

Summary of Ph.D. thesis

**The effects of recreational physical exercise on the cardiovascular system
and on colitis in rats:
the role of heme oxygenase and nitric oxide synthase enzymes**

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Abbreviations

AVP	arginine vasopressine
CO	carbon monoxide
HO	heme oxygenase enzyme
HO-1	heme oxygenase-1 enzyme
MPO	myeloperoxidase enzyme
NO	nitric oxide
NOS	nitric oxide synthase enzyme
cNOS	constitutive nitric oxide synthase enzyme
iNOS	inducible nitric oxide synthase enzyme
SnPP	tin protoporphyrine IX
TNBS	2,4,6 trinitrobenzene sulphonic acid

Introduction

Recreational or leisure-time exercise refers to any moderate-intensity physical activities with the primary purposes of improving or maintaining health and preventing diseases. The beneficial effects of recreational exercise training on chronic diseases such as cardiovascular complications and colon cancer has been demonstrated. On the other hand the underlying mechanisms induced by recreational exercise in the cardiovascular system and in the gastrointestinal tract have not been elucidated.

Moderate-intensity exercise causes low-degree oxidative stress which can induce the antioxidant defence systems, including the heme oxygenase enzyme (HO). HO catalyses the degradation of the pro-oxidant heme into three products: biliverdin, carbon monoxide (CO) and free iron. Biliverdin is subsequently converted to bilirubin by biliverdin reductase. Bilirubin has antioxidant and anti-inflammatory effects. CO is a vasodilator and antioxidant molecule. Heme oxygenase-1 (HO-1), the inducible isoform of heme oxygenase enzymes, is a stress-responsive enzyme, heat shock protein 32, which can be induced by oxidative stress, inflammatory cytokines and many other molecules. HO-1 is thought to play an important role in the protection of tissues from oxidative injuries and inflammation. It has been demonstrated that the upregulation of HO-1 by hemin or hyperthermia is associated with the amelioration of experimental colitis. Furthermore, inhibitors of the activity of HO such as zinc protoporphyrine IX and tin protoporphyrine IX (SnPP) can exacerbate colonic inflammation.

Another enzyme involved in intestinal inflammation and oxidative stress is nitric oxide synthase (NOS), which produces nitric oxide (NO). NO can be beneficial or detrimental depending on the concentration. The constitutive production of NO derived from the constitutive isoforms of the enzyme, namely neuronal NOS (nNOS) and endothelial NOS (eNOS), plays a role in vasodilation. On the other hand, large amount of NO produced by inducible NOS (iNOS) reacts with superoxide to form cytotoxic peroxynitrite and causes oxidative stress.

Aims

1. We aimed to verify the role of HO in the aorta ring model.
2. We addressed the gender differences in aorta ring contraction after inhibition of HO activity;
3. the effects of 6 week-voluntary exercise on the activity of HO in abdominal aorta and left ventricle;
4. the effects of 6 week-voluntary exercise on the activity of constitutive nitric oxide synthase (cNOS) in abdominal aorta and left ventricle;
5. the changes of the body weight of animals during the 6 weeks of exercise and after the induction of colitis;
6. the effects of 3/ 6/ 10 weeks of voluntary exercise on acute colonic inflammation and on the activity of myeloperoxidase enzyme (MPO);
7. the effects of 6 weeks of voluntary exercise on the activity of HO enzyme in the inflamed colon;
8. the effects of 6 weeks of voluntary exercise on the activity of cNOS and inducible nitric oxide synthase (iNOS) enzymes in the inflamed colon.

Methods

We studied the aorta ring contraction induced by arginine vasopressine (AVP; 2.0 µg/ml) after SnPP (30 µmol/kg, s.c., pH 7.4) pre-treatments 24 hours and 1 hour before measurements in male and female Wistar rats.

In further studies, male Wistar rats were used. Rats of the running groups were placed into cages installed with a running wheel. After 6 weeks of voluntary exercise period, abdominal aorta and left ventricle were removed. HO activity and cNOS activity were measured.

To investigate the effect of recreational physical exercise on acute colitis, male rats were randomly divided into four groups: 1, absolute control (non-running non-colitis-induced), 2, running control (running non-colitis-induced), 3, sedentary TNBS (non-running colitis-induced) and 4, running TNBS (running colitis-induced) groups. After 3/ 6/ 10 weeks of voluntary exercise animals were treated with 2,4,6-trinitrobenzene sulphonic acid (TNBS; once 10 mg in 0.25 ml of 50 % ethanol, w/v). Food was withdrawn overnight before induction of colitis. Rats were weighed weekly until the induction of colitis and then daily following the TNBS challenge. 72 hours after the induction of colitis the animals were sacrificed and the distal 8 cm portion of the colon was dissected for the following molecular and biochemical analyses: measurement the of HO, cNOS and iNOS enzyme activities.

Damage score and lesion measurements

The extent of macroscopically apparent inflammation, ulceration and tissue disruption was determined in a randomized manner from the color images, using a proprietary computerized planimetry software, developed in our laboratory (Stat_2_1_1). The area of macroscopically visible mucosal involvement was calculated and expressed as the percentage of the total colonic segment area under study.

The degree of colonic inflammation was scored on a 0-11 scale in a randomized, blinded fashion.

Myeloperoxidase (MPO) activity

O-dianisidine assay was determined spectrophotometrically.

HO activity

HO activity was assessed by measuring bilirubin formation spectrophotometrically.

NOS activity

Nitric oxide synthase activity was determined by quantifying the conversion of [¹⁴C]-radiolabelled L-arginine to L-citrulline.

Protein determination

To determine protein content we used the Bradford microassay.

Data representation and statistical analysis

Statistical comparisons were performed by two-tailed Student's *t*-test. In statistical comparisons, a probability (*P*) value of less than 0.05 was considered significant.

Results

SnPP pre-treatment increased aortic ring contraction induced by AVP in male and female rats. Inhibition of HO activity abolished the gender differences.

The 6 week-voluntary exercises caused a significant augmentation in the activity of HO and cNOS.

After 6 week-running there were no differences in the body weight between non-running and running animals. While there was a progressive fall in body weight after the induction of colonic inflammation in the TNBS treated group, voluntary training attenuated this decrease of body weight in the TNBS challenged rats. 3 week-voluntary exercise did not change the acute colitis induced by TNBS. On the other hand 6 and 10 weeks of running decreased the extent of lesions, severity of mucosal damage and the activity of MPO enzyme. For further investigations we used 6- week running protocol.

6 weeks of running significantly increased HO activity compared to the absolute control group. Treatment with TNBS alone led to even higher HO activity, and there was no significant difference between the running TNBS group and the non-running TNBS animals.

6 weeks of running elevated cNOS activity significantly in the running control and the running TNBS groups compared to the corresponding non-running groups. In the non-running TNBS group we measured a significantly decreased cNOS activity compared to the non-treated control group. In the running TNBS group the voluntary exercise augmented the activity of cNOS, so the TNBS administration reduced this higher value only to the level of the absolute control group. TNBS challenge significantly elevated iNOS activity in the non-running and running groups. Importantly, 6 weeks of running before TNBS treatment significantly reduced this increase of iNOS activity.

Discussion

We demonstrated the role of HO in the regulation of aorta contraction. The inhibition of HO activity abolished the gender differences in aorta ring contractions induced by AVP. These results suggest that males are more susceptible for cardiovascular diseases.

The increased HO and cNOS activities reflect the role of vasoactive gases, carbon monoxide and nitric oxide produced by HO and NOS enzymes in the adaptation of cardiovascular system to physical activity.

The macroscopic parameters of inflammation and results of MPO activity show that 6 and 10 weeks of wheel running have anti-inflammatory effects on acute colitis in rats. 6 weeks of freewheel running alone increased the activity of HO and cNOS and did not change the activity of iNOS. The 6 weeks of running did not alter the HO activity induced by TNBS treatment. Voluntary exercise before TNBS administration increased the activity of cNOS and the reduction of cNOS activity caused by acute inflammation resulted in a control-level of the activity of cNOS in the running TNBS treated group. The elevated enzyme activities (HO, cNOS) induced by voluntary exercise have an anti-inflammatory effects in rat colon during the reduction of iNOS activity.

In our study we demonstrated that 6 weeks of running wheel exercise protocol is effective to activate beneficial mechanisms in the protection of the examined systems.

List of publications

Publications connected to the thesis:

Szalai Z, Szász A, Nagy I, Puskás LG, Kupai K, Király A, Berkó AM, Pósa A, Strifler G, Baráth Z, Nagy LI, Szabó R, Pávó I, Murlasits Z, Gyöngyösi M, Varga C. (2014) Anti-Inflammatory Effect of Recreational Exercise in TNBS-Induced Colitis in Rats: Role of NOS/HO/MPO System. *Oxid Med Cell Longev*. 2014;2014:925981. doi: 10.1155/2014/925981. **IF: 3,393**

Pósa A, Kupai K, Ménesi R, **Szalai Z**, Szabó R, Pintér Z, Pálfi G, Gyöngyösi M, Berkó A, Pávó I, Varga C. (2013) Sexual dimorphism of cardiovascular ischemia susceptibility is mediated by heme oxygenase. *Oxid Med Cell Longev*. 2013;2013:521563. doi: 10.1155/2013/521563. **IF: 3,393**

Conference publications

Szalai Z, Kupai K, Magyariné Berkó A, Pósa A, Szabó R, Varga C (2013) Time-dependent changes in inflammatory markers in a rat model of colitis by leisure sport activity; Magyar Gasztroenterológiai Társaság 55. Nagygyűlése, Tihany, Z Gastroenterol 51: 477; IF: 1,408

Posa A, Szabo R, Szablics P, Berko MA, **Szalai Z**, Orban K, Balogh L, Varga C (2011) Long term recreational physical exercise decreases angina susceptible and reduces myocardial infarct size. XXX Annual meeting of the European Section of the ISHR, Haifa, Israel, Bologna: Monduzzi International Proceedings Division, 2012. p. 39.

Szalai, Z, Berkó, M. A., Pósa, A., Szabó, R., Orbán, K., Király, A., László, F., Varga, Cs. (2011) Long term low intensity recreational physical exercise attenuates colonic inflammation in rats: the role of heme oxygenase system. 13th European Congress of Endocrinology Rotterdam, Nederland. ENDOCRINE ABSTRACTS 26: Paper P340. (2011)

Renáta Szabó, Anikó Pósa, **Zita Szalai**, Péter Szablics, Csaba Varga, Anikó Berkó, Kornélia Orbán, Ferenc László (2011) Long term recreational physical exercise decreases angina susceptibility and induces cardiovascular protective shifts in nitric oxide synthase, heme oxygenase and matrix metalloproteinase enzyme regulation in rats. 13th European Congress of Endocrinology Rotterdam, Nederland. ENDOCRINE ABSTRACTS 26: Paper P581. (2011)