopia de luz e estereologia. Na Ao verificou-se a densidade numérica (QA) de núcleos de células musculares lisas da túnica média, densidade de superfície (SV) de lamelas elásticas e espessura da parede. No MG analisou-se: densidade numérica, densidade de comprimento (LV), densidade de superfície e densidade de volume (VV) de artérias de resistência. As diferenças estatísticas foram testadas pela ANOVA one way e pós-teste Newman-Keuls. Nos grupos WLN e SHRLN, a pressão arterial aumentou 49% e 57%, respectivamente e os animais apresentaram significativa redução do peso corporal. As artérias de resistência do MG mostraram, no SHRLN, aumento maior que 50% do QA e LV e maior que 100% do SV. Na Ao, notou-se redução significativa do QA no grupo WLN em relação ao WC (-32%), mas não houve diferença entre os SHRs tratados com os não tratados. O SV de lamelas apresentou comportamento semelhante (-23,77% no grupo WLN versus WC). **Conclusões:** As alterações quantitativas de artérias de resistência do MG em ratos hipertensos com deficiência de óxido nítrico (ON) sugerem possível indução de neovascularização nesses animais. Na Ao, a deficiência de ON leva a hipertrofia de células musculares lisas nos ratos Wistar, mas não nos SHRs. Os SHRs, sendo geneticamente hipertensos, já apresentariam hipertrofia dessas células antes da inibição da síntese do ON. **Apoio Financeiro:** CNPq, Faperj

04.064

EXPRESSION OF PIN AND NNOS IN HEART AND KIDNEYS FROM SPONTANEOUSLY HYPERTENSIVE RATS

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Introduction: Neuronal nitric oxide synthase (nNOS)-derived nitric oxide (NO) plays an important role in kidney homeostasis by regulating sodium and water excretion and renal medullary blood flow. In the heart, it enhances ß-adrenergic-stimulated contractility by increasing SR Ca^{++} release. Despite the discovery of an endogenous protein inhibitor of nNOS (PIN), little is known about its distribution and regulation in the kidney and heart of spontaneously hypertensive rats (SHR). **Methods:** Male SHR and the normotensive control Wistar rats (14-16 weeks-old) were used. The protein expression of PIN and nNOS in whole kidney homogenates was analyzed by Western blotting and the gene expression of PIN and nNOS in renal cortex and medulla homogenates was analyzed by RT-PCR. **Results:** Our results show that in whole kidney from SHR, protein expression of PIN was significantly decreased (58%, $p<0.01$) and nNOS was significantly increased (72%, $p<0.05$). When we study the renal cortex, the gene expression of PIN was significantly increased (33.31%, $p<0.05$) and no difference was found in nNOS. In the renal medulla we didn't see any difference in the gene expression of PIN and nNOS. On the other hand, in the heart of SHR, we didn't see any difference in the protein expression of PIN. **Discussion:** The observed altered expressions of both PIN and nNOS in SHR kidneys could contribute to hypertensive state in SHR. However, the physiological significance of these alterations still remains to be elucidated. **Supported by:** FAPESP, Pronex and CNPq.