Gender differences of coronary adaptation in normo- and hypertension

Ph.D. thesis

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1. Introduction

Gender differences estrogen vs. testosterone

The presence of gender differences in the cardiovascular system is well-established in many mammals. Gender dimorphism may have an effect on network properties, vessel size, composition and histology as well as several functional characteristics. This study aims to analyze differences in vascular geometry, elasticity and contractility between age-matched adult male and female rat intramural coronary resistance artery segments prepared from the same location with similar inner radii.

2. Objectives

Our series of experiments was designed to answer the following questions:

1) Are there gender differences regarding vessel morphology, geometry, biomechanics and vascular reactivity of the intramural coronaries under normotensive conditions?

2) Are there gender differences regarding vessel morphology, geometry, biomechanics and vascular reactivity of the intramural coronaries under hypertensive conditions?

3) How does angiotensin II induced hypertension effect the vessel morphology, geometry, biomechanics and vascular reactivity of the intramural coronaries in females?

3. Methods

3.1. Ethical approval and animals

3.1.1. Ethical approval

The investigation conformed to the Guide for the Care and Use of Laboratory Animals (NIH Publication No. 85-23, revised 1966) and with the Hungarian Law on Animal Care (Permission Number 36/1999). The investigation conformed to the Principles of Laboratory Animal Care National Institutes of Health publication No. 85-23 (revised 1985) with the Euroconform Hungarian Law on Animal Care (XXVIII/1998) and was approved by the institutional Animal Care Commission (institutional review board approval: Semmelweis University, 61/2003; 22.1/2960/003/2009).

3.1.2. Animals

3.1.2.1. Normotensive groups

Sexually mature, age-matched (10–12 weeks) male (n=10) and virgin female (n=10) Sprague–Dawley rats (Charles River Laboratories, USA/Germany) were used.

3.1.2.2. Hypertensive groups

Sexually mature, age-matched (2-month old) male (n=10) and virgin female (n=10) Sprague–Dawley rats (Charles River Laboratories, USA/Germany) were used.

The females and males were both subjected to subcutaneous implantation of an osmotic minipump performed under anesthesia (by pentobarbital 45 mg/kg) and sterile conditions (Ang II treated female and male groups). The osmotic pump infused 100 ng/body weight kg/min Ang II subcutaneously. The mean arterial pressure values from the hypertensive study groups were found to be the following: AngII-treated females: 131±5 mmHg; AngII-treated males: 134±7 mmHg. The mean relative heart weight (heart weight/body mass) of hypertensive animals were found to be the following; AngII-treated females: 0.387±0.009g/100g; AngII-treated males: 0.306±0.006 g/100g).

3.2 Experimental setup and protocols

Following four weeks of chronic AngII treatment, animals were re-anesthetized (Nembutal 45 mg/kg i.m.). The blood pressure was measured directly with cannulation of the carotid artery. Intramural coronary arteries (approximately 200 mm in diameter, secondary branches from the left anterior descending coronary artery) were isolated and placed into a vessel chamber filled with nKR, and it was cannulated at both ends with plastic microcannulas and extended to its *in vivo* length. Both cannulas were connected to pressure-servo systems (Living Systems, Burlington, VT) and arteries were pressurized in a no-flow condition.

The outer and inner diameters of the arteries were measured by microangiometry. During our measurements, we examined myogenic tone, the vascular effects of U46619, the TxA_2 receptor agonist (10^{-6} M) and bradykinin (10^{-6} M). For vessel morphological comparisons and biomechanical calculations, we used the diameter data of the vessel measured in a calcium-free solution.

3.3 Biomechanical calculations

- Wall thickness $h=r_o-r_i$; where h is the wall thickness, r_i is the inner, r_o is the outer radius.
- Incremental distensibility $D_{inc}=\Delta V/V\Delta P$, where D_{inc} is the incremental distensibility, ΔV is the change in vessel lumen volume in relation to the initial volume of V in response to pressure change of ΔP.
- The circumferential incremental elastic modulus was computed from the following equation: $E_{inc}= (\Delta p/\Delta r_o)*2r_i^2*r_o/(r_o^2-r_i^2)$, where E_{inc} is the incremental elastic modulus, r_i is the inner, r_o is the outer radius, Δr_o is the change in outer radius in response to intraluminal pressure change of Δp.

3.4. Vascular reactivity calculations

- Spontaneous tone T_{nKR} = (r_i Ca^{2+}-free–r_i nKR)/r_i Ca^{2+}-free, where where r_i Ca^{2+}-free, and r_i nKR are the inner radii measured in calcium-free Krebs solution and in normal Krebs-Ringer solution, respectively.
- TxA_2 induced tone TxA_2 = (r_i Ca^{2+}-free–r_i TxA_2)/r_i Ca^{2+}-free, where r_i Ca^{2+}-free, and r_i TxA_2 are the inner radii measured in calcium-free Krebs solution and U46619 /TxA_2-agonist/, respectively.
- Bradykinin induced tone T_{BK} = (r_i Ca^{2+}-free–r_i BK)/r_i Ca^{2+}-free, where r_i Ca^{2+}-free, and r_i BK are the inner radii measured in calcium-free Krebs solution and bradykinin, respectively.

3.5. Statistical analysis

For statistical analysis, the data measurements were compared by two-way ANOVA. The in vitro parameters, plotted as a function of intraluminal pressure between groups, were compared by two-way ANOVA. Paired comparisons for treatment groups were made for the curves. As a post hoc test, Tukey‘s test was used.

A P value<0.05 was uniformly accepted as a significant difference. The data were presented as means (SEM).

4. Results

4.1. Gender differences under normotensive conditions

4.1.1. Gender differences regarding vessel morphology, geometry and biomechanics of the intramural coronaries under normotensive conditions

There were no significant differences in outer and inner radius, these were morphologically identical vessels. However, wall thickness was higher in males compared to females measured in the relaxed state (in Ca^{2+}-free nKR) **(Fig. 1)**.
The values regarding distensibility were measured to be increased significantly in males (mmHg pressure values measured intraluminally 0.034427±0.0059 kPa-1 vs. 0.01537±0.0036 kPa-1 at 50 mmHg), while the elastic modulus (isobaric) remained decreased over pressure values of 30 mmHg (log elastic modulus, 5.4±0.1 Pa vs. 5.8±0.1 Pa at 50 mmHg in the male and also in the female groups, $p<0.001$).

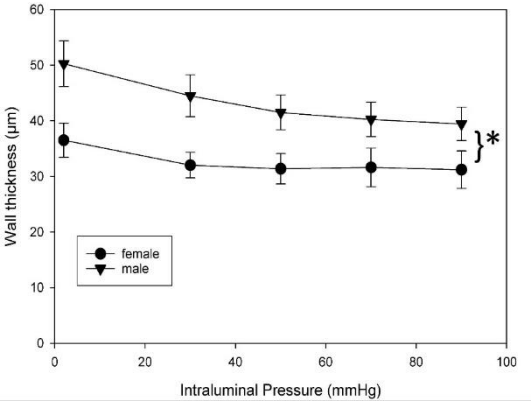

Figure 1. Wall thickness The isolated male (n=10) and female (n=10) coronary artery segments from the Sprague Dawley rats. Geometric parameters measured in the relaxed state (in Ca^{2+}-free nKR). Values of wall thickness as a function of intraluminal pressure. Both mean and ± SEM values are depicted. Significance levels using two-way ANOVA between the two groups are shown. *$p<0.05$.

4.1.2. Gender differences regarding vascular reactivity of the intramural coronaries under normotensive conditions

There were no differences in spontaneous tone and bradykinin induced tone. Maximum values of contractions to vasoconstrictor U46619 of male coronaries were much more vigorous compared to the values measured in the females (30.9±6.6% in the male group, and 14.5±3.3% in the female group - values of the vessels were measured at 50 mmHg pressure values measured intraluminally, $p<0.001$).

4.2. Gender differences in angiotensin II induced hypertension

4.2.1 Gender differences in angiotensin II induced hypertension regarding vessel morphology, geometry and biomechanics of intramural resistance coronaries in Angiotensin II induced hypertension

As we have demonstrated above the prepared segments were identical morphologically. In these segments significant differences were not found regarding the outer radii of the males and the females at p=50 mmHg in nKR solution (female: 130.0±7.6μm; male: 146.5±9.2 μm). Significant differences did not occur in terms of cross-section areas between the groups in nKR solution in 50 mmHg (23,149±3805 μm^2 in females and 20,618±2906 μm^2 in males). The intramural coronary segments harvested from male rats demonstrated significantly higher elastic modulus values than those that were prepared from the heart of the females **(Fig. 2).**

Gender related differences were not found concerning measurements describing parameters of distensibility (0.0295±0.0089 kPa–1 in males vs 0.0288±0.0079 kPa–1 in females in Ca^{2+}-free solution at 50 mm Hg).

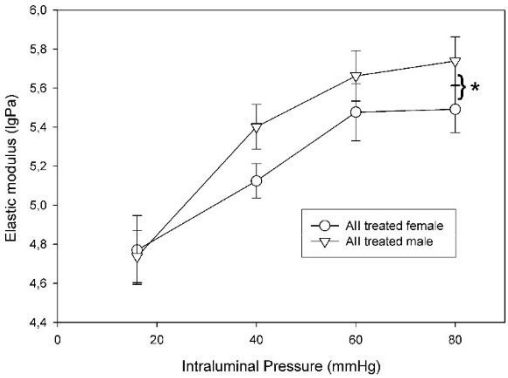

Figure 2. Biomechanical result from the isolated arterioles of males (n = 10) and females (n = 10). Elastic properties were measured in a passive condition (in Ca2+-free Krebs). The logarithm of the incremental tangential elastic modulus is shown as a function of the intraluminal pressure. Data found in in turn expressed as means (SEM) values. Significance levels of two-way ANOVA tests between the gender groups are demonstrated.

4.2.2. Gender differences regarding vascular contractility of intramural resistance coronaries in Angiotensin II induced hypertension

Following identical doses of AngII the coronary segments harvested from male specimens were shown to have significantly higher spontaneous tone values compared to females **(Fig. 3)**.

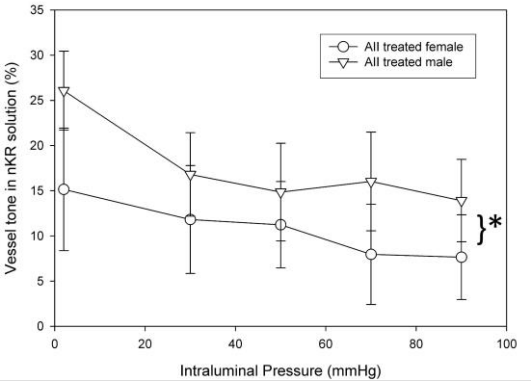

Figure 3. Vascular tone in male (n = 10) and female (n = 10) isolated coronary segments. Contractions are expressed relative to the inner radii measured in calcium-free solution at the same intraluminal pressure. Data are expressed and in turn demonstrated as means (SEM). Contractile response was significantly higher in the male group (two- way ANOVA and Tukey's post hoc test).

Segments from the male rats also demonstrated the increased tone of the vessels that was induced by TxA2 and the increase of vasoconstriction measurements compared to vessels harvested from the females **(Fig. 4)**.

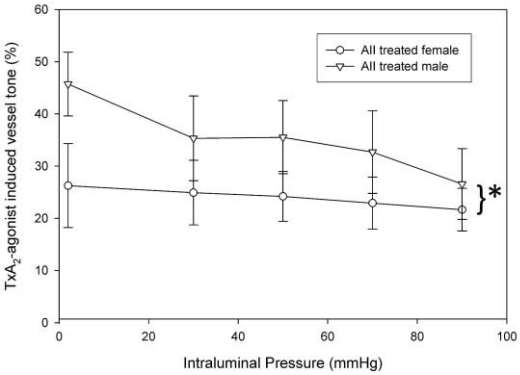

Figure 4. Tone induced by U46619 (1 mM) in male (n = 10) and female (n = 10) intramural resistance coronaries. Contractions are expressed relative to the inner radii measured in calcium-free solution at the same intraluminal pressure. Data are expressed and in turn demonstared as means (SEM). The contractile response was significantly higher in males (two- way ANOVA and Tukey's post hoc test).

4.3. Gender related differences regarding biomechanical and contractile of resistance coronaries in normotensive versus female rats with hypertension induced by Angiotensin II

4.3.1. Biomechanical properties of resistance coronaries in normotension versus Angiotensin II hypertension in female rats – Mechanisms of female hypertension

In the relaxed state the outer vessel radius of the coronaries were matching (in Ca^{2+}-free solution at 50 mmHg AngII 135.4 ±7.5 μm vs. control 142.7 ± 8.9 μm). This measurement confirms that the prepared and dissected blood vessels may indeed be considered similar and comparable. Following AngII inner radius decreased in a significant manner, and wall thickness increased significantly.

As an effect of AngII treatment the ratio of wall thickness to inner radius (lumen) increased significantly. Changes were not observed regarding the calculated cross-section areas of the

vessel segments following the administration of AngII treatment (22037 ± 1671 μm2 vs. 23652 ± 3314 μm2 on 50 mmHg).

Following AngII, distensibility significantly increased at higher pressure levels (in Ca^{2+}-free on 90 mmHg AngII 0.0324 ± 0.0075 kPa-1 vs. control 0.0119 ± 0.0032 kPa-1). Concurrently the values of elastic moduli decreased to a significant extent **(Fig. 5)**.

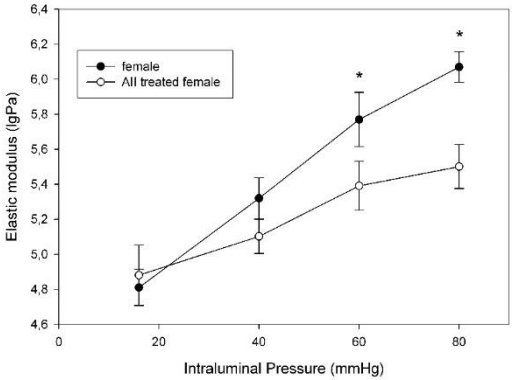

Figure 5. Elastic modulus of the isolated coronary resistance segments a function of intraluminal pressure in passive condition (in Ca2+-free Krebs). Values are shown as mean ± SEM, p<0.0. Control and AngII-treated groups are shown.

4.3.2. Contractility of resistance coronaries in normotension versus Angiotensin II hypertension in female rats – Mechanisms of female HT

There was no significant difference in spontaneous tone and bradykinin induced tone. In hypertension induced by AngII U46619 induced tone was observed to be elevated markedly (**Fig. 6**).

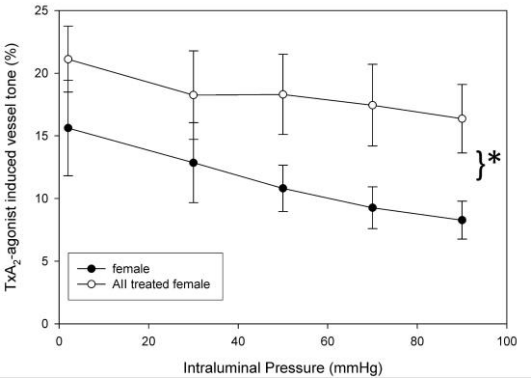

Figure 6. TxA2 induced tone (10-6 M U46619) was expressed as an active strain of rat intramural coronary resistance arteries as a function of intraluminal pressure. Values of AII-treated group (AII treated female) and control group (female) are shown as mean ± SEM, p values indicate statistical significance ($p<0.05$) between Control and AII-treated groups.

5. Conclusions

Our series of experiments was designed to answer the following questions:

1) Are there gender differences regarding vessel morphology, geometry, biomechanics and vascular reactivity of the intramural coronaries under normotensive conditions?

   Under normotensive conditions, as a gender difference, we described a higher wall thickness, lower elastic modulus, lower mechanical load of the vascular wall and higher TxA_2-contraction in males.

2) Are there gender differences regarding vessel morphology, geometry, biomechanics and vascular reactivity of the intramural coronaries under hypertensive conditions?

   In AngII hypertension, as a gender difference, an inward eutrophic remodeling and increased TxA_2 induced contraction were detected in males.

3) How does angiotensin II induced hypertension effect the vessel morphology, geometry, biomechanics and vascular reactivity of the intramural coronaries in females?

   We observed lumen narrowing and vessel wall thickening as an effect of AngII hypertension in female animals which resulted in lower mechanical load of the vascular wall and lower elastic modulus values, TxA_2-induced tone increased in hypertension.

6. Summary

The fact that there are gender differences within the cardiovascular system and also regarding adaptation mechanisms along the cardiovascular system to hypertension is well known, however, little is known about the mechanisms themselves and the agender differences regarding adaptation strategies. Our aim was to analyze gender differences regarding biomechanical properties and the pharmacological reactivity of intramural coronaries - to study the initial steps of hypertensive adaptation. We applied a rat AngII dependent hypertension model (100 ng/bwkg/min for 4 weeks).

As a gender difference, we described a higher wall thickness in males and lower elastic modulus and TxA_2-contraction in females.

We detected lumen narrowing and vessel wall thickening as an effect of AngII hypertension in female animals - which resulted in lower tangential stress and elastic modulus values. There were no differences regarding spontaneous and bradykinin induced tones, however, TxA2-induced tone increased in hypertension.

In females, hypertension caused an inward eutrophic remodeling and increased TxA2 induced contraction.

During our research we found substantial functional gender related differences regarding the biomechanical properties and pharmacological reactivity of intramural coronaries. Our research group also described the hypertensive adaptation of female coronaries. Knowledge of these differences may open the gate to a myriad of clinical studies regarding therapeutic option.

7. Bibliography of the candidate's publications

7.1. Publications related to the thesis:

Matrai M, Mericli M, Nadasy GL, Szekeres M, Varbiro S, Banhidy F, Acs N, Monos E, Szekacs B. (2007) Gender differences in biomechanical properties of intramural coronary resistance arteries of rats, an in vitro microarteriographic study. J Biomech, 40: 1024-1030. IF: 2.897

Matrai M, Szekacs B, Mericli M, Nadasy GL, Szekeres M, Banhidy F, Bekesi G, Monos E, Várbíró S. (2010) Biomechanics and vasoreactivity of female intramural coronaries in angiotensin II induced hypertension. Acta Physiol Hung, 97: 31-40. IF: 1.226

Mátrai M, Hetthéssy J, Nádasy GL, Monos E, Székács B, Várbíró S. (2012) Sex differences in the biomechanics and contractility of intramural coronary arteries in angiotensin II-induced hypertension. Gend Med, 9: 548-556. IF: 1.690

Varbiro S, Matrai M, Szekeres M, Nadasy GL, Szaky E, Mericli M, Banhidy F, Monos E, Szekacs B. (2006) Intramural coronary artery constrictor reactivity to thromboxane is higher in male than in female rats. Gynecol Endocrinol, 22: 44-47. IF: 0.995

7.2. Publications not related to the thesis:

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Jósvai A, Török M, Mátrai M, Hetthéssy J, Monori-Kiss A, Makk J, Székács B, Nádasy GL, Várbíró S. (2020) Effects of Testosterone Deficiency and Angiotensin II-Induced Hypertension on the Biomechanics of Intramural Coronary Arteries. J Sex Med, 17: 2322-2330. IF: 3.802

Mericli M, Nádasy GL, Szekeres M, Várbíró S, Vajo Z, Mátrai M, Acs N, Monos E, Székács B. (2004) Estrogen replacement therapy reverses changes in intramural coronary resistance arteries caused by female sex hormone depletion. Cardiovasc Res, 61: 317-324. IF: 4.575

∑IF: 17.918