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DISSERTATION

Influence of iodinated contrast media on cerebral and renal vascular function

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To the light eternal memory of

the great neurosurgeon and the best father ever

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Summary

Abstract (English)

Iodinated contrast media exert toxic effects on vessels. Patients surviving subarachnoid hemorrhage and vasospasm require angiographic examinations. We hypothesized that contrast media influence cerebral blood flow autoregulation. To test the hypothesis, we developed an *in vitro* model of cerebral vasospasm. Rat superior cerebellar arteries were isolated, pressurized and studied under isobaric conditions. Coagulated blood was used to simulate hemorrhage, and the contrast medium was applied intraluminally. We found that exposition to blood caused development of a stronger myogenic tone and a decrease of the myogenic response compared to control. The contrast medium had no negative influence on myogenic tone and myogenic response in our model of vasospasm post subarachnoid hemorrhage. Thus, this *in vitro* study suggests no severe impairment of cerebral vessel function by iodinated contrast media [P1].

Oxidative stress has been discussed as an important factor in the pathogenesis of contrast media-induced acute kidney injury (CI-AKI). We proposed increase of superoxide and decrease of nitric oxide as consequences of cell damage caused by contrast media. We also hypothesized that oxidative stress impairs tubuloglomerular feedback. Rat thick ascending limbs and rabbit juxtaglomerular apparatus were isolated and perfused with the contrast medium or vehicle. Oxidative stress and cell death rate were estimated by using fluorescence methods. Tubuloglomerular feedback was measured in the double perfused juxtaglomerular apparatus. We found an increased superoxide and a decreased nitric oxide bioavailability, and an increased cell death rate in contrast-perfused thick ascending limbs. Tubuloglomerular response was enhanced in contrast-perfused juxtaglomerular apparatus. In conclusion, application of the contrast medium directly induced tubular cell death and local oxidative stress, and modified tubuloglomerular feedback. These derangements may play a role in CI-AKI pathogenesis [P2].

Females are protected from cardiovascular events before menopause, suggesting sex related differences in cardiovascular control. We tested the hypothesis that renal vessels of female and male mice differ in response to angiotensin II, a main player of renal perfusion control, and checked a possible role of angiotensin II type 2 receptors in this context. Our experiments with isolated renal interlobar arteries mounted in the wire myograph showed stronger

contraction to angiotensin II in males. Blockade of angiotensin II type 2 receptors strengthened the contraction to angiotensin II only in females. Further investigations revealed endothelial nitric oxide as the main mediator of these events. The results suggest a nitric oxide-mediated sex-specific action of angiotensin II via angiotensin II type 2 receptors in renal interlobar arteries [P3].

Abstract (German)

Jodhaltige Kontrastmittel beeinflussen die Gefäßfunktion über ihre toxischen Eigenschaften. Da Patienten mit Subarachnoidalblutung und Gefäßspasmus angiographisch untersucht werden müssen, haben wir einen möglichen Einfluss von Kontrastmitteln auf die Hirnarterienfunktion getestet. Dafür wurde ein *in vitro* Modell des zerebralen Vasospasmus entwickelt. Die Arteria cerebellaris superior der Ratte wurde isoliert, unter physiologischen Druck gesetzt und unter isobarischen Bedingungen studiert. Geronnenes Blut wurde in das Gefäßbad eingebracht, um die Subarachbnoidalblutung zu simulieren. Das Kontrastmittel wurde ins Gefäßlumen appliziert. Die Ergebnisse zeigen, dass geronnenes Blut den myogenen Tonus erhöht und die myogene Antwort beeinträchtigt. Das Röntgenkontrastmittel hat keinen negativen Einfluss auf den Tonus oder die myogene Antwort. Die Ergebnisse dieser *in vitro* Studie zeigen keine wesentliche Beeinflussung der Hirnarterienfunktion durch jodhaltige Kontrastmittel [P1].

Experimentelle und klinische Studien weisen darauf hin, dass oxidativer Stress eine Rolle in der Genese kontrastmittelinduzierten akuten Nierenversagens (CI-AKI) spielen kann. Wir prüften die Hypothese, dass eine erhöhte Superoxidkonzentration und verringerte Stickstoffmonoxidbioverfügbarkeit die Folge kontrastmittelinduzierter Zellzerstörung sind, und dass oxidativer Stress tubuloglomeruläre Signalwege stört. Der dicke aufsteigende Teil der Henle-Schleife (Ratte) bzw. der juxtaglomeruläre Apparat (Kaninchen) wurden isoliert und mit Kontrastmittel perfundiert. Oxidativer Stress und Zellschädigung wurden unter Anwendung von Fluoreszenz-Verfahren erfasst. Die tubuloglomeruläre Rückkopplung wurde mittels Doppelperfusion des juxtaglomerulären Apparates gemessen. Das jodhaltige Kontrastmittel Iodixanol erhöhte die Superoxidkonzentration, verminderte die Stickstoffmonoxidbioverfügbarkeit und beschleunigte den Epithelzelluntergang in dem dicken aufsteigenden Teil der Henle-Schleife. Die tubuloglomeruläre Rückkopplung wurde durch das Kontrastmittel konzentrationsabhängig moduliert. In Verbindung mit oxidativem Stress und Stickstoffmonoxidmangel an den Gefäßen kann dies zur Verminderung des renalen Blutflusses und der Nierenfunktion bei CI-AKI beitragen [P2].

Frauen vor der Menopause sind von kardiovaskulären Krankheiten weniger betroffen als Männer. Dies weist auf eine geschlechtspezifische Herz-Kreislauf-Regulation, –Pathologie, als auch Blutdruckregulation hin. Wir untersuchten geschlechtsabhängige Antworten der Arteria interlobares der Mausniere auf Angiotensin II, welches in der Regulation der

Nierendurchblutung eine große Rolle spielt. Die männlichen Arterien zeigten unter isometrischen Bedingungen eine stärkere Kontraktion durch Angiotensin II im Vergleich zu weiblichen Gefäßen. Die Blockade des Angiotensin II Type-2-Rezeptors verstärkte die Kontraktion durch Angiotensin II nur bei weiblichen Mäusen. Die Gefäßreaktivität war bei weiblichen Mäusen stärker vom Stickstoffmonoxid abhängig als bei männlichen Tieren. Wir schlussfolgern, dass es eine Stickstoffmonoxid-vermittelte, geschlechtsspezifische, Angiotensin II-induzierte Gefäßdilatation via Type-2-Rezeptoren gibt, welche Unterschiede in der Nierenperfusion und damit in der Blutdruckregulation verursachen kann [P3].

Introduction

My thesis is directed to vascular physiology. During my doctorate I studied function of arterial vessels of the brain and the kidney. I learned techniques for their investigation including dissection, mounting, performance of experiments, data analysis, and data interpretation. I isolated vessels of various sizes from different vascular beds of rats and mice. The main methods of my work were (i) isometric investigation by using wire myograph, (ii) isobaric investigation by using pressure myograph and (iii) isotonic investigation by using microperfusion. The main focus of my studies was to investigate the effects of iodinated contrast media on vessel function. Contrast media are commonly used in clinics for the diagnosis and treatment of vessel pathology, but their application may induce critical situations. The first article [P1] tests the hypothesis that contrast media may worsen the function of cerebral vessel with vasospasm. The second article [P2] is related to the contrast media-induced acute kidney injury (CI-AKI). Since the word-number is limited in summary, my first-author [P1] work about contrast media and cerebral vasospasm will be primarily discussed along with the CI-AKI, and less will be talked about gender differences in renal arterial function [P3].

Being very helpful for the clinical work, all contrast media even of last generation possess high biological insalubrity. Many investigations have been done to understand the mechanisms behind contrast media-induced damage, but precisely how contrast media harm tissue is still not well understood. Previous generations of contrast media exerted their toxic effects because of their high osmolality. Reduction of the osmolality and ionicity across generations lowered the side effects, but contrast media still have toxic effects which impair the function of renal vessels and vessels from other vascular beds (Sendeski, 2011).

It has been shown that the cerebral vasculature can be influenced by contrast media (Rosengarten et al., 2003). Hence, the use of contrast media may be fatal for patients who already have cerebral blood flow disturbances. Unfortunately, the literature is rare regarding this important question. One of the most life-threatening brain diseases is subarachnoid hemorrhage. This event goes along with impaired regulation of cerebral blood flow (Koide et al., 2013). Cerebral vasospasm, which usually follows subarachnoid hemorrhage, impairs those patients who survived the first attack. We hypothesized that contrast media application in patients after subarachnoid hemorrhage may further disturb cerebral blood flow

autoregulation and thus also determine the outcome of subarachnoid hemorrhage. To test the hypothesis, we developed an *in vitro* model of acute subarachnoid hemorrhage and studied vessel myogenic tone and myogenic response during contrast media application.

Indeed, the kidney is the most vulnerable organ for contrast media induced injuries. The main factors in pathogenesis of CI-AKI are cytotoxicity and high viscosity of contrast media of the last generation, which cause a decrease of renal perfusion and glomerular filtration rate - a hallmark of AKI. Along with chemical and mechanical effects of contrast media, there are also auto- and paracrine derangements, such as increased production of renal vasoconstrictors, diminished nitric oxide bioavailability, and local oxidative stress, playing a role in CI-AKI development (Seeliger et al., 2012). We hypothesized in our study that contrast media directly damage cells of thick ascending limb of Henle's loop independently of hypoxia and oxidative stress, and that oxidative stress is rather a consequence of cell damage than its reason. We also presume that the tubulo-vascular cross-talk is impaired in CI-AKI. Only few studies have been performed to evaluate a potential role of the tubuloglomerular feedback in the pathogenesis of CI-AKI, but they did not clarify the contribution of this mechanism to the reduced glomerular filtration rate and renal perfusion (Persson and Patzak, 2005). We hypothesized that contrast media influence the tubuloglomerular feedback by inducing oxidative stress in macula densa cells via toxic effects on these cells.

Working with renal vessels, I also performed a lot of experiments with interlobar arteries. Studies show that men have a higher risk for cardiovascular diseases than women. The background of this important observation is not well known. Since postmenopausal women's risk for developing cardiovascular diseases and life threatening event approaches to that of men, the sexual hormones may be important for the protection (Dubey et al., 2002). How the structure and function of the cardiovascular system are influenced by hormones is of large interest. The kidney is integrated in several systems of blood pressure control, where renal blood flow and perfusion pressure are important determinants of kidney function. The renin-angiotensin-system is one of the most powerful systems for the regulation of renal blood flow. Gender differences in the component expression and function of this system have been reported (Reckelhoff, 2001). We hypothesized that there is a gender difference in renal vessel function which is related to the angiotensin II type 2 receptor (AT_2R). Thus, we studied the angiotensin II response of female and male mice, and investigated the signalling pathways of the angiotensin II action.

Methodology

All animal handling and experiments were performed in accordance to the guidelines of the Office for Health and Social Matters of Berlin (Berlin, Germany).

Examination of vessel tone and myogenic response in cerebral arteries

Adult male Sprague Dawley rats were anaesthetized and decapitated. After craniotomy, brains were removed and segments of superior cerebellar arteries were obtained. The arteries were mounted on glass pipets of the pressure myograph's experimental chamber, perfused with physiological saline solution (PSS) and pressurized. Either vehicle or a contemporary contrast medium iodixanol (23 mg iodine/ml) was added to the PSS intraluminally. After mounting of arteries, the intraluminal pressure of 80 mmHg was manually installed [P1, Fig.1].

Subarachnoid hemorrhage was simulated using coagulated rat blood deployed in the experimental chamber, which was then filled with PSS before mounting the dissected arteries. There was no mechanical interaction between the coagulated blood and the vessels. Thus, the vessels were distributed in 4 experimental groups: 1) with PSS inside of the vessel (=intraluminally) and outside of the vessel (=inside of the experimental chamber) 2) with iodixanol intraluminally and PSS in the chamber, 3) PSS intraluminally and with coagulated blood in the chamber, 4) iodixanol intraluminally and coagulated blood in the chamber [P1, Tab.1].

Diameter of arteries was registered in a continuous manner over time using a video camera assembled to an inverted microscope: 1) during one hour of initial stable intraluminal pressure of 80 mmHg, while the vessels develop spontaneous myogenic tone, and 2) during controlled changes of intraluminal pressure in order to examine vessels myogenic response (within 4 steps, each of 5 min: 1 step – from 80 to 40 mmHg, 2 step – from 40 to 80 mmHg, 3 step – from 80 to 120 mmHg, 4 step – from 120 to 80 mmHg) [P1, Fig.2].

For statistics, two-way/repeated measurements ANOVA and Tukey HSD as a post hoc test were used to analyse the myogenic tone. The myogenic response was analysed with the Kruskal-Wallis and the Mann-Whitney-test. P value smaller than 0.05 was considered as statistically significant.

Investigation of contrast media effects in renal tubules

To investigate the effect of contrast media on tubule function, the thick ascending limb of Henle's loop of Spraque-Dawley rats was dissected from the excised kidneys. The tubule was mounted on concentric pipettes of a microperfusion system, cannulated from the proximal end and perfused with PSS.

Either vehicle or iodixanol (23 mg iodine/ml and/or 11 mg iodine/ml) was added to the perfusate. Superoxide and nitric oxide bioavailability was measured in thick ascending limbs of Henle's loop by using fluorescence techniques (dihydroethidium=DHE and 4-amino-5 methylamino-2',7'-difluorofluorescein diacetate=DAF-FM, respectively). Pharmacological interventions using the nitric oxide synthase inhibitor (L-NAME) and the superoxide dismutase mimetic (tempol) provided further information about the role of both signaling systems. Propidium iodide and trypan blue were used to assess cell damage rate [P2, Fig.1, Fig.2].

Measurement of tubuloglomerular feedback in juxtaglomerular apparatus

The juxtaglomerular apparatus of New Zealand white rabbits was dissected from the excised kidneys. The distal tubule and its attached afferent arteriole were double cannulated and simultaneously perfused with PSS, each with a specific composition, by using an arrangement of concentric pipettes (microperfusion system).

Either vehicle or iodixanol (11 mg iodine/ml and 23 mg iodine/ml) was added to the perfusate of the distal tubule. The tubuloglomerular feedback was estimated by measuring of an absolute diameter of the afferent arteriole when changing the NaCl concentration of the perfusate from 10 mM to 80 mM in the distal tubule. Tempol was used in some experiments with iodixanol of 11 mg iodine/ml.

For statistical analysis, ANOVA-like test for repeated measurements of non-parametric data (Brunner's test) was used for all experiments. P value smaller than 0.05 was considered as statistically significant.

Investigation of gender differences in renal interlobar arteries

Renal interlobar arteries were dissected from kidneys of adult male and female C57BL6 mice and mounted in a wire myograph system. The experimental chambers filled with PSS were warmed up and supplied with carbogen. Vessels were normalized and the maximal contractile

function of interlobar arteries was checked by application of 100mmol/l KCl. The developed force served as a reference value in the experiment.

Concentration-response-curves were performed for angiotensin II and phenylephrine in order to evaluate constrictor properties of interlobar arteries. Dilatory properties of interlobar arteries were examined by measuring concentration-response-curves for acetylcholine (endothelium-dependent dilation) and sodium nitroprusside (endothelium-independent dilation). Incubation of vessels with of L-NAME was used to define the role of nitric oxide, incubation with indomethacin – for the role of cyclooxygenase 1 and 2 in the sex differences. The effects of L-NAME on basal vessel tone were recorded. Incubation with the antagonist of AT_2R (PD123,319) was performed to test the influence of the AT_2R on the sex differences. Simultaneous incubation with L-NAME and PD123,319 was done to clarify the role of nitric oxide on the AT_2R vasodilatory function. The endothelium was removed using mice whiskers to check the role of endothelium.

For statistical analysis, ANOVA-like test for repeated measurements of non-parametric data (Brunner's test) was used for all experiments. P value smaller than 0.05 was considered as statistically significant.

Results

Contrast media did not influence the myogenic tone of cerebral vessels

Myogenic tone was expressed as a percent (%) of maximal diameter of the vessel and reported as average and standard error of the mean (average±SEM) in the text.

Cerebral vessels of the control group developed a typical spontaneous myogenic tone (77.1±1.2% of the maximal diameter). The contrast medium iodixanol did not influence the myogenic tone of the control group (77.2±1.7%). Exposition of vessels to coagulated blood significantly enhanced the myogenic tone of the control group (65.7±2.0%) and of the vessels treated with iodixanol (62.1±2.1%). Iodixanol did not change the tone of vessels exposed to blood [P1, Fig.3].

Contrast media did not worsen the myogenic response of cerebral vessels

Myogenic response was quantified in micrometers (µm) as an absolute change in the vessel diameter after each controlled change of the intraluminal pressure, and reported as medians in the text.

Cerebral arteries of our control group demonstrated normal myogenic responses, i.e. dilation in response to decrease of the intraluminal pressure, and constriction in response to the pressure elevation (80–40 mmHg: 11.9 µm; 40–80 mmHg: -13 µm; 80–120 mmHg: 1.4 µm; 120–80 mmHg: 0.6 µm). Vessels treated with iodixanol alone did not have significant changes in the myogenic response (80–40 mmHg: 9.1 µm; 40–80 mmHg: -9.3µm; 80–120 mmHg: -0.1 µm; 120–80 mmHg: 1.7 µm), when compared to the control group. Coagulated blood caused a significant impairment of the myogenic response in all steps of the control group (80–40 mmHg: -3.3 µm; 40–80 mmHg: 1.7 µm; 80–120 mmHg: 10.9 µm; 120–80 mmHg: -6.4 µm). However, treatment of vessels with iodixanol together with coagulated blood caused less functional impairment of the myogenic response at one step of the protocol – when the pressure was increased from 80–120 mmHg (1.1 µm vs. 10.9 µm of its control – the blood group). Responses to the other three pressure manipulations did not significantly differ compared to the blood alone treated vessels (80–40 mmHg: -3.3 µm; 40–80 mmHg: -1.1 µm; 120–80 mmHg: -1.6 µm) [P1, Fig.4].

Contrast media caused oxidative stress and epithelial cell death in renal tubules

All values of the results in thick ascending limb of Henle's loop are expressed as % of the baseline, and reported as average±SEM in the text.

Thick ascending limbs of Henle's loop perfused with iodixanol of 23 mg iodine/ml showed a significantly increased production of superoxide (9.58±1.43%) compared to the control group (1.72±1.01%), as well as compared to thick ascending limbs of Henle's loop perfused with iodixanol at 11 mg iodine/ml (0.22±1.88%). Tempol abolished the contrast media-caused increase of superoxide (0.35±1.33%) [P2, Fig.3].

Thick ascending limbs of Henle's loop perfused with iodixanol of 23 mg iodine/ml showed a significant decrease in nitric oxide bioavailability (-0.55±0.64%) compared to the control group (increase to 1.63±0.34%). L-NAME did not influence the effect of iodixanol and the response significantly differed from the control group (-0.29±0.76%) [P2, Fig.4].

Thick ascending limbs of Henle's loop perfused with iodixanol of 23 mg iodine/ml showed a significant increase in propidium iodide fluorescence [P2, Fig.5], as well as an increased uptake of Trypan blue over time [P2, Fig.6], when compared with the control group. Tempol did not influence the effect of iodixanol on cell death rate.

Contrast media enhanced the tubuloglomerular feedback

All values of tubuloglomerular feedback were expressed as an absolute change in the diameter of the afferent arteriole in response to the increase of the NaCl concentration in the distal tubule, and presented as average±SEM in the text.

In our control experiments, the exchange of NaCl concentration from 10 mM to 80 mM in the distal tubule caused a constriction of the afferent arteriole by 2.8±0.4µm. The lower dose of iodixanol increased the tubuloglomerular feedback: when applying 11mg iodine/ml, the afferent arteriole constricted significantly stronger in response to NaCl in comparison to control (4.5±0.4µm). The higher dose of iodixanol (23 mg iodine/ml) did not significantly change the control tubuloglomerular response (2.3±0.3µm) [P2, Fig.7]. Tempol prevented the effect of iodixanol on the tubuloglomerular feedback (1.8±0.6µm for tempol alone, 1.5±0.6µm for tempol + iodixanol 11mg iodine/ml) [P2, Fig.8].

Gender differences in renal interlobar arteries

All values of interlobar arteries responses were expressed as % of maximal KCl contraction, and reported as average±SEM in the text, if no other stated.

Contractions to KCl (7.2±0.7 and 7.0±0.4 millinewtons for female and male mice, respectively) and to phenylephrine [P3, Fig.2] showed similar behavior in vessels of females and males. Angiotensin II caused more pronounced constriction of male interlobar arteries than females [P3, Fig.1]. Arteries of females and males showed a concentration-dependent response to angiotensin II and phenylephrine.

Treatment with L-NAME and mechanical removal of endothelium significantly enhanced responses to angiotensin II of both groups similarly. The basal vessel tone was slightly enhanced by L-NAME application also in both groups (6.2±1.6% for females and 5.3±0.8% for males). Application of the AT_2R antagonist increased contraction to angiotensin II only in female mice, i.e. the inhibition with PD123,319 abolished the sex differences in the angiotensin II response. Application of L-NAME and PD123,319 together increased the angiotensin II concentration response curve also only in females, and the curve was similar when applying L-NAME alone. In males, application of PD123,319 alone or together with L-NAME did not change the response to angiotensin II [P3, Fig.3].

Interlobar vessels from male mice showed a stronger relaxation to acetylcholine than from females. Application of L-NAME diminished the relaxation to acetylcholine in both females and males, showing more pronounced effect in females. Application of indomethacin did not significantly change the acetylcholine response, but suppressed the sex difference in the acetylcholine response of the control group [P3, Fig.4]. Relaxation to sodium nitroprusside showed no difference between arteries of the two groups [P3, Fig.5].

Discussion

The effects of contrast media on cerebral and renal vasculature

The main aim of my thesis was to ascertain whether a contemporary contrast medium iodixanol could influence the function of cerebral and renal vessels. To answer the question, spontaneous myogenic tone and myogenic response were measured in isolated cerebral vessels during contrast media application in normal conditions and in an *in vitro* model of cerebral vasospasm post subarachnoid hemorrhage. In the kidney, the tubulo-vascular cross-talk was of interest. Therefore the renal tubular cell state and the tubuloglomerular feedback were investigated during application of the contrast medium.

The autoregulation is a very powerful mechanism for cerebral blood flow control. Bayliss was the first who described myogenic response: it is an ability of arteries to respond to an increase of pressure ("stretching force") by contraction, and to a decrease of pressure by dilation, given that the vessels are in a state of myogenic tone (Bayliss, 1902).

We found, that iodixanol did not exert clear negative effects on the myogenic response of cerebral vessels under control conditions as well as in vessels where vasospasm was simulated by using coagulated blood. This is an important finding, since it has been shown that contrast media can disturb regulation of the dynamic cerebral blood flow in *healthy* individuals (Rosengarten et al., 2003). Further, patients surviving subarachnoid hemorrhage have an already impaired cerebral blood flow regulation, and their treatment and diagnostic require application of contrast media. Importantly, in our experiments coagulated blood caused a significant derangement of the entire myogenic response in comparison to control cerebral vessels. In other words, when increasing the intraluminal pressure, the spastic vessels showed dilation instead of constriction, and the vessels constricted in response to a pressure drop. We suppose that this phenomenon reflects the clinical evidence of deranged regulation of cerebral blood flow after hemorrhage. Cerebral vasospasm which develops within few days after subarachnoid hemorrhage usually worsens the clinical picture of the disease (Kellner et al., 2012). This agrees with our observation that the vessels exposed to coagulated blood develop a significantly more pronounced spontaneous myogenic tone than the control vessels. Iodixanol did not influence the development of the myogenic tone in both control and as well as in spastic vessels.

Thus, contrast medium did not negatively influence the vascular tone and the myogenic response of cerebral vessels in our model. Rather, when switching the intraluminal pressure from 80 to 120 mmHg, the vessels which were treated simultaneously with coagulated blood and iodixanol showed less functional impairment than the vessels exposed to blood alone. Since the toxic effects of all types of contrast media on vessels are well known, we do not believe in a real improvement. This effect might be due to a loss of vessel wall elasticity or reduction of nitric oxide bioavailability because of severe endothelial damage (Sendeski et al., 2012). It is considered that the myogenic response does not require the presence of endothelium, but it still can be modulated by nitric oxide (Schubert and Mulvany, 1999). Our observations in renal vessels, which are discussed below, also demonstrate endothelial destruction and reduction of nitric oxide bioavailability by the contrast medium and, in this way, augments the possibility for our cerebral vessels to be damaged by iodixanol.

It has been recently shown, that most types of iodinated contrast media have similar severe toxic impact on cells. This property is very possibly related to iodine, a component which provides their radio-opacity (Sendeski, 2011). Dimeric contrast media of the last generation have a high viscosity, what may cause the reduction of renal blood and tubular flow, and glomerular filtration rate. Contrast media directly causes apoptosis and cell lesion of tubular epithelial cells and endothelium of afferent arteriole and vasa recta. Low glomerular filtration rate results in a longer contact between contrast media and the tissue, what in turn aggravates contrast media induced damage. As a consequence of epithelium damage, vessels constrict, causing medullar hypoperfusion and pronouncing the suffering of tubules. Disbalance in the production of auto- and paracrine factors, such as decreased nitric oxide bioavailability and increased superoxide release, play a final accord in the cascade development of the medullary hypoxia (Persson et al., 2005).

We found that superoxide concentration is significantly increased in the thick ascending limb of Henle's loop when perfused with iodixanol. Increased superoxide concentration in CI-AKI has been related to hypoxia (Heyman et al., 2010). However, our *in vitro* model excludes hypoxia as a reason for oxidative stress, because oxygen partial pressure was within physiological level in our experiments. It is, therefore, very likely that the superoxide concentration in the tubules was increased due to the toxic effect of iodixanol on the epithelial cells. Oxidative stress seems to be the result but not the reason for cell damage in CI-AKI. This may explain why some clinical studies did not show beneficial effects of antioxidant treatment (Stacul et al., 2011). Increased medullar superoxide production may have serious

consequences, because superoxide enhances Na^+ transport and Na^+-K^+-Cl^+ co-transporter function, and diminishes nitric oxide production. At the same time we have observed decreased nitric oxide in our experiments, and this aggravates the derangement of ionic transport, because nitric oxide antagonizes superoxide influence and regulates other ionic channel permeability.

Along with increased superoxide production, an increased cell death rate was demonstrated in our thick ascending limb of Henle's loop, when exposed to higher concentration of iodixanol. However, tempol did not influence the effect of iodixanol. This also supports the idea that the epithelial cell damage goes independently from oxidative stress.

We found that iodixanol enhances the constriction of afferent arterioles in response to an increase of NaCl concentration in the distal tubule only at the concentration of 11mg iodine/ml, but not at 23mg iodine/ml. Thus, the contrast medium can strengthen the tubuloglomerular feedback in our model. Further, we found that the application of tempol suppressed the constrictor effect of contrast medium, suggesting superoxide as the main actor in the enhanced feedback. This result confirms our finding of an increased superoxide concentration in isolated tubules due to contrast medium application. Interestingly, only the lower concentration of iodixanol caused the enhanced tubuloglomerular feedback, whilst only the higher concentration of iodixanol induced oxidative stress in the thick ascending limb of Henle's loop experiments. The differences in the concentration dependency of the contrast medium effects may be caused by the use of different animal species (rat vs. rabbit) and/or different anatomical and physiological conditions. The loss of the increased tubuloglomerular feedback when applying 23mg iodine/ml of iodixanol compared to 11mg iodine/ml might be the result of a severe damage of macula densa cells with less production of superoxide.

In conclusion, contrast media did not show negative influence on the myogenic response and the myogenic tone of healthy and spastic cerebral vessels in our model of cerebral vasospasm post subarachnoid hemorrhage. However, contrast media caused direct cell destruction of renal tubular epithelium, which led to oxidative stress and low nitric oxide bioavailability independently of hypoxia and hypoperfusion, and disturbed renal tubuloglomerular feedback of the juxtaglomerular apparatus. These apparently contradictory findings may have the same background, considering different functional and anatomical characteristics of the experimental settings. Therefore, drawing conclusion for the medical practice requires additional studies on the effects of contrast media in vessels and their underlying mechanisms.

Gender differences in renal interlobar arteries

The results of this study show that interlobar arteries from male mice have a greater response to angiotensin II. In contrast, the same vessels did not reveal any gender differences in response to phenylephrine and KCl. Thus, we presume that the sex differences are related to angiotensin II signaling pathways, where nitric oxide is considered to play a determining role and to balance the constrictor property of angiotensin II (Walsh et al., 2009). After exposition to L-NAME, the vessels from female and male enhanced their constriction to angiotensin II, and, eventually, lost the sex difference which they had in response to angiotensin II alone. This suggests that female vessels have a greater dependency on nitric oxide than the males. Angiotensin II acts on two types of receptors: angiotensin II type 1 (AT_1R) which mainly mediates vessel constriction, and AT_2R which is liable for vessel dilation (Patzak and Persson, 2007). After blockade of the AT_2R, only the vessels from females showed a stronger constriction to angiotensin II compared to the control. The baseline effects of L-NAME without angiotensin II application showed no sex differences in nitric oxide bioavailability. Thus, we can assume, that the lower contraction to angiotensin II of females is related to a higher nitric oxide production by endothelium.

Our results show that nitric oxide is also the main mediator of the acetylcholine induced dilation in renal interlobar arteries. Indomethacin did not affect the acetylcholine response significantly, which indicates no important contribution of COX-dependent pathways to the dilatory response. The absence of gender differences in dilatory responses to sodium nitroprusside suggests that the observed sex differences in response to angiotensin II are not related to the functional changes of vascular smooth muscle cells or vessel remodeling. Rather, they are due to the differences in the endothelial nitric oxide bioavailability.

In conclusion, our results show gender differences in the angiotensin II response of renal interlobar arteries of the mouse, which are related to the release of endothelial nitric oxide via activation of AT_2R. They may influence kidney perfusion and, in this way, play a role in the gender differences of blood pressure regulation.

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Affidavit

I, *Tatiana Nikitina*, certify under penalty of perjury by my own signature that I have submitted the thesis on the topic "*Influence of iodinated contrast media on cerebral and renal vascular function"*. I wrote this thesis independently and without assistance from third parties, I used no other aids than the listed sources and resources.

All points based literally or in spirit on publications or presentations of other authors are, as such, in proper citations (see "uniform requirements for manuscripts (URM)" the ICMJE www.icmje.org) indicated. The sections on methodology (in particular practical work, laboratory requirements, statistical processing) and results (in particular images, graphics and tables) correspond to the URM (s.o) and are answered by me. My contributions in the selected publications for this dissertation correspond to those that are specified in the following joint declaration with the responsible person and supervisor. All publications resulting from this thesis and which I am author of correspond to the URM (see above) and I am solely responsible.

The importance of this affidavit and the criminal consequences of a false affidavit (section 156,161 of the Criminal Code) are known to me and I understand the rights and responsibilities stated therein.

_____

Date Signature

Declaration of any eventual publications

Tatiana Nikitina had the following share in the following publications:

[Publication 1]: *Tatiana Nikitina*, Olga Zavaritskaya, Vladimir Semenyutin, Pontus B. Persson, Andreas Patzak, Mauricio Sendeski. Effects of iodinated contrast media in a novel model of cerebral vasospasm. Accepted in the Arquivos de Neuro-Psiquiatria on 03-Oct-2014.

Contribution of the doctoral candidate: 80% (100% of the experimental work)
The doctoral candidate carried out all experiments. This included development of the experimental model, investigation of vasotonus and myogenic response of cerebral vessels, analyses and interpretation of data, preparation of the figures and drafting the text of the paper, submission and revision of the article.

[Publication 2]: Zhi Zhao Liu, Kristin Schmerbach, Yuan Lu, Andrea Perlewitz, *Tatiana Nikitina*, Kathleen Cantow, Erdmann Seeliger, Pontus B. Persson, Andreas Patzak, Ruisheng Liu, Mauricio Sendeski. Iodinated contrast media cause direct tubular cell damage, leading to oxidative stress, low nitric oxide, and impairment of tubuloglomerular feedback. Am J Physiol Renal Physiol. 2014 Feb; 306: F864–F872.

Contribution of the doctoral candidate: 20%.
The doctoral candidate contributed to this publication in several ways, including performance of experiments, data analyses and data interpretation.

[Publication 3]: Viegas VU, Liu ZZ, *Nikitina T*, Perlewitz A, Zavaritskaya O, Schlichting J, Persson PB, Regitz-Zagrosek V, Patzak A, Sendeski MM. Angiotensin II type 2 receptor mediates sex differences in mice renal interlobar arteries response to angiotensin II. J Hypertens. 2012 Sep; 30(9):1791-8.

Contribution of the doctoral candidate: 20%.
The doctoral candidate took part of practical work, such as dissection of vessels, performance of experiments, data analyses and their interpretation.

Signature, date and stamp of the supervising University teacher

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Signature of the doctoral candidate

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Publication 1

EFFECTS OF IODINATED CONTRAST MEDIA IN A NOVEL MODEL OF CEREBRAL VASOSPASM

Tatiana Nikitina, Olga Zavaritskaya, Vladimir Semenyutin, Pontus B. Persson, Andreas Patzak, Mauricio Sendeski

Arquivos de Neuro-Psiquiatria 2015; 73(2):125-131.

http://dx.doi.org/10.1590/0004-282X20140222

Publication 2

IODINATED CONTRAST MEDIA CAUSE DIRECT TUBULAR CELL DAMAGE, LEADING TO OXIDATIVE STRESS, LOW NITRIC OXIDE, AND IMPAIRMENT OF TUBULOGLOMERULAR FEEDBACK

Zhi Zhao Liu, Kristin Schmerbach, Yuan Lu, Andrea Perlewitz, Tatiana Nikitina, Kathleen Cantow, Erdmann Seeliger, Pontus B. Persson, Andreas Patzak, Ruisheng Liu, Mauricio Sendeski

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Publication 3

ANGIOTENSIN II TYPE 2 RECEPTOR MEDIATES SEX DIFFERENCES IN MICE RENAL INTERLOBAR ARTERIES RESPONSE TO ANGIOTENSIN II

Viegas VU, Liu ZZ, Nikitina T, Perlewitz A, Zavaritskaya O, Schlichting J, Persson PB, Regitz-Zagrosek V, Patzak A, Sendeski MM.

J Hypertens. 2012 Sep; 30(9):1791-8.

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Curriculum Vitae

Mein Lebenslauf wird aus datenschutzrechtlichen Gründen in der elektronischen Version meiner Arbeit nicht veröffentlicht.

Complete list of publications

1. *Nikitina T,* Zavaritskaya O, Semenyutin V, Patzak A, Sendeski M. Effects of iodinated contrast media in a novel model of cerebral vasospasm.

   Arquivos de Neuro-Psiquiatria 2015; 73(2):125-131.

2. Liu ZZ, Schmerbach K, Lu Y, Perlewitz A, *Nikitina T,* Cantow K, *et al.* Iodinated contrast media cause direct tubular cell damage, leading to oxidative stress, low nitric oxide, and impairment of tubuloglomerular feedback.

   Am J Physiol - Ren Physiol 2014; 306:F864–F872.

3. Viegas VU, Liu ZZ, *Nikitina T,* Perlewitz A, Zavaritskaya O, Schlichting J, Persson PB, Regitz-Zagrosek V, Patzak A, Sendeski M. Angiotensin II type 2 receptor mediates sex differences in mice renal interlobar arteries response to angiotensin II.

   Journal of Hypertension 2012; 30(9):1791–1798.

4. Kaufmann J, Martinka P, Moede O, Sendeski M, Steege A, Fähling M, Hultström M, Gaestel M, Moraes-Silva IC, *Nikitina T*, Liu ZZ, Zavaritskaya O, Patzak A. Noradrenaline enhances angiotensin II responses via p38 MAPK activation after hypoxia.re-oxygenation in renal interlobar arteries.

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