PLASMA-LEVELS OF ENDOTHELIN-1 AND ANGIOTENSIN-II AND REACTIVITY OF ARTERIAL BLOOD PRESSURE TO EXOGENOUS SYMPATHOMIMETICS AND VASOACTIVE PEPTIDES IN RAT MODEL OF MALIGNANT RENAL HYPERTENSION

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PLAZMATICKÉ HLADINY ENDOTELÍNU-1 A ANGIOTENZÍNU-II A REAKTIVITA ARTERIÁLNEHO TLAKU NA EXOGÉNNE SYMPATOMIMETIKÁ A VAZOAKTÍVNE PEPTIDY NA ANIMÁLNOM MODELI MALÍGNEJ RENÁLNEJ HYPERTENZIE

Abstract

Mislovicova M, Kettmann V, Moncek F, Drimal J Jr, Drimal D, Doherty AM, Drimal J:
Plasma-levels of endothelin-1 and angiotensin-II and reactivity of arterial blood pressure to exogenous sympathomimetics and vasoactive peptides in rat model of malignant renal hypertension
Bratisl Lek Listy 2000; 101 (3): 123–129

Background: **There is still considerable uncertainty regarding sensitivity of arterial blood pressure to endogenous peptides in renal hypertension. Many pathological processes including hypertension have been shown to be associated with release of endothelin-1 (ET-1). However the role of ET-1 in regulation of arterial blood pressure in hypertension is still controversial.**
Ob*jectives:* **The role of endothelin-1 (ET-1) and angiotensin-II (AT-II) in malignant phase of renovascular hypertension has been assessed on the basis of arterial blood pressure increase and ET_A receptor density measurements in Glodblatt-hypertensive rats (RVH).**
Results: **The arterial blood pressure response to sympatomimetic amines, vasopressors, the plasma ET-1 and AT-II levels as well as renal subtype-ET_A receptor density were significantly increased in RVH rats with malignant hypertension. The dominance of vasopressor ET_A receptors in RVH rats suggest the contribution of endothelin peptides to malignant renovascular hypertension.** ***(Tab. 1, Fig. 7, Ref. 25.)***
Key words: endothelin-1, angiotensin-II, hemodynamics, hypertension, endothelin ET(A) receptor.

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Abstrakt

Mislovičová M., Kettmann V., Monček F., Dřímal J. Jr., Dřímal D., Doherty A.M., Dřímal J.:
Plazmatické hladiny endotelínu-1 a angiotenzínu-II a reaktivita arteriálneho tlaku na exogénne sympatomimetiká a vazoaktívne peptidy na animálnom modeli malígnej renálnej hypertenzie
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Pozadie problému: V otázke senzitivity arteriálneho tlaku krvi na vazoaktívne peptidy pri renálnej hypertenzii je veľa nejasností. Mnohé ochorenia, i artériová hypertenzia, sú sprevádzané zvýšeným uvoľnením endogénnych pôsobkov. Úloha endotelínu-1 (ET-1) pri malígnej hypertenzii je sporná.
Cieľ: Objasnenie úlohy ET-1 v malígnej fáze renálnej hypertenzie.
Metódy: V chronických pokusoch na animálnych modeloch Goldblattových renálne-hypertenzných potkanoch (RVH) sa merali plazmatické hladiny ET-1 a PRA, v rádioligandových štúdiách denzita renálnych ET_A receptorov a po podaní exogénneho ET-1, angiotenzínu-II (AR-II) a sympatomimetík a in vivo i hemodynamika.

Výsledky: Odpoveď arteriálneho tlaku na vazopresorické látky, plazmatické hladiny ET-1 a AT-II a i denzita renálnych ET_A receptorov boli signifikantne zvýšené pri RVH na rozdiel od normotenzívnych (NTZ) potkanov.

Závery: Dominancia ET_A vazopresorického podtypu receptorov a zvýšenie reaktivity arteriálneho tlaku na presory v skupine RVH svedčí o účasti ET-1 na malígnej renálnej hypertenzii. *(Tab. 1, obr. 7, lit. 25.)*

Kľúčové slová: endotelín-1, angiotenzín-II, hemodynamika, hypertenzia, ET_A receptor.

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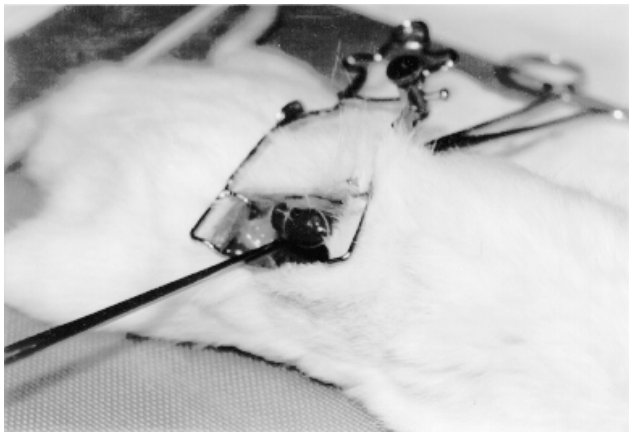

Fig. 1. Photograph of the Goldbtatt/Grollmann procedure on solitary rat kidney. Only a half of the "figure of eight" ligature is completed). For explanation see the text.
Obr. 1. Záber operačného výkonu podľa Goldblatta/Grollmanna na obličke: naložená je len časť „osmičkovej ligatúry", na povrchu solitárnej obličky, po kompletácii sa pri hypertrofii obličky sutúra zúčastňuje na hypoperfúzii.

The mortality studies have confirmed that disorders of the heart and blood vessels icluding all forms of heart attacks and cerebral stroke, represent the leading cause of diseases and deaths in industrialized countries. The arterial hypertension and atherosclerosis and their complications in coronary and cerebral vasculature, manifested as ischemic heart disease, myocardial infarction, heart failure and cerebral stroke, participated on more than 50 % causes of deaths in productive population. From 16 to 20 % of young inhabitans in Slovakia suffer with increased blood pressure and the occurence of essential hypertension increases with age (1). The essential hypertension and congestive heart failure may lead to renal failure, or occasionally, the primary defect of kidneys is a cause of accelerated, malignant form of hypertension (2). The mortality of patients with malignant form of hypertension is higher than 90 % at present (3). Evidence has accumulated suggesting that the excess of endogenous vasopressor peptide and comitogen endothelin-1 (ET-1) may be the cause of hypertension (4) myocardial infarction and congestive heart failure (5). The release of endothelin from endothelial and vascular smooth muscle play an unsubstitutable role in the homeostasis of arterial blod pressure (6), while reduction in the production of nitrogen oxide, potent vasodilatory mediator and overexpression of endothelium-derived constrictive factor (EDCF, presumably ET-1), participate on the development of serious cardiovascular disorders (7). The vasoactive peptide ET-1 (21-amino acid) is produced by endothelial and vascular smooth muscle cells, isopeptides are also produced in the heart, brain, kidney and other tissues.The primary place of production of ET-1 in kidneys are epitelial cells of tubuli colligens and the innermost layer of renal pulp (8). In tubular epithelial cells there is also the highest density of ET-1 receptors (9). The constitutional and also stimulated release of ET-1 may provoke pathological renal vasoconstriction. The effects of ET1 are mediated by two different subtypes of endothelin receptors (ET(A) and ET(B)) and both subtypes are present abundantly in vascular wall and also in the myocardium (10, 11). The existence of two subtypes of endothelin ET(B) receptors (ET(B1) and ET(B2)) was also proposed, their existence however, is questionable, because results have shown endothelin ET(B) receptor as product of a solitary gene (12). The endothelin ET(A) and presumably also ET(B2) receptors mediate vasoconstriction, the endothelin ET(B1) receptors are presumably vasodilatory (13). The native, human ET-1 is very effective agonist that binds preferentially and with high affinity to ET(A) receptors in vascular smooth muscle and with affinity many times lower to ET(B) receptors (14). Contrary to the endothelin-ET(A) receptor the ET(B)-subtype is quickly phosphorylated on serine and threonine residues after stimulation with ET-1 and resultant complex of ligand and receptor is internalized in cells (15). Recently much attention has been payed to genes and transcription factors responsible for cleavage of vasopressor and proliferative peptides. In defiance of an exeptionally intensive vasopressor and mitogenic action of endothelin peptides, there is much disagreement in question of sensitivity of arterial blood pressure to endothelin-1 in arterial hypertension. Similarly, also diligent effort to ellucidate the basic mechanism and ethiology of essential hypertension was vaining for the time being. Presupposition however exists that important role additionally to the given genetic, life style and risk factors may play the release of endogenous vasoconstrictor peptides.

The present study was designed to investigate possibility that plasma levels of endogenous endothelin and angiotensin and the reactivity of systemic arterial blood pressure are causally related. The aim of this study was to investigate this possibility with using animal model of chronic renal hypertension and exogenous administration of sympathomimetic amines, and peptidic vasopressors. The concentrations of endogenous peptides endothelin and angiotensin in plasma, the reactivity of arterial blood pressure, heart rate response and the maximal density of renal endothelin ET(A) receptors (mediating vasoconstriction and proliferation in solitary kidney) were measured in maligannt phase of hypertension in Goldblatt renal hypertensive rats (RVH).

Methods

The experimental protocol of this study was approved by the local ethics committee and conformed to the guidelines for the care and use of laboratory animals published by the US National Institutes of Health (Institute of Laboratory and Animal Resources, Commision on Life Science,National research Council (USDHHS,PHS,NIH Publication No 85-23, revised 1985). Conventional male Wistar (Hann) Dobra Voda rats (from 200 to 250 g) were used.

Operation

After induction of anesthesia (Pentobarbital 55 mg/kg intraperitoneally) and under aseptic conditions two parellel incisions (an approximately 20 mm in length) were were made in dorsalvertebrocostal angle. In the first (control) group after light exterioration of kidney from renal capsula both kidneys were repositioned to the retroperitoneum and wounds were closed (each incision with five sutures). In the second group after release from renal capsula the renal artery and vein were tied and kidney was excised. A "figure of eight" suture was loaded on contralateral kidney (Fig. 1). The suture was softly tight to be at the surface of

kidney, but not to the point of cutting of the parenchyma of kidney (the method according to (16) in modification of (17)). Penicillin G was administered intraperitoneally and Depot Penicillin was injected intramuscularly. Incisions were sutured, the wounds were treated with Ajatin and rats (each in a separate cage) were placed in Animal House. A special care was given with tape water and food ad libitum.

Hemodynamic measurements

Four weeks after operation , similarly in both groups, rats were anesthetized with pentobarbital (50 mg/kg intraperitoneally) and after incision and preparation of femoral artery and vein blood vessels vere cannulated with the specially adjusted catheters (Portex 196 with the maximal internal diameter 0.4 mm and a dead space 0.04 ml). The catheters were filled with anticoagullant (Heparin 500 IU). Direct measuremens of diastolic arterial blood pressure was made with arterial blood pressure receptors Tesla LDP and arterial blood pressure and three standard ECG leads were recorded on Mingograph 1600 Siemens-Elema.

Renal section

Four weeks after operation, after induction of ether anesthesia, and middle incision, the solitary kidney was relieved from concrescences. After incision and opening of kidney the four or six segments were dissected each in 0.5x5 mm with drill and sliced in a series of parallel segments in thickness 1 mm. Renal sections of control normotensive (NTZ) and renal-hypertensive rats (RVH) were placed in 96 Wells Cluster-Plates(Costar) containing 350 µl of HEPES-buffered physiological salt solution (PSS), (in mmol/l: NaCl 135; $CaCl_2$ 1.25; $MgCl_2$ 1.0; KH_2PO_4 0.44; NaH_2PO_4 0.34; $NaHCO_3$ 2.6; HEPES 20; glucose 5.5; containing protease inhibitors: PMSF 0.2 (following in µg/ml): leupeptin, antipain, pepstatin and aprotinin (each in 10.0, pH=7.51 and temperature at 25 ˚C). For identification of the total number of vasopressor, specific endothelin-ET(A) receptors in renal papillary sections with high affinity for endothelin-1 we used endothelin-ET(A) selective radioligand [^{125}I]PD151242 in saturation studies.

Ligand binding studies

Renal sections were incubated for 60 min with 8 gradually increasing concentrations (in the range from 0.05 to 1.05 nmol/l) of endothelin-ET(A)selective antagonist in two groups of experiments (n=12), in renal slices from control, normotensive (NTZ), sham operated rats and from rats with malignant renal hypertension. The nonspecific binding was determined in the presence of PD151242 in concentration 1.0 µmol/l. After the end of incubation, bound ligand was separated from free fraction by rapid filtration of renal sections on GF/C Whatman glass microfibre filters, followed by three subsequent 1.5 ml washes with ice-cold incubation medium and free ligand was isolated from bound corpuscular by vacuum filtration. After drying on filters, renal sections were immersed in 10 ml scintillation solution (Bray, Spolana, Czech Republic). Radioactivity bound on samples was determined by scintillation spectro-metry in Packard Tricarb 300 CD, Packard Instruments, Downers Growe Il., U.S.A.

Other measurements

Plasma levels of endothelin-1 and angiotensin-II were determined by commercial kits. Binding data were analyzed by the curve fitting program Ligand and Inplot and Hill analysis predicted the probability of a single binding site versus two sites of population of endothelin receptors. The significance of differences between groups of data was assessed by Student's t-test.

Chemicals

Angiotensin-II (Ciba), Acetylcholine (Roche), human, porcine Endothelin-1 (Sigma), Heparin (Spofa), HEPES, IRL1620 (Calbiochem), Norepinephrine (Sigma), PD-151242 (Parke-Davis), Pentobarbital (Spofa), Penicillin-G, Pendepon (Biotika). Radiochemical: [^{125}I]PD151242(Amersham Life Sci, Buckinghampshire, England).

Results

Plasmatic renin activity and levels of endothelin-1 in plasma

Four weeks after unilateral nephrectomy and ligature on solitary kidney (1K, 1L) in our experiments the plasmatic renin activity (PRA) was not significally different from control group (1.05±0.2 ng/ml). When compared with control group of NTZ rats the plasma levels of ET-1 however, were significantly higher in group of rats with renal hypertension (RVH=3.66±0.05 pg/ml, versus NTZ=0.21±0.05 pg/ml, $p<0.05$, n=12).

The effects of exogenous vasopressors on hemodynamics

The initial vallues of diasolic arterial blood pressure in the group of RVH rats were significantly increased from fourth up to seventh day after the procedure. The increase in diastolic arterial blood pressure four weeks after nephrectomy reached to RVH=17.6±0.9 kPa, what in comparison with control NTZ group represented the significant increase +3.8±0.2 kPa. When compared with control group (NTZ=376±12 beats/min) the heart rate of RVH rats was slightly higher (RVH=398±11 b/min) by 22 b/min ($p<0.05$). After infusion of norepinephrine (20,40 and 80 µg/kg/30 s) there was a dose dependent pressor reaction and the response to middle dosis in NTZ rats was +33±6 %. The response of arterial blood pressure to 40 µg/kg/30 s was markedly higher in RVH group (+48±8 %, $p>0.05$) (Fig. 2). Immediately after infusion of norepinephrine in the RVH rats in the present study there were dysrhythmias, mostly extrasystoles or extrasysoles in salvos. The arrhythmias were seen an approximately up to 30 seconds after infusion and only after norepinephrine. Disturbances were represented by short cycle of ventricular extrasystoles followed by sinus rhythm. Analysis of ectopic activity in group of RVH rats is summarized in Table 1. At the infusion of norepinephrine in control group of normotensive (NTZ) rats there was no ectopic activity. After infusion of endothelin there was an initial extrasystolia only in one of six experiments and the ectopic activity in this case was less then 3 %. The heart rate after infusion of norepinephrine was only slightly reduced in control group. The more expressive reduction in heart rate was observed in RVH group,shortly after infusion of medium dose of norepinephrine (-11.2±5, $p<0.05$). The infusion of acetylcholine (20 and 40 µ/kg/30 s) resulted in a decrease in diastolic arterial blood pressure in both groups, (NTZ=-68±5 %) while the response to acetylcholine was significantly reduced in the group of RVH rats (-38±3 %) (Fig. 3).

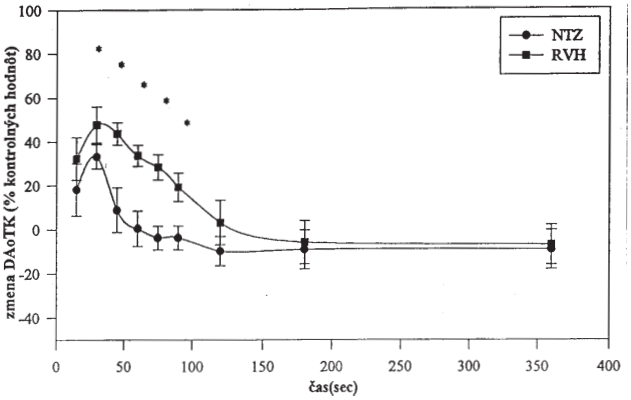

Fig. 2. Effect of intravenous norepinephrine infusion (40 μg/kg/30 s) on diastolic arterial blood pressure (DAoBP) on control, normotensive (NTZ) and renal-hypertensive (RVH) rats (n=12). Ordinata: time (t sec), abscissa: change in diastolic arterial blood pressure (DAoBP) in % of control. Values are: mean±standard error of the mean, * statistical significance p<0.05.
Obr. 2. Vplyv intravenóznej infúzie noradrenalínu (40 μg/kg/30 s) na diastolický arteriálny tlak normotenzívnych (NTZ) a renovaskulárne hypertenzných (RVH) potkanov (n=12). Na osi X čas v sekundách, na osi Y zmena diastolického arteriálneho tlaku v aorte (DAoTK) v % východiskových hodnôt. Priemerné hodnoty a stredné chyby priemeru, * p<0.05.

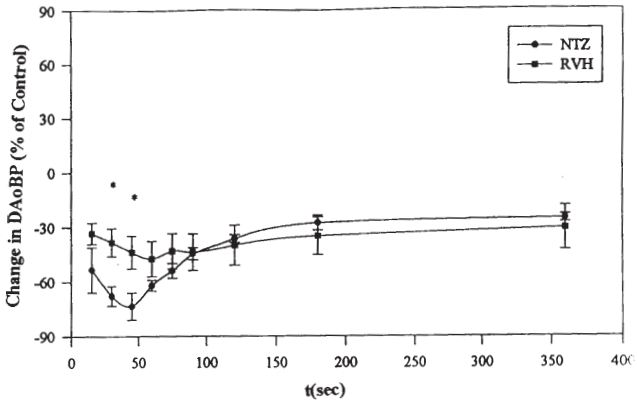

Fig. 3. Effects of intravenous infusion of acetylcholine (20 μg/kg/30 s) on DAoBP. Explanation and symbols as in the preceding figure.
Obr. 3. Účinky intravenóznej infúzie actetylcholínu (20 μg/kg/30 s) na DAoTK. Objasnenie a symboly ako na predchádzajúcom obrázku.

After infusion of acetylcholine there was a decrease in the heart rate in our experiments, however, there was no significant differences between both groups. Significant differences in diastolic arterial blood pressure were recorded after infusion of antiotensin-II (20 μ/kg/30 s). The response of arteral blood pressure was phasic, with the initial depression (-31±9 %) that was followed with presser response (+17±7 %) in control group. The initial depression was absent in RVH group with an immediate presser response (+52±20 %) (Fig. 4). The heart rate response after infusion of angiotensin in both groups contrasted by degree of changes. NTZ rats responded to angiotensin-II with significant bradycardia while the RVH group of rats responded only slightly. The control group responsed to intravenous infusion of endothelin (100 ng/kg/30 s) with small pressor response, after initial increase the diastolic blood pressure returned rapidly to its initial levels. RVH group of rats responded to endothelin infusion with meaningly higher increase in arterial blood pressure (+31±11 %, p<0.05) (Figs 5 and 6), while the relatively shorter duration of the presser response to exogenous endothelin was not sigificantly different form control group. Differences in the heart rate after infusion of endothelin were not significant.

Tab. 1. Ectopic activity (EA), (% of ectopic (aberrant) beats from the total number of beats) in an interval 30 s after infusion of norepinephrine. Rats with established malignant renal hypertension (RVH) four weeks after operation procedure (Solitary kidney + "figure of eight" suture).
Tab. 1. Ektopická aktivita (% ektopických porúch z celkového počtu systol) v intervale 30 sekúnd po podaní infúzie. Potkany s etablovanou renovaskulárnou hypertenziou (RVH) 4 týždne po operačnom výkone (solitárna oblička + „sutúra").

Experimental code Kód experimentu	Ectopic activity Ektopická aktivita (EA %)	Cardiac rhythm Rytmus srdca
RVH12/05/98	10,1	ST
RVH14/05/98	68,4	VT
RVH22/05/98	25,8	SR
RVH22/07/98	15,1	SR
RVH23/07/98	27,3	SR
Mean±SEM Priemer±stredná chyba	37,2±11 (p<0,05)	

ST – simus tachycardia (sínudová tachykardia)
VT – ventricular tachycardia (ventrikulárna tachykardia)
SR – sinus rhythm (sínusový rytmus)

Specific binding of [^{125}I]PD151242 on renal sections

The results of saturation binding experiments with on sections of renal papillae are summarized in Fig. 7. The characteristics of specific binding of [^{125}I]PD151242 in control sections showed the high affinity specific binding to one type of specific bindig sites with B_{max}=755±171 dpm/mg w.w. and a reasonably low K_D=0.67±0.29 nmol/l. The more than two times higher were the values of density of ET_A receptors obtained in sections isolated from RVH rats with established form of renal hypertension (B_{max}=1515±263 dpm/mg w.w., p<0.01) at unchanged afinity (K_D=0.42±0.17).

Discussion

The results obtained in this study clearly document significant increase in endogenous peptide endothelin-1 in plasma, increased plasmatic renin activity, increased total number of specific endothelin ET_A receptors in renal papillary sections, as well as significantly higher reactivity of systemic arterial blood pressure

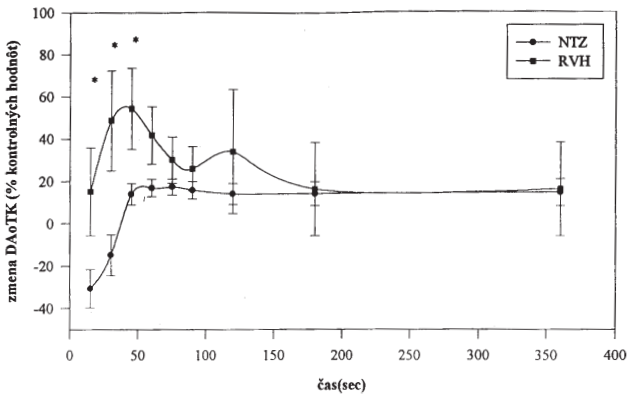

Fig. 4. Effects of angiotensin-II (20 μg/kg/30 s) on DAoBP (n=12).
Obr. 4. Účinky angiotenzínu-II (20 μg/kg/30 s) na DAoTK (n=12).

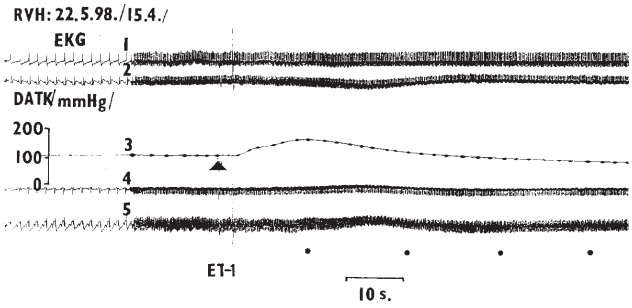

Fig. 5. An example of the response in Goldblatt renal-hypertensive (RVH) rat with one kidey, one suture (IK, IL), 4 weeks after induction of hypertension to intravenous infusion 100 ng/kg/30 s of human/porcine endothelin-1 (ET-1). Recording 2.5 mm/s. Code indicate the date of the experiment with measurements of hemodynamic parameters, arterial blood pressure, heart rate and standart leads of ECG, in parenthesis is the date of G/G procedure inducing hypertension.
Obr. 5. Príklad odpovede potkana s osmičkovou sutúrou na solitárnej obličke a renálnou hypertenziou (RVH, 4 týždne po indukcii renálnej hypertenzie). Kód značí deň merania hemodynamických veličín, arteriálneho tlaku a tepovej frekvencie, spolu so štandardnými zvodmi EKG, ďalší údaj (15.4.) značí dátum operačného výkonu) na intravenóznu infúziu 100 ng/kg/30 s humánneho/porcinného endotelínu-1 (ET-1). Pozn.: Krátke trvanie presorického účinku ET-1. Záznam rýchlosťou 2,5 mm/s.

to sympathomimetic amines, and peptides angiotensin and endothhelin-1 manifested on experimental model of renal-hypertensive rats, representing new findings on the Goldblatt type of renal hypertension. Data on the increased reactivity of systemic arterial circulation of RVH rats in our study correspond well with the results of our previous studies (18) and with findings of increased plasmatic levels of norepinephrine and increased density of α1 adrenergic receptors in the arterial wall of great arteries documented in a similar experimental model seven days after induction of hypertension (19). In accordance with these findings our results documented significantly higher maxima and also longer duration of presser effect of norepinephrine in the group of RVH rats 4 weeks after establishment of renal hypertension. Simply, from the standpoint of increased occurence of extrasystolia this response of RVH rats can be characterized as being markedly sensitive to sympathomimetic amines. Undoubtely, the relative short occurence of extrasystolia after infusion of noradrenaline in the present study, is connected also with significant changes in hemodynamics in this group of experiments, however, the disturbance did not represent limiting factor for the evaluation of diastolic arterial blood pressure, since the arterial blood pressure after norepinephrine administration increased considerably slower as the immediate appearance and duration of ectopic disturbances. Invariably important are our findings of reduced responsiveness of diastolic arterial blood pressure to acetylcholine in RVH rats clearly documented in our study, suggesting significant inhibition of production of nitric oxide in RVH rats 4 weeks after induction of renal hypertension. A relatively short duration of presser action after infusion of endothelin in rat vasculature support the view of an intensive degradation of endothelin peptides presumably by neutral endopeptidases and carboxypeptidases after the first passage by pulmonary and portal circulation. Similar mechanism was reported also in human tissues (20).

Renal injury and analysis of vasoconstrictive endothelin-ET_A receptor subtype

The progressively increased total peripheral resistance in established renal-hypertension in Goldblatt rats induced by ligature on solitary kidney in our experiments is produced by congestion of renal parenchyma and leads to marked reduction of glomerular filtration. Chronic hemodynamic and humoral alterations which come out in RVH rats in the phase of chronic high blood pressure lead to inicipient heart failure. Solitary kidney with the "figure of eight" ligature most probably is not capable to excrete quanta of sodium and fluids with the resultant heart failure. Our measurements of vasoconstrictive ET_A subtype of endothelin receptors in RHR rats were realized exactly in this phase of disturbances. The [^{125}I]PD151242 is a selective endothelin-1 ligand, the endothelin ET_A receptor antagonist (with the index of afinity approximately 1 nmol/l), binding exclusively and with high affinity to the ET_A subtype of endothelin receptors (21).After having published several papers with this ligand we believe that [^{125}I]PD151242 is ligand suitable for identification this subtype of endothelin receptors.Even though, the significant increase in the specific binding of [^{125}I]PD151242 in segments of renal papillae observed in the present study is not possible to score exclusively to renal-canalicular, endothelial or to vascular smooth muscle-vasoconstrictor component. According to the present state of knowledge of endothelin peptides and endothelin receptor classification there is ET_{B1} endothelin receptor present on endothelial and endothelial-like type (endocardial layer) of heart cells, the ET_{B2} subtype is expressed mostly on vascular smooth muscle cells, and mediate contraction (22). Significant increase in specific binding of ET_A selective peptidic [^{125}I]PD151242 observed in the present study suggests the magnification of vascular vasoconstrictive component. When considering that in vascular endothelial layer are expressed mostly endothelin ET_{B1} receptors, then significant increase in ET_A receptors in renal sections in our experiments with RVH rats ref-

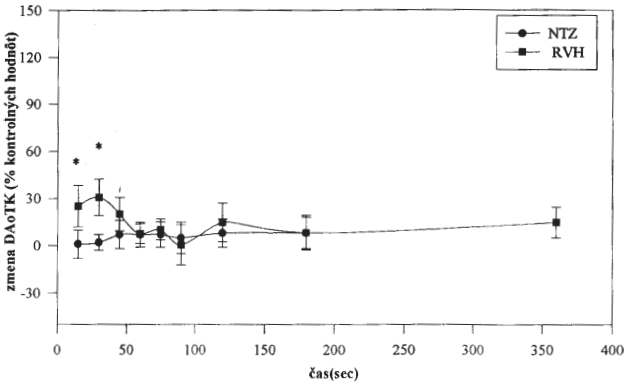

Fig. 6. Effect of intravenous infusion of endothelin-1 (100 ng/kg/30 s) on diastolic arterial blood pressure (n=12). Explanation and symbols as in the preceding figs. Note short duration of presser action of infused ET-1 indicate either an effective degradation of the peptide in tissues, or its effective binding. The notion of quasi irreversible binding of endothelin-1 in vascular smooth muscle seems to be fallacious.
Obr. 6. Účinky infúzie endotelínu-1 (100 ng/kg/30 s) na diastolický arteriálny tlak (n=12). Symboly a objasnenie ako v predchádzajúcom obrázku. Krátke trvanie presorického účinku ET-1.

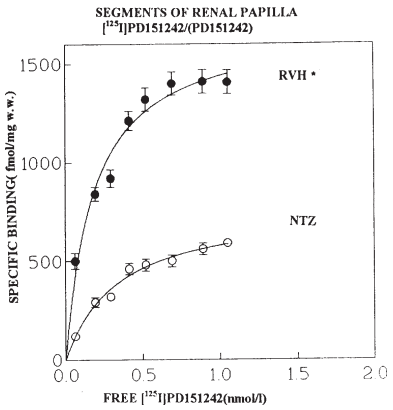

Fig. 7. The comparison of the saturation binding isotherms obtained in experiments with renal canalicular sections (renal papillae, see Methods) and the endothelin-ET_A selective peptide-type ligand [^{125}I]PD151242 in normotensive (NTZ) and in renal hypertensive (RVH) rats (n=12). Free radioligand concentrations (nmol/l) are on X axis and specific binding (in fmol/mg w.w.) is on the Y axis. Note the significant increase in the B_{maX} (the maximal density of ET_A specific binding sites) in papillary sections isolated from RVH rat hypertensive kidneys.
Obr. 7. Porovnanie saturačných izotermických kriviek získaných v saturovaných rádioligandových štúdiách s renálnymi rezmi (papilou obličky) a s ET_A-selektívnym rádioligandom peptidického typu [125I]-PD151242 u normotenzívnych (NTZ) a renálne hypertenzívnych potkanov (n=12). Na osi X sú koncentrácie voľného rádioligandu (nmol/l), na osi Y špecifická väzba (fmol/mg v/v). Výynamné zvýšenie celkového počtu špecifických miest väzby (B_{max}).

lects the preference of vasopresor subtype of endothelin receptors of canalicular or most probably vascular elements. Whether the increase in ET_A endothelin receptors in Goldblatt rats is conditioned by generalized expression of mRNA coding this subtype of endothelin receptors was not proven yet. This is an attractive "working hypothesis" and aim of our future experiments. If the relevance of this regularity would be proven then it would be possible to generalize the interconnection of subtype-ET_B receptor down-regulation with the expression of endothelin-ET_A receptors as malignant mediators of renal disease. Similarly, also in other experimental models of hypertension conditioned by increased admission of salt, as it is in the case of DOCA-rats, or in Dahl-sensitive rats (23) and with heavy hypertension in stroke-prone rats (24) is endothelin increased especially in the vascular wall, and partially also in endothelium. In conclusion, following our results with the renal-hypertensive rats the exogenous endothelin-1, norepinephrine as well as angiotensin-II induced significantly higher response of diastolic arterial blood pressure in RVH rats, and in correlation with increased responsiveness there is our finding of significantly higher density of endothelin ET_A subtype of receptors in papillary sections of solitary kidneys of renal hypertensive rats. In this connection, assuming that increased reactivity is result of ET_A receptor predominance than also preference of vasoconstrictive response in RVH rats is the result of this predominance. The reduction in vascular reactivity to acetylcholine infusion may reflect an alteration in the proportion of total number of ET_A and ET_B receptors and the ET_A preference. It is very plausible that the preference of ET_A receptors and malignant increase in arterial blod pressure cohere with the suppression of endothelial function and inhibition of production of nitric oxide, mediator of reactive vasodilatation in systemic circulation.

Conclusion

The response of rats with chronic, malignant renal hypertension to exogenous acetylcholin, sympathomimetic amines and peptides angiotensin-II and endothelin-1 and also increased levels of vasopressor peptides in plasma of Goldblatt rats, as well as increased density of pressor endothelin-ET_A receptor subtype in solitary kidney of rats with malignant hypertension, clearly documented in this study suggest that endogenous peptide endothelin-1 could be the pathological modulator of renovascular hypertension. Quo ruit et lethum...*

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