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Anyagcsere betegségek molekuláris genetikája, patomechanizmusa és klinikai vonatkozásai
című program

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Gender differences of coronary adaptation in normo- and hypertension

PhD thesis

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LIST OF ABBREVATIONS

AAS	anabolic androgen steroid
ACE	Angiotensin-converting enzyme
AngII	angiotensin II
ANOVA	Analysis of variance
AT-1	Angiotensin II receptor type 1
AT-2	Angiotensin II receptor type 2
BK	bradykinin
BP	blood pressure
Ca^{2+}	calcium ion
cAMP	cyclic adenosine monophosphate
CO_2	carbon dioxide
CV	cardiovascular
D_{inc}	Incremental distensibility
E_{inc}	incremental elastic modulus
ET-1	endothelin 1
h	wall thickness
HDL	high-density lipoprotein
LAD	left anterior descending artery
mRNA	messenger ribonucleic acid
N_2	nitrogen
nKR	Normal Krebs-Ringer
NO	nitrogen-oxide
O_2	oxygen
P	pressure
PCOS	Policystarian ovarian syndrome
PVAT	perivascular adipose tissue
r	radius
SEM	Standard error of the mean
SHR	spontaneous hypertensive rats
SOD	superoxide dismutase

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TGF	transforming growth factor factors
TxA_2	Thromboxane-A2 receptor
V	volume

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1. INTRODUCTION

1.1. The cardiovascular effects of estrogen

Estrogen plays a key role in maintaining cardiovascular health in women. Female cardiovascular risk increases to such a degree following menopause, that menopause in itself may be considered to be a completely independent risk factor regarding cardiovascular disease (1). Most of this phenomenon may be considered to be the effect of the decrease in estrogen levels and the consequential decrease in its cardiovascular protective effects. There have been studies that have shown that estrogen administered as replacement therapy - started in a timely fashion and applied transdermally – may decrease cardiovascular risk in women following menopause (2, 3).

Estrogen has an effect on vascular vasodilation, as it increases it in the arteries and impedes the response to vascular injury at the cellular level, thereby aiding the prevention of atherosclerosis. Arterial vasodilation is regulated by fast, non-genome dependent mechanisms and is therefore unaffected by changes in gene expression. More time is required for genome-dependent mechanisms to evolve and these effect vascular tone, cellular response to injury and the development of atherosclerosis through changes in gene expression (2).

1.1.1. Direct vascular effects of estrogen

We differentiate between early and late-onset effects regarding the effects of estrogen taking place directly on the arterioles. The most fundamental genome-independent mechanisms of estrogen responsible for early onset rapid vascular relaxation are: the release of nitrogen-oxide (NO) from the endothelium, which in turn offsets the vasoconstrictive effects of endothelin; hyperpolarization of the smooth muscle; it also acts as a direct inhibitor on the calcium ion ($Ca^{2+)}$ channels of the vascular smooth muscle cells, and increases the cyclic adenosine monophosphate (cAMP) signal in the smooth muscle cells (4).

The cardioprotective effects linked to estrogen take effect through gene expression mediated by estrogen receptors (5). Estrogen is also a key factor regarding long-term role in the regulation of vessel diameter by increasing the production of vasodilator enzymes

(prostaglandin-cyclooxygenase, prostacyclin-synthase, nitrogen oxide-synthase). It also has an effect on the composition of the extracellular matrix of the vessel wall, the ratio of connective tissue elements, on smooth muscle cell proliferation through growth factors (transforming growth factor (TGF-β1) and protooncogen proteins (c-fos) it modifies the lipid profile, coagulation and it also modifies the effects of sexual steroids (4).

1.1.1.1.Endothelial effects

The direct effect of estrogen on the endothelium plays a key role in its cardioprotective effects. Studies comparing menopausal women to fertile ones showed that serum NO/endothelin-1 ratio decreased in the menopausal group (6). Estrogen deceases the level of vasoconstrictor endothelin-1, and in vitro studies have demonstrated that growth factors play a role as well. Not all, but most studies confirm, that estrogen increases endothelial prostacyclin levels and decreases the production of thromboxane (7). Both effects lead to vasodilation, and decreased thrombocyte aggregation. Flow-mediated vasodilation was significantly smaller in spontaneous hypertensive rats (SHR) female rats following ovariectomy compared to both control and ovariectomy plus hormone replacement groups (8).

In summary we may state, that estrogen maintains NO-dependent flow-induced vasodilation, which leads to decreased tangential stress along the vascular wall. This may also be a component of the cardiovascular protective effects of estrogen. Oxygen derived free radicals associated with atherosclerosis may inactivate NO – however, the antioxidant effects of estrogen may counteract these effects and promote vasodilation (9).

1.1.1.2.The effects of estrogen on the smooth muscle arterial cells

The proliferation of smooth muscle cells plays a fundamental role in the occurrance of vessel wall lesions to mechanical, chemical and immunological factors. Estrogen inhibits the proliferation of smooth muscle cells by modifying genetic control of the life-cycle of the cell (growth factors protooncogens). Modifications local TGF ß-1 production also has atheroprotective effects, as do the changes caused by the effects of the growth hormones themselves on the smooth muscle (10). Another study pinpoints to the c-fos protooncogen as the main effector estrogen on smooth muscle proliferation (11).

The inhibition of collagen synthesis is also a well-known effect of estrogen – this may play a cardinal role in vascular remodeling following injury to the vascular wall. This

type of remodeling occurs at every stage of the atherosclerotic process. The direct effect of estrogen on collagen and elastin transcription has also been established. Low concentrations have antiatherosclerotic effects, while high concentrations lead to the development of vascular lesions. The subtle modification of the extracellular matrix explains why depletion or deficiency of certain hormones and hormone replacement effect the biomechanical alteration of the arterioles in hypertension even before changes in wall thickness occur (12).

1.1.1.3.The effects of estrogen on angiotensin II induced hypertension

The most common underlying cause of hypertension in elder patients is the decreased elasticity of the large caliber arteries. The alterations occurring at the arteriole levels also play a cardinal part in the fact that hypertension develops and progresses. Due to the fact that impedance (namely hydrodynamic impedance) increases from the large caliber arteries to the smaller ones, the changes occurring in the biomechanical parameters of resistance arteries play a more and more cardinal role in the progression of hypertension. Both hypertension and angiotensin II (AngII) alter the biomechanical parameters of the arteries: the increase wall thickness and contractility and the alter the viscoelastic parameters of the vessels as well (13, 14). Chronic AngII therapy lead to a significant decrease in vessel diameter in hormone depleted animals, while this do not occur in hormone replaced animals. Simon et al also observed AngII effects in male rats that were dependent on dose (15). In consequence hormone replacement therapy may counteract the vicious cycle of decreasing diameter, which in turn would lead to an increase in peripheral resistance and elevated blood pressure levels (16, 17).

The administration of 17-ß estradiol therapy significantly decreased the vasoconstrictor effects to AngII independent of the intactness of endothelium (18). In the absence of estrogen Angiotensin II receptor type 1 (AT-1) receptor density showed significant increase without any modification to receptor affinity (19).

In contrast estrogen therapy leads to messenger ribonucleic acid (mRNA) dependent down regulation of the AT-1 receptor overproduction. Therefore, AT-l activation may be considered a key factor in blood pressure regulation, fluid balance and smooth muscle cell proliferation. Based on the above estrogen deficit and hypertension increases the risk of atherosclerosis. Transcription of angiotensin in the liver cells is increased through mechanisms controlled by estrogen receptors (20). Due to the direct smooth muscle

effects of estrogen increased AngII levels do not have vasopressor effects, but AngII primarily affects volume regulation. Estrogen decreases the production of Angiotensin-converting enzyme (ACE) through receptorial mechanisms by reducing vasoconstrictor AngII levels and increasing bradykinin levels (21).

1.2. The cardiovascular effects of testosterone

Following menopause cardiovascular risk increases for women significantly, which may be consequence of losing the cardioprotective effects linked to estrogen. In the past few years, the hormone-deficit of elderly men – namely andropause – had gained significant interest (22). Healthy middle-age/elderly men who had lower levels of testosterine demonstrated a higher degree of age-related endothelial dysfunction, which in turn is associated with higher oxidative stress and inflammation. This data suggests, that low testosterone concentration may contribute to the acceleration of vascular aging in men (22).

Epidemiological studies have not shown correlation between high androgen levels and atherosclerosis. Moreover, many studies have demonstrated that hypoandrogenisms are associated with coronary disease, atherogenic lipid profiles, metabolic syndrome, type II diabetes, systolic and diastolic hypertension, visceral obesity and elevated fibrinogen levels (23-25). Hypoandrogenism is frequent - 10% of men between the ages of 40 and 60 and 25% of men between the ages of 60 and 80 were found to have low free testosterone levels. Therefore hypoandrogenism – instead of hyperandrogenism – is considered as a non-specific contributing factor of atherosclerosis (26). However not all studies have confirmed this. Because low testosterone level is considered to have adverse effects, supplementation of testosterone may be beneficial. This was confirmed by studies analyzing safety of treatment. These studies also recorded the positive effects of hormone supplementation in cardiovascular risk i. e. less visceral fat, increased insulin sensitivity and glucose control and the positive effects on hyperlipidemia also (27, 28). Regression of angina symptoms and ischemic echocardiography has also been attributed to the direct effects of testosterone (27). However Whitsel et al found that following testosterone supplementation high-density lipoprotein (HDL) cholesterol concentration showed a dose-dependent decrease (29). Similarly, Meriggiola and Wu also found that while in

younger men administration of exogenous testosterone decreased HDL levels, it did not have any effect on HDL levels in elderly men (30, 31).

There are studies that have shown adverse androgenic effects regarding hypertension and ischemic stroke. Reckelhoff found testosterone to be a pro-hypertensive agent, while Hawk et al found testosterone was associated with worsening in the acute phases of stroke (32, 33). Therefore, should testosterone supplementation be considered, these effects should be weighed as well.

There are several cardiovascular (CV) diseases associated with the administration of anabolic androgen steroid (AAS). Self-administration of these steroids may induce vasospasm, sudden cardiac death, thrombocyte aggregation, the activation of the coagulation cascade and abnormal left chamber function and hypertrophy (34). Administration of anabolic steroids for 8-14 weeks has an adverse effect on lipid profile (35). In women surplus androgen has been shown to have adverse effects regarding cardiovascular risk. Insulin resistance is the most commonly studied risk factor, and this may also develop as a consequence of androgen effects. In 2005 Korytkowski et al demonstrated surplus androgen in postmenopausal women diagnosed with type 2 diabetes compared to women of the same age who do not suffer from diabetes (36). Policystarian ovarian syndrome (PCOS) is another example of correlation between high androgen levels and CV disease. Women suffering from PCOS have sustained high androgen levels. PCOS is accompanied by endothelial dysfunction, obesity, metabolic abnormalities such as insulin resistance and dyslipidemia, therefor the risk of atherosclerosis increases multifold in this condition (37).

We may conclude, that exogenous testosterone therapy leads to hyperlipidemia in both sexes – it increases blood pressure and risk of stroke in men, but it also decreases the size of plaques and insulin resistance, while the opposite occurs in women. Hormone replacement therapy has a positive effect through aromatization, especially when physiological testosterone levels are low. In contrast testosterone is detrimental regarding CV risk factors especially when concurrent diabetes is present.

Coronary flow was increased by administering intraluminal testosterone on patients with coronary atherosclerosis following Acetylcholine induced contraction (38). A study performed by Malkin et al. (28) revealed that administering testosterone therapy in the higher physiological bands for a period of three months increases vasoconstrictive

responses to noradrenaline and concurrently reduces dilatative response to acetylcholine and sodium nitroprusside in isolated resistance arteries (isolated from the subcutaneous layer of gluteal biopsy tissue samples) in androgen-deficient men compared to the pre-treatment levels. The vasodilatative reactivity of large coronaries to testosterone decreased in elderly rats compared to values from younger ones. Testosterone influences not only the ion channels (as it is well established) but also endothelial function (through its effects on NO release) (39).

1.3. Cardiovascular damage caused by hypertension

Reduced coronary flow and left chamber hypertrophy – that develop a consequence of coronary sclerosis – have a detrimental effect on the circulation of the heart muscle itself. Hypertension leads to a relative deterioration of coronary blood flow, because greater effort is required to pump blood through the peripheral vasculature due to elevated resistance. The contributing factors of hypertension also have a direct detrimental effect on heart function. The consequential degenerative changes (increased collagen production, decreased cardiomyocyte reduction, fibrosis) and frequent concurrent left chamber hypertrophy may lead to a decrease in both systolic and diastolic function. Decreased elasticity of the left chamber damages diastolic function, leading to an increase in intracardial pressure leading to subendocardial ischemia. The ischemic damage to the conduit system and the increase in inracardial pressure has a significant detrimental effect on impulse conduction, which leads to an increase in conduction time. Heart muscle ischemia leads to acidosis, which escalates the risk of arrhythmias (extrasystole, atrial fibrillation) (40, 41).

The presence of gender dimorphism in well-established in the literature along different vessel sgements in the cardiovascular system of several mammals including humans. These differences have pathological consequences. Lower prevalence is found in premenopausal women regarding cardiovascular disease - including ischemic heart disease and myocardial infarction –compared to men of the same age. This gender-dependent difference may develop as a result of a myriad of factors, including left chamber mass discrepancies, the differences in the severity of atherosclerosis on the coronaries and peripheral arteries and the differences regarding the reactivity to vasoactive, circulating mediators between the genders (42).

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The intramural resistance arteries of the left chamber are considered to have a fundamental role regarding blood supply to the heart muscle¸ they have a direct effect on tissue perfusion, but they are rarely studied in vitro due to methodological reasons – and especially due to the demanding technical requirements of dissection (43). Intramural coronary segments may not be compared to epicardial coronaries due to differences in caliber and the special relationship of these vessels to the surrounding tissue bed (contracting heart muscle) leading to highly particular flow conditions (44). Direct comparison of male and female coronaries has only been performed on larger caliber subepicardial and coronary arteries (45).

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2. OBJECTIVES

There is an abundance of literature available regarding vascular adaptation of intramural coronary arteries in males, however publications on female adaptation to hypertension are scarce. The objective of our study was to gain experimental data on gender related differences in the adaptation mechanisms of resistance arterioles of the coronary network. Our study also targeted female specific adaptation.

Vessel morphology, geometry, biomechanics and vascular reactivity, of the intramural coronaries was analyzed based on the following animal model.

Normo- and hypertensive male and female animals were analyzed. Gender differences regarding these parameters were analyzed using male vs. female normotensive and hypertensive rats. The effects of angiotensin II on these same parameters was examined by comparing normo- and hypertensive female rats.

Hypertension was achieved via subpressor dose of Angiotensin II administered by a subcutaneous osmotic minipump (100 ng/kgbdwth/min)

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

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3. METHODS

3.1. Ethical approval and animals

3.1.1. Ethical approval

Our experimental series 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 study 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).

Surgical or other complications did not occur. Conventional rat chow and tap water were provided *ad libitum.* The investigation conforms with the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health.

3.1.2. Animals

3.1.2.1. Normotensive groups

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

3.1.2.2. Hypertensive groups

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

Subcutaneous implantation of an osmotic pump was performed under anaesthesia on both the male and the female group (pentobarbital was used to anaesthesize the animals in a dose of 45 mg/kg). Sterile conditions were ensured. The osmotic minipump subcutaneously infused continually infused 100 ng/body weight kg/min AngII. Previous work demonstrated that this method leads to chronic blood pressure elevation following 2 to 3 weeks, with no observable acute pressure effects. This was the rationalse to use this model to study gender differences regarding early hypertensive vessel alterations (12, 46).

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) (47).

3.2. Chemicals

Anaesthesia was performed using Pentobarbital (Nembutal, Phylaxia–Sanofi, Budapest, Hungary). Penicillin – in the dose of 100 000 IU penicillin (TEVA–Biogal, Debrecen, Hungary) was administered i.m. We used the minipump (Alzet 2 ML4, Durect co., USA) containing angiotensin II acetate (AngII) from Sigma-Aldrich Co. (St. Louis, MO, USA and Budapest, Hungary).

Normal Krebs-Ringer (nKR) solution was administered for the studies. Temperature was maintained at 37 °C, and oxygenated and bubbled with 5% CO_2, 21% O_2, 74% N_2. This assured pH at 7.4. Thromboxane A2 receptor agonist U46619 and bradykinin (BK) were used to test vascular reactivity (Sigma-Aldrich Co. (St. Louis, MO, USA and Budapest, Hungary).

3.3. Experimental setup and protocols

Animals were re-anesthesized after four weeks of AngII treatment. Blood pressure was measured via cannulation of the carotid. The heart was removed and intramural coronary arteries - approximately 200 mm in diameter (representing secondary branches of the left anterior descending argtery (LAD)) were isolated according to previous studies (43, 48-50). The isolated segment was placed into a vessel chamber into nKR, it was cannulated and extended to its *in vivo* length. It was connected to a pressure-servo system (Living Systems, Burlington, VT) and the arteries were pressurized under no-flow conditions.

Both the inner and the diameter of the vessel segments was measured via microangiometry. A microcomputer evaluated the signals and automatically positioned two light markers aligning with the wall of the vessel. During our series we measured myogenic tone and vasoreactivity. Biomechanical calculations were performed using data from the microangiometry and data from the calcium-free solution measurements.

3.4. Biomechanical calculations

- Wall thickness $h=r_o-r_i$; h - wall thickness, r_i - inner, r_o - outer radius.
- Incremental distensibility $D_{inc}=\Delta V/V\Delta P$, D_{inc} - incremental distensibility, ΔV - change in vessel lumen volume in response to a pressure value change of ΔP.
- Circumferential incremental elastic modulus: $E_{inc}= (\Delta p/\Delta r_o)*2r_i^2*r_o/(r_o^2-r_i^2)$, E_{inc} - incremental elastic modulus, r_i - inner, r_o - radius, Δr_o -change in outer radius following a pressure value change of Δp.

3.5. Vascular reactivity calculations

- Spontaneous tone T_{nKR} = (r_i Ca^{2+}-free–r_i nKR)/r_i Ca^{2+}-free, e r_i Ca^{2+}-free, and r_i nKR - inner radii measured in calcium-free Krebs vs. normal nKR.
- TxA_2 induced tone TxA_2 = (r_i Ca^{2+}-free–r_i TxA_2)/r_i Ca^{2+}-free, r_i Ca^{2+}-free, and r_i TxA_2 - inner radii in calcium-free Krebs and following the administration of U46619 /TxA_2-agonist.
- Bradykinin induced tone T_{BK} = (r_i Ca^{2+}-free–r_i BK)/r_i Ca^{2+}-free, r_i Ca^{2+}-free, and r_i BK -inner radii measured in calcium-free Krebs and following the administration of bradykinin.

3.6. Statistical analysis

Two-way ANOVA was used for comparison. Paired comparisons were made for to calsulate the curves for different treatment groups. Tukey‘s test was our choice for post hoc test. Significant difference was accepted at $P<0.05$. Data is shown as means (SEM).

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4. RESULTS

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

4.1.1. Gender related differences regarding the geometrical parameters of intramural resistance coronary arteries under normotensive conditions

Under control conditions the mean value of the outer radius of the male intramural coronary arteries was found to be 146±9 μm, in female vessels this was 130±8 μm (no significance). In nKR solution and in a relaxed state (Ca^{2+}-free solution) the inner radius of male arteries also did not differ significantly different from female vessels (in nKR solution at 50 mm Hg intraluminal pressure 100±8 μm by female and 102±8 μm by male; at 50 mmHg in Ca^{2+}-free solution 111±9 μm by female and 116±10 μm by male). Based on these results we may state that preparing similar caliber blood vessels from male and female animals was successful. These vessels from the male and female groups demonstrated differences regarding geometry at each pressure level studied - the segments from the male demonstrated significantly greater values regarding the thickness of the vessel wall compared to the female group (e.g., in the male rat coronary group 41.5±2.9 μm vs. 31.4±2.7 μm in the female rat coronary group, under control conditions at 50 mmHg, **Fig. 1**, $p<0.05$). In male vessels the ratio of the thickness of the wall compared to the inner radius increased significantly also: in nKR-Solution at 50 mmHg intraluminal pressure: female h/r_i-ratio: 0.37±0.05 and male 0.44±0.03.

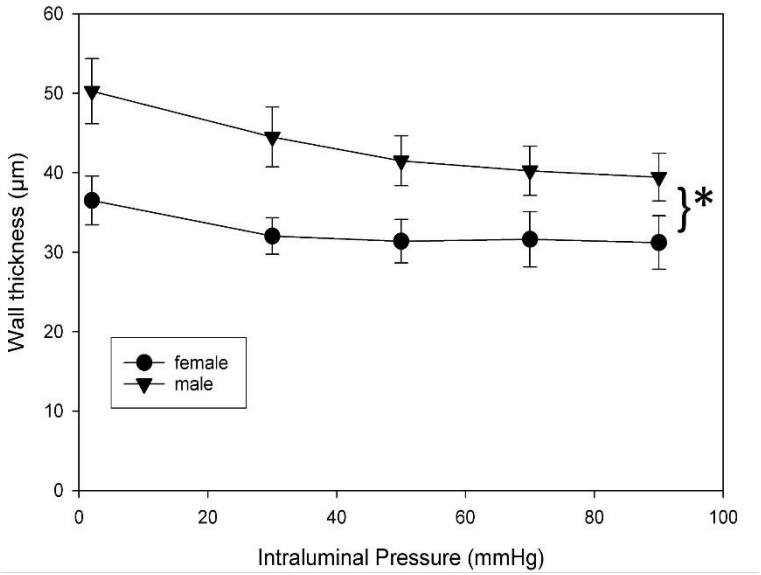

Fig. 1. The isolated male (n=10) and female (n=10) coronary artery segments from the Sprague Dawley rats were harvested from the intramural vasculature of the heart and were cannulated and measured via angiometry in a tissue bath. Geometric parameters measured in the relaxed state (in Ca^{2+}-free nKR). 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. *$p<0.05$. (51)

4.1.2. Gender related differences regarding biomechanical parameters of intramural coronary arteries under normotensive conditions

Fig. 2 demonstrates the properties representing elasticity of the vessels under passive/relaxed conditions. The values regarding distensibility were measured to be increased significantly in males (e.g., mmHg pressure values measured intraluminally 0.034427±0.0059 kPa^{-1} vs. 0.01537±0.0036 kPa^{-1} at 50, **Fig. 2A**, $p<0.05$,), while the elastic modulus (isobaric) remained decreased over pressure values of 30 mmHg (e.g., log elastic modulus, 5.4±0.1 lgPa vs. 5.8±0.1 lgPa at 50 mmHg in the male and also in the female groups **Fig. 2B,** $p<0.001$).

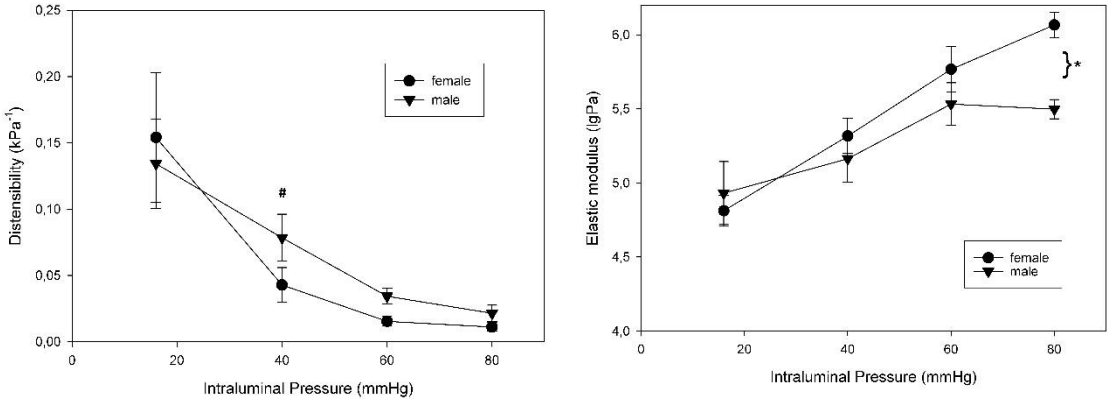

Fig. 2A and 2B. Elastic properties were measured in the male (n=10) and female (n=10) groups measured in the passive condition (in Ca^{2+}- free Krebs solution). (A) Incremental distensibility is shown. (B) Logarithm of incremental tangential elastic modulus is shown. Mean±SEM values. Significance between the two groups is marked. The # symbol represents statistical significance ($p<0.05$) between the two groups at the given pressure level with the Tukey post-hoc test. (51)

4.1.3. Gender related differences regarding parameters representing the contractility of the vessels and vascular reactivity of intramural resistance coronaries under normotensive conditions

Following an incubation period all of the segments demonstrated a marked spontaneous tone with a limited but very present response of stretch. This response is characteristic for resistance arteries of the coronary network in this range of caliber size. Spontaneous contractions were not statistically significantly different between the male and the female groups (e.g., 9.8±2.8% vs. 10.8±2.6% tone in nKR at 50 mmHg intraluminal pressure in the male and female group, respectively). 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, **Fig. 3.** $p<0.001$).

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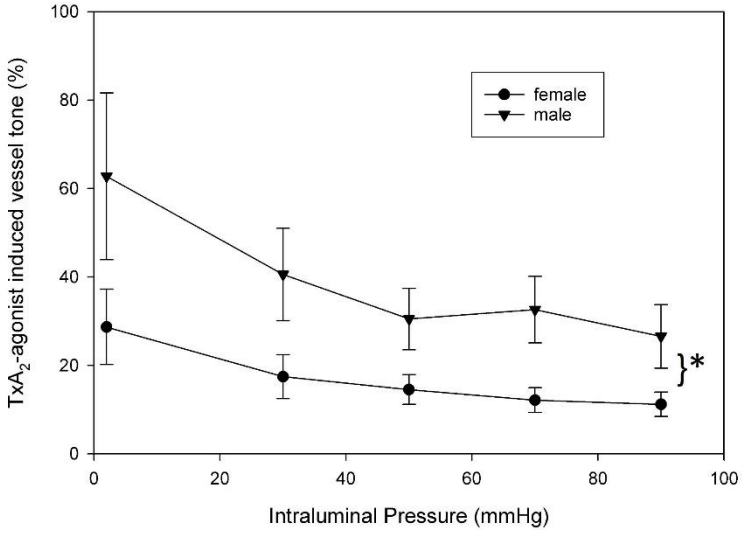

Fig. 3. Thromboxane-A2 (TxA_2) receptor agonist U46619 (1 mM) induced tone was compared between the male (n = 10) and female (n = 10) isolated vessel segments. Contractions are marked compared to the inner radii values from calcium-free solution using measurements takenat the same level of intraluminal pressure. Data are marked as mean values (SEM). Significantly higher results were seen in the male groups compared with the female group (Two-way ANOVA and Tukey's post hoc test; factors: sex, intraluminal pressure). (52)

Extraluminal BK induced endothelial dependent dilation was not different between the male and female groups (e.g., pressures measured intraluminally were respectively 8.1±2.3% vs. 7.7±2.0% at 50 mmHg). When BK was administered both extra- and intraluminally, dilations between male group and the female group vessel segments did not differ significantly (e.g., 6.4±2.2% versus 3.5±1.3% at 50 mmHg pressure values measured intraluminally).

4.2. Gender differences regarding vessel biomechanics and contractile parameters of intramural resistance coronaries in hypertension induces Angiotensin II

4.2.1. Gender differences regarding geometrical parameters of intramural resistance coronaries in hypertension induced by Angiotensin II

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 (23149±3805 μm^2 in females and 20618±2906 μm^2 in males).

4.2.2. Gender related differences regarding biomechanical parameters of the intramural resistance coronaries in hypertension induced by Angiotensin II

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. 4**; $P<0.05$).

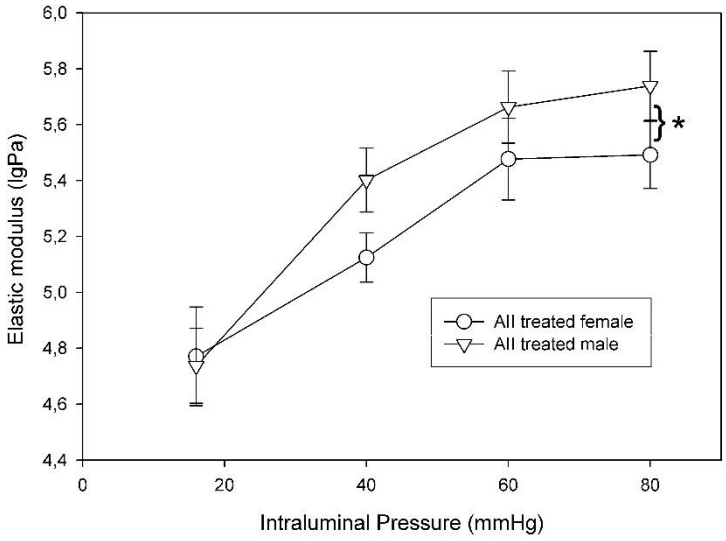

Fig. 4. Biomechanical result from the isolated arterioles of males (n = 10) and females (n = 10). Elastic properties were measured in a passive condition (in Ca^{2+}-free Krebs). The logarithm of the incremental tangential elastic modulus is shown as a function of the intraluminal pressure. Data found in in turn expressed as means (SEM) values. Significance levels of two-way ANOVA tests between the gender groups are demonstrated. (47)

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.

4.2.3. Gender related differences regarding contractile parameters of the vessels and endothelial function of the resistance coronaries in hypertension induced by Angiotensin II

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. 5**; $P<0.05$).

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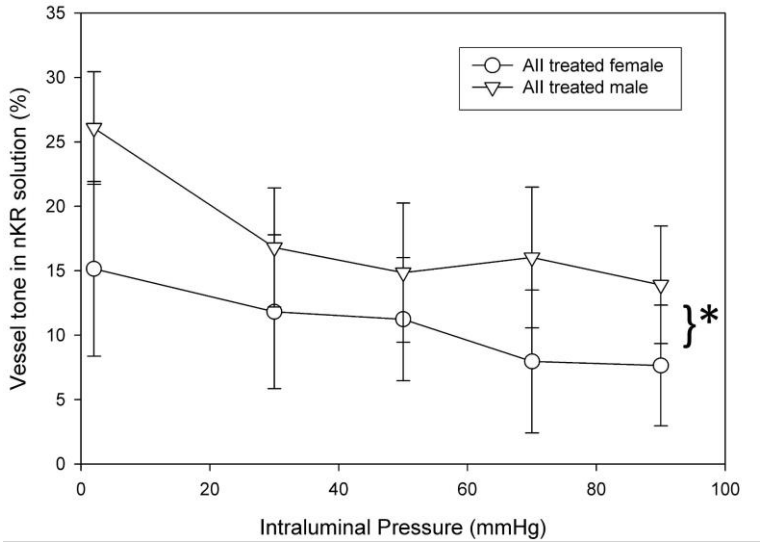

Fig. 5. Vascular tone in male (n = 10) and female (n = 10) isolated coronary segments. Contractions are expressed relative to the inner radii measured in calcium-free solution at the same intraluminal pressure. Data are expressed and in turn demonstrated as means (SEM). Contractile response was significantly higher in the male group (two- way ANOVA and Tukey's post hoc test). (47)

Segments from the male rats also demonstrated the increased tone of the vessels that was induced by TxA_2 and the increase of vasoconstriction measurements compared to vessels harvested from the females (**Fig. 6**; $P<0.05$).

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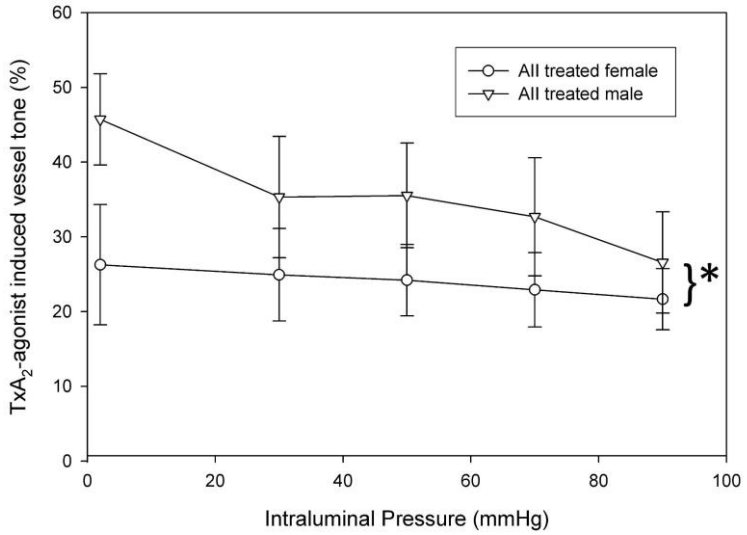

Fig. 6. Tone induced by U46619 (1 mM) in male (n = 10) and female (n = 10) intramural resistance coronaries. Contractions are expressed relative to the inner radii measured in calcium-free solution at the same intraluminal pressure. Data are expressed and in turn demonstared as means (SEM). The contractile response was significantly higher in males (two- way ANOVA and Tukey's post hoc test). (47)

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. Differences regarding vessel geometry of coronary arteries in normotensive versus Angiotensin II–induced hypertensive female rats

In the relaxed state the outer vessel radius values 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 **(Fig. 7A)**, and wall thickness increased significantly **(Fig. 7B).**

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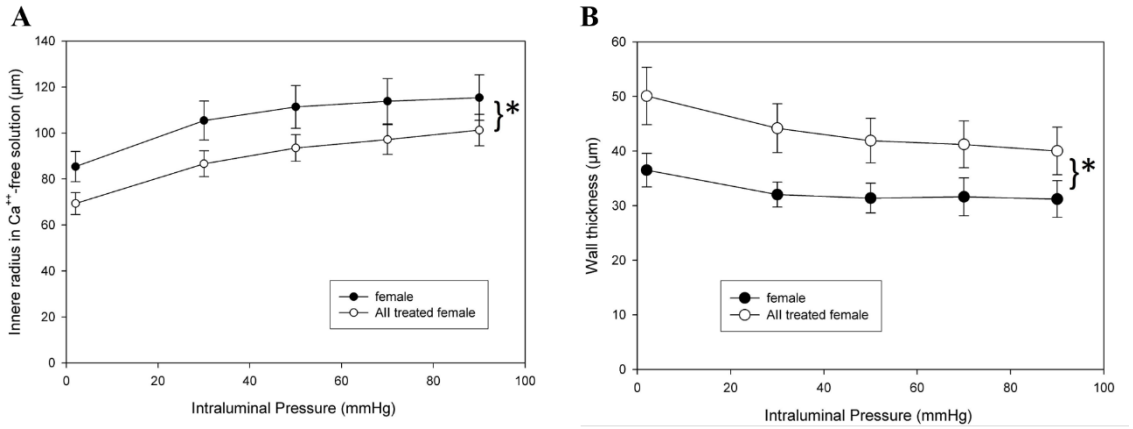

Fig. 7A and 7B. Inner radius and wall thickness was measured in the AngII female and control groups in the passive condition (in Ca^{2+}- free Krebs solution). Values of the AngII and the control group are shown as mean ± SEM, p is considered significant at $p<0.05$. (53)

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 μm^2 vs. 23652 ± 3314 μm^2 on 50 mmHg).

4.3.2. Biomechanical parameters of intramural resistance coronaries in normotension versus Angiotensin II induced hypertension in female rats

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}, **Fig. 8**). Concurrently the values of elastic moduli decreased to a significant extent (**Fig. 9**).

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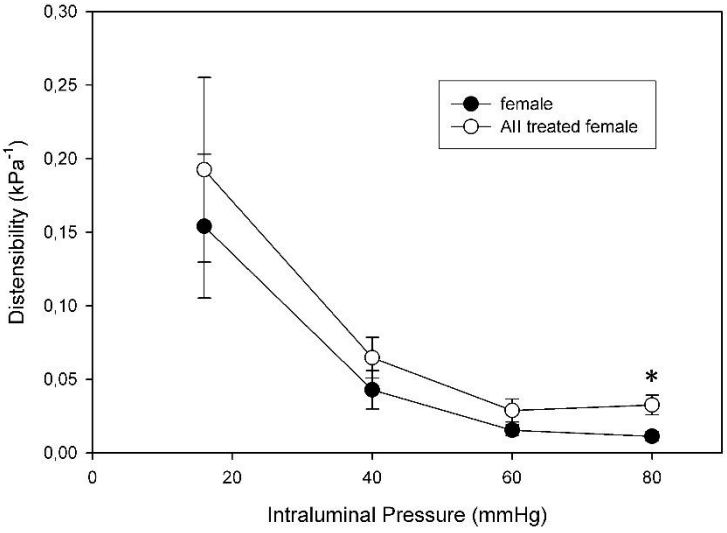

Fig. 8. Distensibility of the isolated coronary segments as a function of intraluminal pressure in the passive condition (in Ca^{2+}-free solution). Values are shown as mean ± SEM, $p<0.05$ Control and AngII-treated female groups are shown. (53)

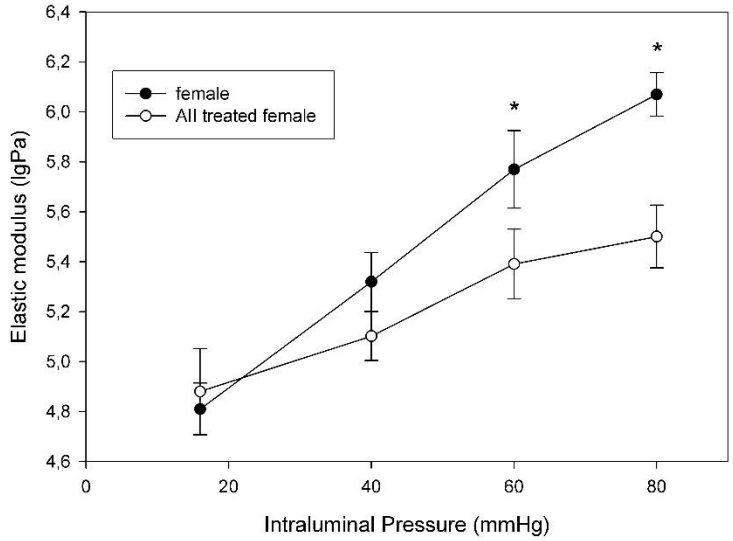

Fig 9. Elastic modulus of the isolated coronary resistance segments a function of intraluminal pressure in passive condition (in Ca^{2+}-free Krebs). Values are shown as mean ± SEM, $p<0.0$. Control and AngII-treated groups are shown. (53)

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4.3.3. Gender related differences regarding the contractile values of coronaries in normotensive versus female rats with hypertension induced by Angiotensin II

Spontaneous myogenic tone was unaffected by AngII (in Krebs solution at 50mmHg AngII 9.8 ± 2.8% vs. control 7.6 ± 1.6%). In hypertension induced by AngII U46619 induced tone was observed to be elevated markedly (**Fig. 10**).

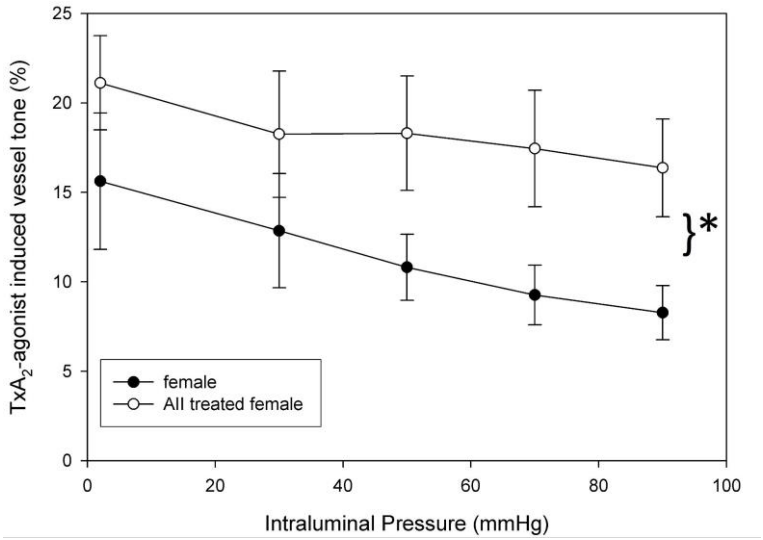

Fig 10. TxA_2 induced tone (10^{-6} M U46619) was expressed as an active strain as a function of intraluminal pressure in the isolated coronary segnents. Values shown as mean ± SEM, $p<0.05$. Control and AngII-treated groups are shown. (53)

Significant differences were not seen regarding relaxation induced by the administration of BK following either extra- or (AngII 5.0 ± 1.3% vs. control 6.1 ± 1.3% on 50 mmHg in Krebs solution) intraluminal administration (control: 3.5 ± 1.3 % versus AngII: 4.8 ± 2.8 %, non-significant on 50 mmHg intraluminal pressure, in Krebs solution).

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5. DISCUSSION

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

The presence of gender rekated differences along the cardiovascular system is well-descibed in many mammals. Gender dimorphism may have an effect on network properties, vessel size, composition and histology as well as several functional characteristics (54-60). This study revelaed the differences regarding vascular geometry, elasticity and contractility between age-matched adult male and female rats using isolated intramural coronary resistance artery segments prepared from the same location with similar inner radii.

Regarding geometry, thickness of the wall and wall-thickness-to-inner-radius ratio were found to be larger in male compared with values from female specimens. In males the greater wall thickness and unaltered inner radius resulted in a decrease in wall stress (isobaric) in the male versus the female group. Based on coronary ultrasound smaller coronary artery size is found in human females (57) even following normalization for left ventricular mass (61, 62). Krus demonstrated that intimal thickness was decreased in women, while the mean number of myocytes calculated to 1 mm^2 is equivalent in both genders (63, 64). Histological composition has not demonstrated gender differences in terms of coronary thickness in previous studies (63, 65). At higher pressures the isobaric elastic modulus of male vessel specimens smaller, while the calculated values regarding distensibility was increased when measured at physiological pressures compared to females. Based on these results we may state that male coronaries may be considered to be more elastic at higher intraluminal pressures than the corresponding female vessel specimens. In contrast to our results, Török et al. found no difference in the outer and inner diameter and wall thickness of resistance coronary arterioles of Wistar rats. In the 24-week-old rats, the wall tension was already higher in the males, while the gender difference in the elastic modulus and contractility observed in the younger rats was no longer observed (66). The intramural coronary network in remodels with age (67), this reorganization may differ in male and female animals. We can not rule out the possibility

that the rate of aging may differ in the two sexes. As a result of the remodeling the position of the vessels (external diameter of 200 micrometers) changes within the network (in older animals, the 200 micrometer branches are in a more peripheral position). Furthermore, a study regarding elastic moduli of epicardiac coronary arteries found that these values were higher in men than in women (65). Reduced isobaric wall tension may offer a partial explanation for the lesser rigidity of male vessel segments. Considering that the log elastic modulus of the male coronary resistance arteries was less even when plotted against tangential wall stress, we hypothesize a real difference either regarding the amount or in the interconnection of tissue elements responsible for passive elasticity. Based on previous biomechanical measurements performed on male coronary resistance arteries, pressure-dependent wall stresses and isobaric elastic modulus have been proven to become consequently smaller towards the peripheral segments of the network (50).

The changes observed in the intramural coronary vessel walls were not restricted to passive characteristics only. In our series, we found that spontaneous myogenic tone was similar in both genders; in females the maximum of active isobaric vasoconstriction was much more expressed in the male versus the female group. Males demonstrated an increased wall thickness compared to females which may explain the agonist-induced contractile response. Kingma et al. investigated the spontaneous tone of septal coronary arteries (<200 μm) and responses with and without NO blockers to endothelin-1 in male, female and ovariectomized animals using wire-myograph (68). Spontaneous tone was significantly higher in males, and the male rats exhibited greater sensitivity to the vasoconstrictive effects of endothelin 1 (ET-1). Following exposure to NO blockade vessel responses to ET-1 normalized in both male and female groups ovariectomized group was not studied. However, gracilis muscle arterioles have been found to demonstrate a gender-dependent difference regarding myogenic response. This may be attributed to enhanced release of NO associated with the presence of estrogen (55). Greater pressure-induced myogenic tone was observed in vessel segments harvested from male or ovariectomized female rats compared similar arteries control females or animals receiving estrogen replacement (69). The differences in the regulation of endothelial ecNOS and Ca^{2+} between the genders may explain the lower myogenic tone in women demonstrated by Knot et al. (58). Bowles et al. also found that gender has an effect on coronary L-type Ca^{2+} current in smooth muscle cells in large coronary arteries harvested

from miniature swine (45). Voltage- dependent activation of Ca^{2+} current shifted toward more negative membrane potential values in males. Similar mechanisms may play a role in the greater vascular contractility demonstrated in males in our series.

In our study the BK-induced vasodilation of the resistance coronary arteries did not differ between the genders. Mainly through the endothelial release of NO, BK is a potent vasodilator in intramural coronary arteries (49, 70). BK also induced NO-dependent vasodilation in both genders although differences in extent were observed (49). These findings are supported by the data of Barber and Miller (71). Their series on swine coronaries confirmed that the activity of calcium-dependent and calcium-independent NO synthase was similar in both genders.

Gender-dependent differences were found in our experiments of intramural coronaries regarding TxA_2 induced vasoconstrictor responses. Males compared to females demonstrated increased constrictor reactivity. TxA_2 was chosen for its physiological role in this vessel region. This is key as most vasoconstrictors are ineffective or have minimal effects on coronaries. Other agents may have vasoconstrictor properties but they fail to reach sufficiently high concentrations locally in this region (angiotensin II, prostaglandin). Coronary arteries are also not equipped with sympathetic vasoconstrictor innervation. At the same time, perivascular adipose tissue (PVAT)-mediated relaxation may be an underlying cause of the reduced contractility observed in females. In the presence of adherent PVAT, contractions to vasoconstrictor thromboxane agonist (U46619) and endothelin-1 were significantly reduced in porcine coronary arteries from females, but not in males (72).

The result from our series may be interpreted as follows. The demonstrated differences regarding geometry (increased wall thickness/decreased vascular lumen) and/or differences regarding contractility of the vascular smooth muscle (73-76) may be responsible for the varying vasoconstrictor responsiveness of the same coronary vessel segment in males versus females. The gender difference is not due to the effects of alteration in blood pressure, as this parameter was matched in both groups. There is a possibility that there is an increased sensitivity in the male coronary smooth muscle cells to vasoconstrictor TxA_2 compared to females.

Exploring the clinical correlation of these results may be promising. The release or production of platelet-derived thromboxane may be increased as a result of the presence

of pathological regulators or damage to the arterioles themselves. Considering the fact that intramural arteries are responsible for blood supply of the heart muscle, vasoconstriction of these arteries will likely be exaggerated in males compared to females.

Previous investigations have revealed gender differences related to TxA_2-induced vasoconstriction in both main branches of the coronary arteries and subepicardial vessels also (75). Based on these prior results regarding non-intramural coronaries, conclusions could not be derived regarding gender differences in intramural coronary TxA_2 sensitivity. TxA_2 sensitivity is in part related to the type of vascular environment surrounding the vessel i.e., consequential associated cyclical mechanical stress, and differences in the vasoactive reactivity of various coronary beds.

The exact extent and type of pathological consequences of these gender differences may be difficult to gauge correctly at the present level of our knowledge base. Both diagnostic and prognostic relevance of the parameters affected by gender difference (i.e., vascular elasticity and contractility) was supported by measurements performed on of large caliber human coronaries designed to evaluate coronary vascular disease (77, 78). Hemodynamic function is essentially determined by the passive and active biomechanical characteristics of the vessel wall segment (79).

Another field for further analysis is the potential mechanisms underlying these gender differences. Greater wall thickness and more vigorous maximal contractions observed in male intramural coronaries may be the result of female and male sexual hormones on the wall of microvessels. Sex hormones may induce elevated synthesis of wall material including contractile proteins. Earlier studies have elaborated on the direct vascular effects of sexual hormones on coronaries and on the presence of estradiol and androgenic receptors that may be the basis for these effects (80-82). An earlier publication from our laboratory demonstrated that radius-to-wall thickness ratio and contractility changed along the vascular segments of the arterial intramural coronary network.

Possible underlying causes of the gender-dependent differences regarding TxA_2 induced vasoconstrictor responses in our series of intramural coronaries may be explained by the differences in the sensitivity of the vascular components to TxA_2 and also by the complex geometric changes seen in the lumen that are not related to the vessel wall. TxA_2 sensitivity may also readily be the result of genetic or male/ female sexual hormone effects. Previous experiments in our laboratory have shown that estrogen decreased TxA_2-

induced vasoconstriction in the same intramural coronary arterial segments of female rats following ovariectomy. Increased TxA_2 sensitivity has been observed previously in larger epicardial coronary arteries and peripheral arteries, so conclusions should be drawn with caution from these studies (75).

5.2. Gender difference in hypertension

The early gender-related steps of vascular adaptation to hypertension in the intramural coronary arteries was described in our studies. Inward eutrophic remodeling was accompanied by the increased relative heart mass in females. Increased wall strain, elastic modulus, and contractility was found in males with decreased relaxation capacity. Morphological stability of the vessel walls remained intact initially - which according to data in the literature only destabilizes after a longer period of hypertension.

In contrast to our series regarding normotensive animals, higher wall thickness values were observed in AngII hypertensive females. In the normotensive groups males developed thicker walls and produced consequently lower isobaric wall stress values. This affected isobaric elastic modulus and male segments - originally less rigid in normotensives - increased their rigidity compared to females with AngII hypertension. Even though data is not available regarding resistance vessels, Park et al (83). found greater arterial elasticity in hypertensive females compared to males during physical exercise; these differences however disappeared when measurements were normalized to body surface. Hypertensive female rats demonstrate lower activation levels regarding the AngII-AT-1-ACE axis and a higher level of activation regarding ACE2-Ang-(1-7)-Mas axis compared to males. The AngII receptor depressor affects the response to chronic AngII infusion in female but not male hypertensive rats (84). Genetic differences seen in hypertension have been thought to be carried by sex chromosomes. Genetic differences regarding the sympathetic nervous system and the renin–angiotensin system have been directly linked to the Y chromosome (85). Considering the fact that angiotensin-converting enzyme inhibitors are proven to be the most effective antihypertensive medications in women’s, we may safely hypothesize that AngII is the most important hypertensive stimulus in females (86). Underlying molecular mechanisms include c-fos and c-jun pathways. These are associated with estradiol and AngII reaction pathways (87).

Decreased expression of AT-1 receptor as an effect of estradiol has also been established (88) long-term studies have demonstrated that ovariectomy was followed by elevated blood pressure levels and decreased endothelium-dependent relaxation. These adverse consequences may be prevented by the administration of estradiol. Underlying causes include changes in both the nitric oxide and the renin–angiotensin systems (89). In an intrauterine retardation hypertension model, castration of the male offspring rats normalized increasing blood pressure, while ovariectomy performed on the female offspring led to increased blood pressure (90); these processes were mediated via the renin–angiotensin system and through testosterone (91). Controversy surrounds the effects of sex hormones on the vascular system; in summary it has been established that testosterone dominantly affects the renin–angiotensin system and thereby increases sympathetic tone. This in turn leads to vasoconstriction and the progression of atherosclerosis (92). Our biomechanical data showed that wall thickness was greater in the segments harvested from females, and this led to relatively lesser mechanical loading. Meanwhile in males the vessel walls were more rigid, and the wall material demonstrated less hypertrophy, meaning that the mechanical loading was greater. Hypertensive adaptation mechanisms demonstrate that optimizing mechanical loading was key in females, whereas prevention of vessel wall thickening was most important in males.

When the adaptation mechanisms of these vascular segments (responsible for the blood supply of the heart) are analyzed from a biomechanical point of view we may state that hypertrophy of the wall to normalize wall stress was more important in females while the key adaptation mechanism was an increase in wall rigidity in males in this vascular segment. Bonacasa et al. (93) observed decreased remodeling of the coronaries in SHR rats, following methoxyestradiol treatment of castrated females and intact males.

The previously described mechanism might be complemented by the fact that pressure loading may adversely affect the microvessel network of the heart more directly. This may be considered an underlying reason regarding both myocardial infarctions and mechanical gender differences.

Males demonstrated increased spontaneous vessel tone compared to females in this study. Differences in vessel tone were retained in case of maximal TxA_2 constriction and BK relaxation. A previous study described that endothelin-1A receptor density is higher in male hypertensive rats than in female. Because the endothelin-1A receptor is the

predominant receptor subtype along the vasculature, this gender difference in vasoconstrictor tone may be a mechanism contributing to gender differences in the prevalence of hypertension in middle-aged and older adults. This may be explained by the fact that estrogens suppress ET-1 expression, while testosterone promotes the release of ET-1 (84). Furthermore, female SHR have both greater NO production and less scavenging of NO resulting in greater NO bioavailability, which may contribute to more decresed tones compared to males (94). Hypertension in males is mediated by increased superoxide and loss of bioavailable NO, perhaps due to inadequate superoxide dismutase (SOD). In females, the data suggest that prepubetal development of hypertension may be superoxide mediated since tempol (SOD mimetic) is able to prevent the increase in blood pressure (BP) with adulthood. Maintenance of hypertension in females either in adults or following cessation of estrous cycling (menopause) is refractory to tempol (adult and postcycling) but is attenuated with chronic vitamins E and C (postcycling), suggesting that, if the hypertension in mediated by oxidative stress, the oxidants may be peroxynitrite mediated. However, it is also possible that vitamins E and C affected BP via reductions in inflammation rather than oxidative stress per se and that BP in females, regardless of age, plays no role in their hypertension (95). Another explanation for the lower tone of females may be that female macrovessels express more dilatory β1- and β3-adrenoreceptors than male vessels with a predominant endothelial localization. This gender-specific difference is functionally relevant in young adults and is attenuated with aging (96).

Nominal vessel tone showed no difference between the genders within the working pressure range in both sexes was nominally the same. However, this value shifted toward contraction in males. However, male spontaneously hypertensive rats (SHR) have greater AT-1 receptor mRNA and protein expression in the vasculature and the kidney, while females demonstrate greater Angiotensin II receptor type 2 (AT-2) expression. Female SHR also have greater renal Ang (1–7) levels compared with males. Sex differences in the expression levels of RAS components in hypertension are paralleled by gender differences in CV responses. Greater AT-1 receptor expression in male SHR vs. greater AT-2 receptor expression in females translates into enhanced constriction to AngII in isolated aorta and mesenteric microvessels from males (97).

Increased contractile states were observed in males and remodeling in females in our stabilizing hypertension model. We observed the presence of similar mechanisms in our previous studies, as we were able to evoke greater contractures normotensive males, eanwhile no difference was observed regarding spontaneous myogenic tone. Therefore, we may state that hypertensive males demonstrated "morphologically stable vasoconstriction," while females achieved a state of equilibrium through remodeling (hypertrophy of the vessel wall compensated for the increase in pressure loading). Based on our data our study group links these differences to the effects of testosterone and estrogen levels (90). Men over middle-age have more pronounced endothelin receptor A vasoconstrictor control than women (98). Whole cell clamp technique used on miniature swine coronary smooth muscle cells showed Ca^{2+} current density to be higher in males compared to females (45). Female rat coronary smooth muscle cells responded to different stimuli with less contraction than male rats (99). Our results were consistent with these observations.

Several studies found that female gender and estradiol treatment stimulates endothelial nitric oxide release and vasodilation. This mechanism was observed in coronary arteries, even in in states of hypertension (59, 100, 101). Following BK-induced endothelial dilation in long-term AngII–infused female rats, our series demonstrated less remaining tone compared to males. The key local vasodilators of the coronaries are BK and adenosine in rat; therefore, Acetylcholine was not administered to characterize endothelial vasodilation in our series (49, 102).

Gender Differences regarding Alterations of Cardiac Mass

Using this AngII hypertension model (single common etiology hypertension, AngII, age-matched male and female animals), cardiac remodeling occurred in females, while this did not develop in males (47). No gender differences were observed in the normotensive state regarding cardiac mass (0.324±0.018 g/100 g body weight in males vs 0.319±0.023 g/100 g body weight in females); therefore, this difference in relative heart weight was caused by the adaptation mechanisms corresponding to hypertension. Hypertrophic remodeling did not occur in the coronaries but in the cardiac muscle only in females.

However, regarding remodeling (vs. hypertrophy) the heart demonstrated remodeling in both the cardiac mass and the vessels. Through these mechanisms females demonstrated an increased vulnerability in the hypertensive state. The marked dominance of angiotensin-dependent mechanisms suggested that gender differences may occur to pharmacological treatment as well. In our series we found differences in the contractile re- activity of the intramural coronaries. The degree of TxA_2-induced vasoconstriction was greater in male intramural coronaries than females. TxA_2 is a strong vasoconstrictor on the coronary arterial segments and it may reach high concentrations in ischemia related cardiac events, whereas other vasoconstrictors are ineffective or do not reach concentrations high enough to have vasoconstrictor effects. This limited coronary reactivity to vasoconstrictors may be considered a self-preserving property of the coronary arteries, protecting them from an overload of vasoconstriction. TxA_2 however, is a potent vasoconstrictor on the coronaries.

Gender differences regarding the effects of TxA_2 were observed in the epicardial coronaries. Compared to the main branches the secondary mesenteric arteries showed markedly stronger contractions as a response to TxA_2 agonists (103). Different segments of the coronary network demonstrated different levels of vasoconstrictive reactivity (50). Along the cardiovascular system long-term angiotensin treatment led to the development of hypertension and inward hypertrophic remodeling. The following effects were predominantly linked to the angiotensin I receptors: vasoconstriction, vasopressin induction, vascular smooth muscle cell proliferation, activation of the sympathetic nervous system, and increased cardiac contractility. In contrast, AngII receptor activity led to vasodilation, inhibition of cell growth and proliferation, and apoptosis (104). A 10-day AngII treatment in males led to up- regulation of the NAD(P)H oxidase gp 67 phox subunit in the mesenterial vessels, resulting in mesenterial vascular dysfunction (105). In hypertension, the G protein- coupled receptor kinase 5 has partial effects on β-adrenergic receptors in males and AngII receptors in females (106).

5.3. Angiotensin II induced hypertension in females

Using the work of Simon et al. as a basis we created a model, where the early phase of hypertension was induced by administering a low dose of AngII (12, 46, 107). This model was appropriate to analyze the mechanisms of early adaptation. In our series we used this

model to track biomechanical changes occurring in the intramural coronary arteries. This initial “stabilizing” hypertension model has been used on several segments in several studies (12, 46).

Eutrophic remodeling

According to our data the early phase ‘stabilizing’ hypertension induced by AngII resulted in inward eutrophic remodeling, meaning that significant narrowing of the lumen and thickening of the vessel wall occurred without alterations regarding wall cross section area in the intramural arterioles of the left ventricle (108, 109). The type of vascular bed surrounding the intramural coronaries also has an effect on adaptation mechanisms via stretch-triggered and local blood flow (44, 110).

Our working group previously studied that coronary arteries were remodeled not only in isolated blood vessels, but also at the network level in angiotensin-induced hypertension in female animals (111). Four weeks’ moderate angiotensin infusion and consequent blood pressure elevation induced characteristic alterations in the geometrical properties of the intramural coronary resistance artery network of the rat. Morphological deformations appeared, such as broken course of a larger branch, branches running parallel along a longer course, branchings with multiple daughter branches emerging very close to each other, branches crossing each other, with potentially mutually exchanged supplying areas and sometimes even uneven diameter.

Mulvany et al. demonstrated the types of remodeling that may occur regarding cross section compared to the physiological state. Remodeling may be hypertrophic (increase of cross-sectional area), eutrophic (no alteration in the cross-sectional area), or hypotrophic (decrease of cross-sectional area). Remodeling may inward (reduction in lumen diameter), or outward (increase in lumen diameter) (109).

Biomechanical parameters

Initial ‘stabilizing’ hypertension induced by AngII resulted in characteristic alterations in the vascular biomechanical parameters. The intramural coronaries harvested from the

female rats demonstrated a significant reduction in inner diameter and elastic modulus. Considering the differences regarding mean blood pressure between control and hypertensive rats.

Wall stress stabilizes in the early stages of hypertension. This complies with the theory of Rodbard, who suggested that wall stress stays permanent in vivo (112). In accordance, previous studies have demonstrated stabilization of the wall stress values of saphenous artery isolated segments in fertile male (113) and also in ovariectomized female animals (12). This may be interpreted as a more general adaptation mechanism during early phase stabilizing hypertension.

Vessel tone induced by myogenic and vasoactive agents

In our present series myogenic tone and the degree of bradykinin relaxation remained unaltered, while constrictive reactivity increased in the vessel segments harvested from female rats.

Along the coronary network spontaneous myogenic tone is markedly determined by the morphological inner diameter of the vessel (49, 50). Therefore, vessels with similar inner diameters from the same region of the vessel network are proposed to have similar myogenic tone. However, stabilized or severe chronic hypertension, may lead to elevated spontaneous myogenic tone (108, 114).

Increased vasoconstrictor reactivity - measured in our series regarding intramural resistance arteries - is in contrast with that measured on epicardial coronaries (115). As we have mentioned before it is also well established that the increased release of TxA_2 alters the blood supply of coronaries significantly in certain pathological conditions (116, 117). Following administration of angiotensin TxA_2 release may increase (118). AngII also has an effect on the intravascular balance of vasodilatator/constrictor prostanoids, the NO- and cyclooxygenase-2 pathways and the reactive oxidative supernatant production (119-121).

A myriad of associations between hypertension and endothelial dysfunction are well established - following a four-week administration of subpressor dose AngII, vasodilator response did not alter significantly in our model.

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The increased response of the intramural coronary segments to TxA_2 found in the hypertensive rat group was not accompanied by a change in bradykinin induced vasodilatation.

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6. CONCLUSIONS

Based on our experiments and data from the literature we may conclude that there are indeed previously undescribed yet clinically potentially relevant gender related differences regarding geometrical and biomechanical parameters, elastic and contractile properties of the intramural resistance coronaries. We may also draw the conclusion that this gender dimorphism -that may play a key role in characteristic patomorphological mechanisms - may also be observed in other mammals.

Using our hypertension model on Sprague Dawley rats isolated resistance coronary segments of similar inner radii prepared from the same intramural region of the network have been demonstrated to show characteristic gender related differences regarding vessel geometrical parameters, elastic and contractile properties between age-matched sexually mature males and females. Our analysis confirmed that both the thickness of the vessel walls and the ratio of the wall thickness to the inner radius is greater in males than in females. Regarding vessel biomechanics, tangential stress and elastic moduli were larger in females, however distensibility was greater in males. Spontaneous tone and bradykinin-induced relaxation were similar in both groups, however, a marked difference - dependent on gender - was found regarding vasoconstrictor response elicited via TxA_2 in the analyzed vessel segments. Me may conclude that males demonstrated greater TxA_2 tone.

Based on our series we may conclude that there are significant differences in terms of adaptation to hypertension induced by the administration of AngII males versus females. Even though measurements regarding vascular reactivity remained unaltered due to remodeling of the vessel wall itself, there was an increase in terms of the relative weight of the heart (normalized to body mass) in females. Meanwhile remodeling was prevented at this stage in males following an increase in mechanical loading. Increased constrictor reactivity of the vessels - also found in males - may be a potential protective mechanism it may prevent damage in the tissues caused by an increase in the perfusion pressure. However, at the same time it also may be considered a risk factor regarding thrombus formation during an infarction event.

Vessel segments of equal caliber (based on inner radius measurements) were prepared and isolated in the hypertension model we used (hypertension induced by the administration of AngII): no difference was found regarding measurements of the outer radius of the vessels. However, females demonstrated decreased inner radii values, while wall thickness increased. As a consequence, both tangential wall stress and elastic modulus values decreased. Meanwhile in the male group we found that both spontaneous tone and tone induced by TxA_2 increased.

Using the rat model where early phase hypertension was achieved through administration of the subpressor dose AngII regime we were able to demonstrate the clinically relevant initial adaptive phases of vascular adaptation in the intramural segments of the resistance coronaries branching from the LAD. The analyzed vessel segments play a key role in the blood supply of the heart tissue, namely the heart muscles of the left ventricle. In this location the flow conditions provided by the vascular bed differ significantly regarding intramural and epicardial vessels as their flow cycles are affected differently by contractions of the heart muscle. It is a well-established fact that the vasculature adapts through different mechanisms in different locations along the coronary network. In our series, where intramural coronary resistance arteries were studied, early phase AngII induced hypertension lead to the development of inward eutrophic remodeling in the vessels. Eutrophic remodeling is characterized by the determinant goal seen ‘in vivo’ of keeping both tangential wall stress values and elastic moduli properties on constant level. This appears to be of primary importance and may be considered a self-protective maybe even a pathomorphological preventive measure in females. Both eutrophic remodeling and hypertrophic remodeling may be present along the different segments in the vasculature of the same animal. Eutrophic type remodeling appears to be a characteristic in hypertension induced by the administration of angiotensin II, while hypertrophic remodeling is usually linked to endothelial function. The type of adaptation that occurs within a vessel segment is also determined by local effects – these effects and in turn the adaptation mechanisms may play a significant role in determining target organ damage.

When the ratio of wall thickness increases (increase in vascular resistance), this may appear to be a self- and target organ protective mechanism as it buffers short-term

pressure load. However, this apparently ‘protective’ mechanism actually adds to and may even accelerate the various ‘vicious cycles’ observed in the early phases of hypertension.

Hypertensive females demonstrated increased tone to TxA_2-induced, while endothelial function remained unaltered. Based on these results we may hypothesize that the inward type eutrophic remodeling may be considered an initial morphological step of adaptation of a more vigorous vasoconstrictor response preceding vasodilatative dysfunction.

We consider the following results as orginal from our series:

1- 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- In AngII hypertension, as a gender difference, an inward eutrophic remodeling and increased TxA_2 induced contraction were detected in males.
3- 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.

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7. 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 machanisms themselves and the gender 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, TxA_2-induced tone increased in hypertension.

In females, hypertension caused an inward eutrophic remodeling and increased TxA_2 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 provides a basis for clinical studies regarding therapeutic options.

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9. BIBLIOGRAPHY OF PUBLICATIONS

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.

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