MEDICAL SCHOOL - UNIVERSITY OF PECS
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VASCULAR SMOOTH MUSCLE CONTRACTILE CAPACITY AS A FUNCTION OF AGE

From newborn to senescence morphological and functional remodeling leads to increased contractile capacity of arteries

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Summary of Doctoral (Ph.D.) Thesis

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Abbreviations:

cAMP – cyclic adenosine monophosphate

CNS – central nervous system

MABP – mean arterial blood pressure

NE – norepinephrine

PACAP - pituitary adenylate cyclase-activating peptide

PACAP6-38 – PAC1R antagonist

SMC – smooth muscle cell

1. INTRODUCTION

The circulatory system is essential to maintain the supply of oxygen and nutrition to the organs and tissues. From birth the organisms are growing and changing, blood circulation and hemodynamic forces are also constantly undergoing changes in order to adapt to the changes in the environment (20). The importance of this issue was brought up by Björn Folkow (4): **does the remodeling-induced increased vasomotor tone of arteries (due to the hypertrophy) occur first, which is then followed by increases in mean arterial blood pressure (MABP) or *vice versa*?**

1.1 Age-related changes in blood pressure

Evidence shows that with aging there is a gradual change in MABP of humans (7). Several factors can influence the MABP: some of them can be managed with dietary and lifestyle, medical drugs, and those, which cannot be (or difficult to) managed, like genetic and environmental factors (6). The combination of these factors influences the level of hemodynamic forces. In healthy subjects (human and animals) systolic and diastolic pressures change with aging: after initial increase until adulthood, where in elderly they remain unchanged. If the system fails to adapt, it will result in pathological conditions (hypotension or hypertension).

1.2 Vascular remodeling and aging

The main function of vascular smooth muscle is to establish the vascular tone and to regulate the caliber of blood vessels, which can be changed in response to different stimuli (11). These changes will result in vascular remodeling. Vascular remodeling begins in embryogenesis (20). There are "early" remodeling (development of vasculature to be self-sustainable) and "late" remodeling (already developed vasculature needs to additionally adjust for "new" conditions). In both types of remodeling, the principle mechanism is the same: through mechano-transduction (signals) vasculature responds with mechano-adaptation (remodeling) (11).

Changes which occur with aging are both morphological and functional (3, 11, 18). These changes are independent of other pathological conditions (6). Human studies showed that with aging there were increases in wall mass, wall thickness and lumen diameter of arteries, but reduction in wall tension (16, 23). Importantly, most studies investigated only a limited age range or only two-time points (2, 8, 13, 25), precluding characterization the overall effect of healthy aging.

1.3 Vasoactive substances in the blood stream

Vascular stimuli participate in the vasomotor activity and balance between constrictors and dilatators resulting in the steady-state vascular tone. They can be divided into two main groups by the pathways they activate:

1) Receptor-independent responses (ions, which changes membrane potential; e.g. KCl).
2) Receptor-dependent responses (signaling mechanism pathway; e.g. NE, PACAP).

KCl - it was shown that 60mM KCl is the most potent non-receptor-dependent vasoconstrictor (3, 10). Thus age-related changes in receptor availability should not interfere with the assessment of KCl-induced contractile capacity, as it was found to be the case for angiotensin II (12, 27).

NE - it acts through the receptor-dependent pathway, activating α adrenergic receptors. Experiments showed age-dependent NE-induced vascular contraction (9, 15). However, data are still missing for very old and senescence ages.

PACAP - PACAP1-38 is a neuropeptide with a diverse array of biological functions (29). In the cardiovascular system, PACAP1-38 stimulates cAMP activity in the SMC (17) resulting in vasorelaxation (30). PACAP signaling may play a role in the development of age-related diseases of the CNS (19). Age-dependent vascular functions are not well characterized.

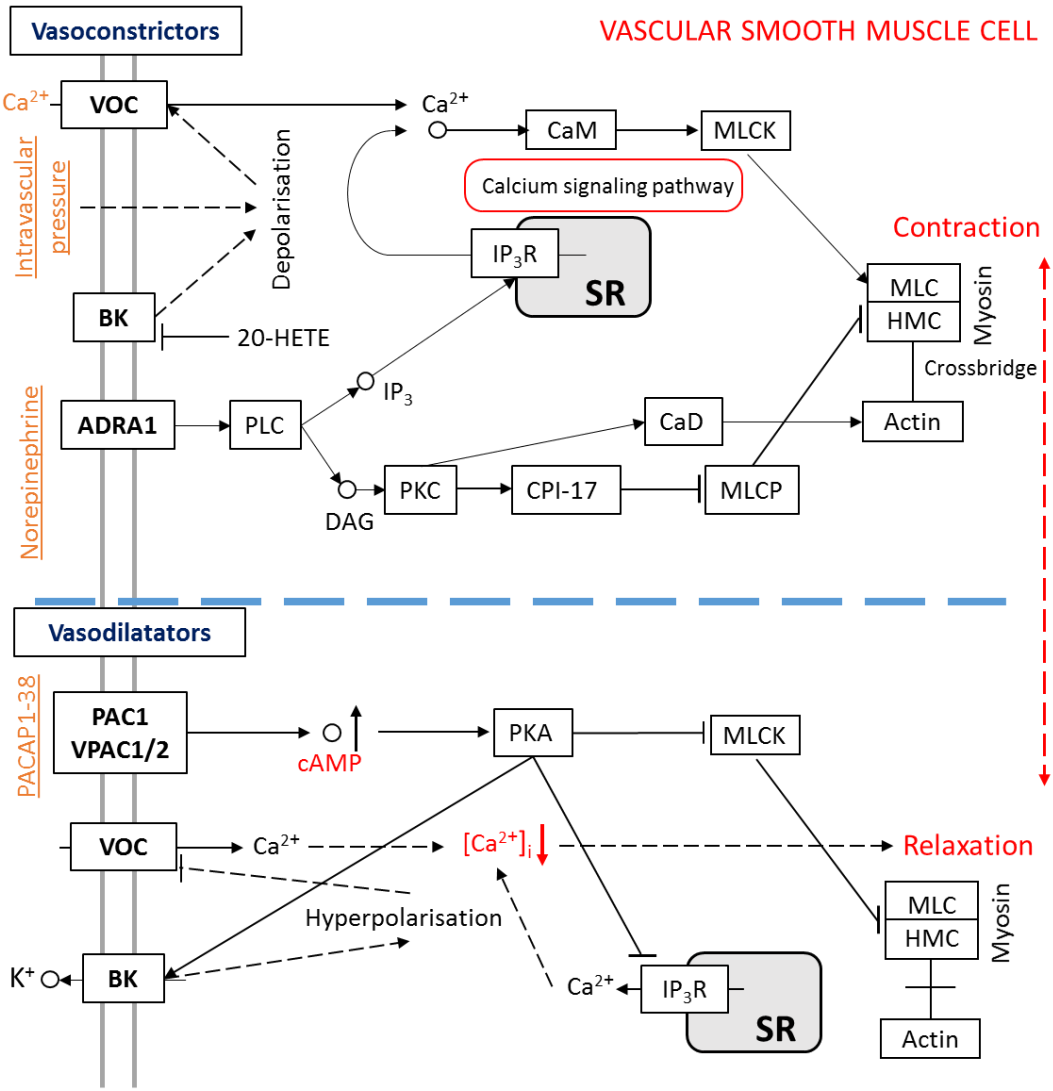

Figure 1. *Simplified graphical presentation of vasoconstrictor (upper section) and vasodilator (lower section) pathways in the development of vascular tone. Graphical section is recreated according to Kanehisa Laboratories© (www.genome.jp).*

2. HYPOTHESIS AND AIMS OF STUDY

It has been shown that blood pressure and vasomotor properties of arteries are age-dependent. Until now, only a few studies have addressed the issues of aging-induced morphological and functional changes in arteries of rats, in a wide range of age groups. Thus our understanding of their contribution to the development of peripheral vascular resistance remains limited.

Thus, we **aimed** to measure from newborn to senescent rats:

(i) the level of mean arterial blood pressure
(ii) morphological characteristics of arteries
(iii) functional characteristics of arteries using KCl-, NE- and PACAP-induced responses

3. METHODS

3.1 Animal model

Ninety-eight male Wistar Kyoto (WKY) rats aged 8 days (0.25 month), 1, 2, 6, 9, 12, 19, 24, and 30 months were used. These animals represented different age groups: newborn, juvenile, young, adult, late adult, middle-aged, late middle-aged, old, and senescent**.** Rats were kept in standard cages, exposed to 12h of light and 12h of dark. They had 24h access to water and food.

3.2 Measurement of mean arterial blood pressure

Rats mean arterial blood pressure (MABP) was measured by two different methods: 1) tail cuff (1) and 2) with the direct manner, by carotid artery cannulation (27). Comparable data could be obtained by the two methods.

3.3 Surgery

From rats, the common carotid artery was isolated by using a surgical microscope under anesthesia induced by a ketamine–xylazine (78 mg/kg Calypsol, Richter + 13 mg/kg Sedaylylan, Eurovet). One carotid artery was ligated proximal and distal point to “keep” prevailing blood pressure inside the ligated section. This section was quickly removed and inserted into fixation solution. Shortly after, the second artery was removed and segments were dissected into 2 - mm long rings and mounted. After the removal of the arteries, the animal was euthanized with an injection of pentobarbital.

3.4 Vascular wall histology

After removal of the artery, this section was quickly removed and inserted into fixation solution, samples were embedded in paraffin and stained with hematoxylin and eosin (H&E). The sections were examined with video-microscopy under different magnifications.

3.5 Isometric force measurements

Measurement of the changes in the isometric force of the vessel rings was conducted as described before (27). In brief, in a four chambers wire-myograph system (DMT 610M) vascular rings were mounted on tungsten wires connected to fixed force recorder on one side and the regulator of distance on other side. The chambers contained 5 ml Krebs solution (continuously perfused with O_2 95% and CO_2 5%; at 36.9 ± 0.1°C). After normalization, the rings were allowed to stabilize for 60 minutes before the start of the experimental protocol.

Two different protocols were conducted: 1) vasoconstriction, where maximal arterial contraction was tested with 60mM KCl or 10^{-6} M NE; and 2) vasorelaxation, where after maximal response with 60mM KCl, the cumulative dose of PACAP1-38 (10^{-9} 10^{-6} M) was performed.

3.6 Calculations of parameters

From the values taken from morphological images of the carotid artery, we calculated tree different parameters: 1) vessel radius, diameter and surface lumen; 2) vascular wall tension and wall/lumen ratio; and 3) smooth muscle contractile capacity.

3.7 Statistical analysis

All data are expressed as means ± SEM. The curve is fitted under the data to provide the more relevant physiological meaning of observed data. Data were compared with one-way ANOVA (Tukey *post hoc* test). Statistical significance was accepted at $p < 0.05$.

4. RESULTS

4.1 Changes in the mean arterial blood pressure

The MABP of rats increased from newborn until the age of 6 months, and it is unchanged until the age of 30 months. Comparable results were obtained by the tail-cuff method.

4.2 Changes in the carotid vascular wall thickness as a function of age

Summary data show that the total wall thickness of carotid arteries increases with aging in three phases: 1) rapidly increased until the age of 2 months, 2) then slowly increased until the age of 19 months and 3) then again gradually increased to senescence. *Media* is constantly increasing, while *adventitia* until the age of 19 months.

4.3 Diameter of carotid artery in histological preparations

Vessel diameter significantly increased until the age of 30 months, while lumen diameter increased significantly until the age of 19 months.

4.4 Wall-to-lumen ratio (W/L)

The W/L ratio shows a non-significant increase from newborn to senescence.

4.5 Vascular wall tension of carotid artery

Wall tension shows rapid decrease until the age of 2 months, and then slowly decreases until the age of 30 months.

4.6 Changes in isometric force of isolated carotid arteries in response to KCl and NE as a function of age

KCl-induced contraction of carotid arteries exhibit three phases: 1) a rapid increase until the age of 2 months, 2) then slow, but sustained increase to the age of 19 months, and 3) further increase until the age of 30 months. Unlike KCl, the NE-induced contraction exhibit 2 phases: 1) vasomotor response increased sharply until the age of 6 months; 2) then it did not change further until the age of 30 months.

4.7 Assessing contractility by normalization of vasomotor responses of carotid artery to media thickness as a function of age

Because the *media* is the contractile layer of the vessel, the isometric force generated by the vessels was normalized to *media* thickness (separately for each age group). The normalized force produced by KCl (contractility) have 2 phases: first there was a significant increase until the age of 2 months and then it did not change significantly until senescence. However, contractility induced by NE also has 2 phases, but in a different pattern: first there is a significant increase until the age of 2 months followed by a significant decrease until the age of 30 months.

4.8 Effect of dose-dependent administration of PACAP1-38 on the relaxation of carotid artery

Data showed that PACAP1-38 induced a dose-dependent relaxation of arteries. Induced relaxation was greatest in young animals, whereas the PACAP-induced relaxation in adult and old age was significantly and substantially reduced (as compared to young animals).

4.9 Effect of dose-dependent administration of PACAP1-38 on the relaxation of carotid artery in the presence of PAC1R antagonist

Data showed that in the presence of PACAP6-38 there was no change in vasomotor response of arteries to the cumulative administration of PACAP1-38 in any age group.

5. DISCUSSION

Cardiovascular system and vascular resistance are greatly affected by aging (14). KCl-induced vascular contraction and MABP are increased until middle age, whereas in senescence age

contraction is further increased, but blood pressure is maintained, suggest a different contribution during aging, possible protection of vasculature.

The thickness of the arterial wall is increasing with aging (most noticeable thickening was recorded in *media*). A similar tendency was found by others (22, 26). This could be interpreted that hypertrophy compensates for the degeneration of elastic and fibrotic layers of media to maintain or improve the vasomotor function of arteries (6, 16). Compensatory thickening of the arterial wall prevents the circumferential wall stress from increasing (23). Although both wall thickness and lumen radius increase with aging, there is no change in wall-to-lumen ratio - outward remodeling of arteries with aging (2).

Another interesting key parameter is wall tension, which is reduced with aging. Van Bavel's group suggested that increased wall tension (increased MABP) leads to induced constriction, whereas low wall tension reduces contraction (28). Our findings confirm this observation. This is of importance during aging, where increases in systolic and reductions in diastolic pressure could be - in part - due to increased contractile tone and reduced compliance of the vessel in older age (6, 7).

KCl-induced contractile ability of arteries increases with aging. This is important, in order to provide the means to control vascular resistance, when MABP and cardiac output are increased (21). This is most likely due to the aging-induced arterial remodeling, which leads to increase in effectiveness of contractile function (18). Such mechano-adaptation is an initial step in eutrophic remodeling to maintain a reduced diameter without the need for maintenance of muscle activity (5). Although vascular hypertrophy is usually connected to hypertension, this finding suggests that hypertrophy "compensates" for increasing lumen and stabilizes blood pressure (2). Interestingly, unlike KCl, NE-induced contractility increased until the age of 6 months and then decreased. This could be due to the aging-induced reduction of the density of adrenergic receptors (24). The MABP did not increase which suggests that vascular remodeling is counterbalanced by other mechanisms to prevent increases in peripheral vascular resistance (27).

PACAP-induced relaxations decline with aging. This could be due to high levels of Ca^{2+} ions, which may interfere with relaxation or due to the inability of the smooth muscle to relax (due to excessive hypertrophy). Another possibility is that density and distribution of PAC1 receptor decline with aging (29).

5.1 Clinical importance of our findings

From clinical aspects, once the pathological condition is developed, it is a challenge how the condition can be stabilized, and if possible, to be reversed and cured. A clear distinction between physiological age-related changes and symptoms of superimposed diseases are therefore of great general importance, but it becomes more and more difficult in older age. Our findings provide additional information for the integration of structural and functional remodeling of arteries in age-dependent manner, which contributes importantly to the regulation of the blood pressure in normal and diseased conditions, such as hypertension.

5.2 Conclusion

In conclusion, the findings are that during healthy aging arterial wall thickness substantially increases (especially *media*), in a phasic manner (young, middle and old age), which may contribute to the increased contractile and reduced relaxation abilities of SMC in older age. These changes seem to be independent of changes in MABP. It seems that other systemic and/or local blood pressure regulatory mechanisms counterbalance the increased smooth muscle contractility, thereby maintaining normal blood pressure in older ages. It is likely, however, that when these mechanisms became impaired the increased contractility of arterial smooth muscle can lead to increased vascular tone and thus peripheral vascular resistance, resulting in hypertension. This conclusion seems to support the earlier concept of Bjorn Folkow: "*an increase in the wall-to-lumen ratio (due to increased smooth muscle layer, leading to increased contractility) could be an important factor in the pathogenesis of hypertension* (5), especially if other mechanisms fail to counterbalance the remodeling of arterial vessels.

6. SUMMARY OF NOVEL FINDINGS

The important novel findings of the present study are that from newborn to senescence:

(i) MABP initially increased until age of 6 months, then it did not change further
(ii) vascular wall thickness - especially the media layer - increased
(iii) wall tension decreased
(iv) the magnitude of the receptor-independent vasomotor agent, KCl-induced contractions gradually increased, whereas the contractility - after an initial increase in early age - did not change further
(v) magnitude of receptor-mediated vasomotor agent, NE - induced contractions first increased, then it did not change in older ages
(vi) PACAP1-38 induced a significantly dose-dependent relaxation
(vii) PACAP1-38 induced a significant age-dependent reduction in relaxation
(viii) the magnitude of SNP-induced relaxation did not change after PACAP1-38 administration and was not affected by age
(ix) nonselective PAC1 receptor antagonist (PACAP6-38) completely diminished PACAP1-38 induced a vasomotor response in all age groups

7. PEER – REVIEWED PUBLICATIONS OF THE AUTHOR

The thesis is based on the following publications:

1. **Ivic I.**, Vamos Z., Cseplo P., Koller A. From newborn to senescence morphological and functional remodeling leads to increased contractile capacity of arteries. J Gerontol A Biol Sci Med Sci. 2016 (IF: 5.416) doi: 10.1093/gerona/glw085

2. Vamos Z., **Ivic I.**, Cseplo P., Toth G., Tamas A., Reglodi D., Koller A. Pituitary adenylate cyclase-activating polypeptide (PACAP) induces relaxations of peripheral and cerebral arteries, which are differentially impaired by aging. J Mol Neurosci. 2014 (3):535-42. (IF: 2.343) doi: 10.1007/s12031-014-0349-9

Other publications:

3. Vamos Z., Cseplo P, **Ivic I.**, Matics R., Hamar J., Koller A. Age determines the magnitudes of angiotensin II-induced contractions, mRNA, and protein expression of angiotensin type 1 receptors in rat carotid arteries. J Gerontol A Biol Sci Med Sci. 2014. 69(5):519-26. (IF: 5.416) doi: 10.1093/gerona/glt128

4. Sirovina D., Orsolic N., **Ivic I.**, Novak S., Gajski G., Garaj-Vrhovac V., Koncic Z. M. Antioxidative and antidiabetic effects of naringin and curcumin in vitro and in vivo. Phytomedicine. 2015. (IF: 1.230) doi: 10.5138/ijpm.v7i2.1679

5. Cseplo P., Vamos Z., Torok O., **Ivic I.**, Toth A., Buki A., Koller A. Hemolyzed blood elicits - calcium antagonist and high CO_2 reversible - constrictions via elevation of Ca^{2+} in isolated cerebral arteries. J Neurotrauma. 2016. (IF: 4,377) doi: 10.1089/neu.2015.4365.

6. Cseplo P., Vamos Z., **Ivic I.**, Torok O., Toth A., Koller A.: The beta-1-receptor blocker nebivolol elicits dilation of cerebral arteries by reducing smooth muscle $[Ca^{2+}]i$; PLoS One. 2016. (IF: 3,234) doi: 10.1371/journal.pone.0164010

7. **Ivic I.**, Solymar M., Pakai E., Rumbus Z., Pinter E., Koller A. and Garami A. Transient receptor potential vanilloid-1 channels contribute to the regulation of acid- and base-induced vasomotor responses. J Vasc Res. 2016. (IF: 2.186) doi: 10.1159/000452414

Abstracts in peer-reviewed journals:

1. **Ivic I.**, Vamos Z., Tamas A., Reglodi D., Koller A. Alternating relaxation to PACAP 1-38 and 1-27 in arteries of PACAP deficient mice. Journal of Vascular Research 52, 37-38. European Vascular Biology Organization (EVBO). 2015.

2. **Ivic I.**, Solymar M., Pakai E., Pinter E., Koller A., Garami A. A tranziens receptor potenciál vanilloid-1 (TRPV1) csatorna limitáló szerepe savi és bázikus hatások indukálta vazomotor válaszban. Évi Konferenciája, Szeged, 2015.

3. Vamos Z., Cseplo P., **Ivic I.**, Matics R., Koller A. Changes in norepinephrine induced vasomotor response and vascular alpha 1-receptor expression as a function of age. Acta Physiologica 211, 183-184. 2014

4. Cseplo P., Vamos Z., **Ivic I.**, Toth G., Tamas A., Reglodi D., Koller A. Pituitary adenylate cyclase-activating polypeptide (PACAP) induces location-and age-related relaxations of isolated arteries. Acta Physiologica 211, 97-97. 2014

5. Vamos Z., Cseplo P., **Ivic I.**, Belak M., Molnar T., Koller A. The Ca (2+)-binding protein S100b elicits a concentration-dependent relaxation on isolated cerebral arteries. Journal of Neurotrauma 31 (5), a47-a47. 2014.

6. **Ivic I.**, Vamos Z., Cseplo P., Szollosi R., Reglodi D., Tamas A., Koller A. Pituitary adenylate cyclase-activating peptide (PACAP) induces age-dependent changes in vasomotor responses on isolated rat arteries. Journal of Neurotrauma 31 (5), a46-a47. 2014.

7. **Ivic I.**, Pakai E., Solymar M, Koller A., Garami A. Role of the transient receptor potential vanilloid-1 (TRPV1) in the development of hydrogen chloride (HCl)-induced vasomotor response in isolated rodent carotid arteries. 2014.

8. Cseplo P., Vamos Z., Torok O., **Ivic I.**, Kalinics P., Solymar M., Szollosi R., Koller A. A vér komponenseinek szerepe a subarachnoidális vérzés indukálta. Vasospasmus kialakulásában. A Magyar Hypertonia Társaság XXII. Kongresszusa: Absztrakt könyv. Konferencia helye, ideje: Siófok, Magyarország, 2014.p. 16.

9. **Ivic I.**, Solymar M., Vamos Z., Cseplo P., Koller A. Effect of Fe^{3+} on the vasomotor tone of arteries. Role of reactive oxygens species. A Magyar Hypertonia Társaság XXII. Kongresszusa: Absztrakt könyv. Konferencia helye, ideje: Siófok, Magyarország, 2014.p. 17.

10. Cseplo P., **Ivic I.**, Vamos Z., Reglodi D., Tamas A., Toth G., Koller A. Pituitary adenylate cyclase-activating peptide (PACAP) induces location-and age-dependent changes in vasomotor responses on isolated rat arteries. Journal of Molecular Neuroscience 51, s224-s225. 2013

11. Vamos Z., Dancs K., **Ivic I.**, Cseplo P., Koller A. Subcellular mechanisms of AT1-receptor mediated vasomotor responses change with aging. Congress of Croatian Physiological Society and 1. Regional Congress of the Physiological Societies, Rijeka, September 13-15., 2013. In: *Periodicum Biologorum* 115: Suppl2, 58, 2013.

12. **Ivic I.**, Vamos Z., Cseplo P., Koller A. During physiological aging the contractile force of arteries increases. Congress of Croatian Physiological Society and 1. Regional Congress of the Physiological Societies, Rijeka, September 13-15., 2013. In: *Periodicum Biologorum* 115: Suppl2, 29, 2013.

13. Novak S., Sirovina D., **Ivic I.**, N. Orsolic. Antioxidative and anti-inflammatory activity of Croatian propolis preparations in alloxan induced diabetes in mice. International Congress of Physiological Sciences - IUPS 2013.

14. Vamos Z., Cseplo P., Batai Z., Torok O., **Ivic I.**, Matics R., Hamar J., Koller A. Changes in Angiotensin II-induced vasomotor function from newborn to senescence: correlation with expression of at1 and AT-2 receptors. The FASEB Journal 27, 1165.4. 2013.

15. **Ivic I.**, Novak S., Cosic A., Cavka A., Grizelj I., Loncaric Z., Koller A., Drenjancevic I. Altered vascular response to acetylcholine and reduced pO_2 in isolated carotid arteries in rats fed by chow containing varied zinc and selenium content. International Congress of Physiological Sciences - IUPS 2013

16. **Ivic I.**, Yusif L., Sorokin L., A. Koller. Aging-induced structural changes in arteries. Role of collagen and laminin isoforms. Proceedings of the Physiological Society. The Physiological Society 2013

17. **Ivic I.**, Vamos Z., Cseplo P., Kosa D., Torok O., Hamar J., Koller A. Vascular contractility increases from newborn to senescence. Cardiovascular Research 93, s122-s122. FCVB, London, 2012.

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9. REFERENCES

1. **Bagi Z, Erdei N, Toth A, Li W, Hintze TH, Koller A, and Kaley G**. Type 2 diabetic mice have increased arteriolar tone and blood pressure: enhanced release of COX-2-derived constrictor prostaglandins. *Arterioscler Thromb Vasc Biol* 25: 1610-1616, 2005.
2. **Cantini C, Kieffer P, Corman B, Liminana P, Atkinson J, and Lartaud-Idjouadiene I**. Aminoguanidine and aortic wall mechanics, structure, and composition in aged rats. *Hypertension* 38: 943-948, 2001.
3. **Docherty JR**. Cardiovascular responses in ageing: a review. *Pharmacol Rev* 42: 103-125, 1990.
4. **Folkow B, Grimby G, and Thulesius O**. Adaptive structural changes of the vascular walls in hypertension and their relation to the control of the peripheral resistance. *Acta Physiol Scand* 44: 255-272, 1958.
5. **Folkow B, Hallback M, Lundgren Y, Sivertsson R, and Weiss L**. Importance of adaptive changes in vascular design for establishment of primary hypertension, studied in man and in spontaneously hypertensive rats. *Circ Res* 32: Suppl 1:2-16, 1973.
6. **Folkow B, and Svanborg A**. Physiology of cardiovascular aging. *Physiol Rev* 73: 725-764, 1993.
7. **Franklin SS, Gustin WT, Wong ND, Larson MG, Weber MA, Kannel WB, and Levy D**. Hemodynamic patterns of age-related changes in blood pressure. The Framingham Heart Study. *Circulation* 96: 308-315, 1997.
8. **Gaballa MA, Jacob CT, Raya TE, Liu J, Simon B, and Goldman S**. Large artery remodeling during aging: biaxial passive and active stiffness. *Hypertension* 32: 437-443, 1998.
9. **Handa RK, and Duckles SP**. Age-related changes in adrenergic vasoconstrictor responses of the rat hindlimb. *Am J Physiol* 253: H1566-1572, 1987.
10. **Ivic I, Vamos Z, Cseplo P, and Koller A**. From Newborn to Senescence Morphological and Functional Remodeling Leads to Increased Contractile Capacity of Arteries. *J Gerontol A Biol Sci Med Sci* 2016.
11. **Jones EA, le Noble F, and Eichmann A**. What determines blood vessel structure? Genetic prespecification vs. hemodynamics. *Physiology* 21: 388-395, 2006.
12. **Konishi C, Naito Y, Saito Y, Ohara N, and Ono H**. Age-related differences and roles of endothelial nitric oxide and prostanoids in angiotensin II responses of isolated, perfused mesenteric arteries and veins of rats. *Eur J Pharmacol* 320: 175-181, 1997.
13. **Mandala M, Pedatella AL, Morales Palomares S, Cipolla MJ, and Osol G**. Maturation is associated with changes in rat cerebral artery structure, biomechanical properties and tone. *Acta Physiol (Oxf)* 205: 363-371, 2012.
14. **Marin J**. Age-related changes in vascular responses: a review. *Mech Ageing Dev* 79: 71-114, 1995.
15. **Martinez-Lemus LA, Hill MA, Bolz SS, Pohl U, and Meininger GA**. Acute mechanoadaptation of vascular smooth muscle cells in response to continuous arteriolar vasoconstriction: implications for functional remodeling. *FASEB J* 18: 708-710, 2004.

16. **Mirea O, Donoiu I, and Plesea IE**. Arterial aging: a brief review. *Rom J Morphol Embryol* 53: 473-477, 2012.

17. **Miyata A, Arimura A, Dahl RR, Minamino N, Uehara A, Jiang L, Culler MD, and Coy DH**. Isolation of a novel 38 residue-hypothalamic polypeptide which stimulates adenylate cyclase in pituitary cells. *Biochem Biophys Res Commun* 164: 567-574, 1989.

18. **Mulvany MJ, Baumbach GL, Aalkjaer C, Heagerty AM, Korsgaard N, Schiffrin EL, and Heistad DD**. Vascular remodeling. *Hypertension* 28: 505-506, 1996.

19. **Reglodi D, Kiss P, Lubics A, and Tamas A**. Review on the protective effects of PACAP in models of neurodegenerative diseases in vitro and in vivo. *Curr Pharm Des* 17: 962-972, 2011.

20. **Sadler TW**. *Langman's Medical Embryology*. Wolters Kluwer Health, 2015.

21. **Sandoval RJ, Injeti ER, Williams JM, Georthoffer WT, and Pearce WJ**. Myogenic contractility is more dependent on myofilament calcium sensitization in term fetal than adult ovine cerebral arteries. *Am J Physiol Heart Circ Physiol* 293: H548-556, 2007.

22. **Simon G, and Danneman KJ**. Dilation and reduced distensibility of rat carotid artery with aging. *Clin Exp Hypertens* 27: 459-466, 2005.

23. **Tanaka H, Dinenno FA, Monahan KD, DeSouza CA, and Seals DR**. Carotid artery wall hypertrophy with age is related to local systolic blood pressure in healthy men. *Arterioscler Thromb Vasc Biol* 21: 82-87, 2001.

24. **Toda N, and Shimizu I**. Neuroeffector function in mesenteric arteries isolated from beagles of different ages. *J Pharmacol Exp Ther* 240: 223-227, 1987.

25. **Tschudi MR, and Luscher TF**. Age and hypertension differently affect coronary contractions to endothelin-1, serotonin, and angiotensins. *Circulation* 91: 2415-2422, 1995.

26. **Tumer N, Toklu HZ, Muller-Delp JM, Oktay S, Ghosh P, Strang K, Delp MD, and Scarpace PJ**. The effects of aging on the functional and structural properties of the rat basilar artery. *Physiol Rep* 2: 2014.

27. **Vamos Z, Cseplo P, Ivic I, Matics R, Hamar J, and Koller A**. Age determines the magnitudes of angiotensin II-induced contractions, mRNA, and protein expression of angiotensin type 1 receptors in rat carotid arteries. *J Gerontol A Biol Sci Med Sci* 69: 519-526, 2014.

28. **VanBavel E, and Mulvany MJ**. Role of wall tension in the vasoconstrictor response of cannulated rat mesenteric small arteries. *J Physiol* 477 (Pt 1): 103-115, 1994.

29. **Vaudry D, Falluel-Morel A, Bourgault S, Basille M, Burel D, Wurtz O, Fournier A, Chow BK, Hashimoto H, Galas L, and Vaudry H**. Pituitary adenylate cyclase-activating polypeptide and its receptors: 20 years after the discovery. *Pharmacol Rev* 61: 283-357, 2009.

30. **Wilson AJ, and Warren JB**. Adenylate cyclase-mediated vascular responses of rabbit aorta, mesenteric artery and skin microcirculation. *Br J Pharmacol* 110: 633-638, 1993.