Tonic action of endothelin type B and dopamine D3 receptors in SHR and DOCA hypertensive rats: effects of intrarenally applied selective antagonists

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Tonic action of endothelin type B and dopamine D3 receptors in SHR and DOCA hypertensive rats: effects of intrarenally applied selective antagonists

Short title: Intrarenal blockade of endothelin type B and dopamine D3 receptors

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Abstract

Endothelins and renal dopamine contribute to control of renal function and arterial pressure in health and various forms of experimental hypertension, the action is mediated by tonic activity of specific receptors. We determined the action mediated by endothelin type B and dopamine D3 receptors (ETB-R, D3-R) in anesthetized spontaneously hypertensive (SHR) and in DOCA-salt hypertensive rats. In rats of both hypertension models infused during 60 min into the interstitium of in situ kidney were either ETB-R antagonist, BQ788 (0.67 mg kg\(^{-1}\) h\(^{-1}\)) or D3-R antagonist, GR169031 (0.2 mg kg\(^{-1}\) h\(^{-1}\)). Arterial pressure (MAP), renal artery blood flow (RBF, Transonic probe) and renal medullary blood flow (MBF, laser-Doppler) were measured along with sodium, water and total solute excretion (U\(_{Na}\)V, V, U\(_{osm}\)V). Experiments with ETB-R blockade confirmed their tonic vasodilator action in the whole kidney (RBF) and medulla (MBF) in both hypertension models. In SHR only, the first evidence was provided that ETB-R specifically increases transtubular backflux of non-electrolyte solutes, such as urea. In DOCA-salt rats ETB-R blockade caused an early decrease in water and salt transport whereas an increase was often reported from many previous studies. The most striking effect of D3-R blockade in SHR was a selective increase in MBF, which strongly suggested tonic vasoconstrictor action of these receptors in the renal medulla; this speaks against prevailing opinion that D3 receptors are virtually inactive in SHR. In our model variant of DOCA-salt rats D3-R blockade clearly caused a rapid major increase in MAP in parallel with depression of renal haemodynamics

Key-words: arterial hypertension; dopamine D3 receptors; endothelin B receptors; renal excretion; renal haemodynamics; rat hypertension
Introduction

Extensive research of the past four decades clearly established that beside the sympathetic nervous system (SNS) and the renin-angiotensin system (RAS), two other humoral systems importantly contribute to the renal control of body fluid status and arterial pressure in health and various forms of hypertension: endothelins, especially endothelin-1 (ET-1), and renal dopamine (dopaminergic) system. Both control body homeostasis via receptors located in many organs and tissues, especially in the kidney where their activity alters tubular transport of salt and water and the tone of resistance vessels. For endothelin two types of receptors were described and characterized: ETA and ETB. For dopamine two classes of receptors are distinguished: D₁-like (D₁ and D₃) and D₂-like (D₂, D₃, D₄).

The past two decades have brought considerable intensification of the research of all aspects of function of ETB and D₂-like (especially D₃ subtype) receptors at all levels (classical functional, molecular, clinical, genetic etc.) [1, 4, 6, 10, 21, 34, 37]. More recently, much interest is given to interaction of ETB and D₃ receptors, especially in the kidney [36, 38]. The progress in knowledge was possible owing to the development of selective receptor agonists and antagonists. However, in vivo studies in normotensive and hypertensive animals, especially using experiments performed on the in situ kidney, were relatively few in number. This, in our opinion, results in some uncertainties and even gaps of knowledge, especially regarding the tonic action of ETB and D₃ receptors in different forms of arterial hypertension. It should be emphasized that the tonic influence cannot be proved just by showing that a receptor antagonist abolishes the effect of the administered agonist: this is relevant to pharmacologic rather than physiological or pathophysiological aspects of the action.

The aim of the present study was to provide robust information on the tonic influence of ETB and D₃ receptors in vivo and in the in situ kidney. In the studies focused on the role of the receptors located in the kidney, to avoid systemic effects receptor agonists and antagonists were often infused to the renal artery [36]. However, since a part of receptors in question are located on the surface of kidney cells we decided to deliver the receptor antagonists to the renal interstitium. Furthermore, since many findings related to the presence and function of ETB and D₃ receptors indicate their important role in the renal medulla, best documented for the former receptor species [11, 14, 20, 25, 31], we decided to deliver the blockers to its outer layer, yet being aware that substantial amount of the agent would diffuse both to the inner medulla and the cortex, so that information would be provided on the changes throughout the kidney.
Considering the potential translational value of the information on the exact role the endothelin and the renal dopamine system in arterial hypertension we wished to compare effects of ETB and D3 receptor blockade in two different rat models: on one side a rather mild form of DOCA-salt hypertension, with the features of body sodium retention and inhibition of renin release and, on the other side, genetically determined pronounced hypertension (SHR) with the known complex pathogenetic background. Regarding the endothelin system, the vascular ET-1 gene expression is enhanced in the former but normal or lowered in the latter [30]. There is evidence of increased dopamine synthesis in SHR [1], and D1 receptor expression is greatly increased, especially in the renal medulla [29] however, D3 receptors appear inactive [24, 33, 34, 36]. On the other hand, in the DOCA-salt model, renal dopamine synthesis is lowered and the dopamine system is “dominantly suppressed” [6, 18]. Inhibition of the receptors was done by intramedullary infusion of established selective antagonists: a peptide BQ788 for endothelin ETB subtype [19] and a non-peptide GR103691 [2] for dopamine D3 receptors. The study was designed to examine early effects of the blockade so the duration of blocker infusion was only one hour, preceded and followed by control and recovery observations.

**Material & Methods**

*Ethical approval.* The procedures used in experimental rats were approved by the extramural Second Local Ethical Committee for Animal Experimentation, Warsaw (WAW2/121/2021) and conform with EU legislation regarding ethical aspects of animal experimentation.

The rats were derived from animal house of the Mossakowski Medical Research Institute, Polish Academy of Sciences, Warsaw.

Studies were performed using two models of rat hypertension:

1. Male spontaneously hypertensive rats (SHR) aged 10-12 weeks, with established hypertension, weighing 270-320 g, maintained on standard sodium diet (0.25% Na⁺ w/w, SSNIFF, GmbH, Soest, Germany), with free access to water.
2. DOCA-salt hypertension induced in male Sprague-Dawley rats aged 12-13 weeks, weighing 280-340 g. Three weeks before proper experiments the rats’ drinking water was replaced with 1% NaCl solution and the pellets containing deoxycorticosterone acetate (DOCA, 50 mg/pellet, Innovative Research of America, Sarasota, USA) were implanted subcutaneously under inhalation anaesthesia with isofluran/O₂ mixture. The pellets were designed to release DOCA over 21 days.
The aim of the study was to examine how administration of a selective endothelin-1 ETB receptor inhibitor (BQ 788, Santa Cruz Biotechnoloy, Inc., Dallas, Texas, USA) or dopamine D3 receptor inhibitor (GR 103697, MedChemExpress EU, Sollentuna, Sweden), infused directly to the renal medulla in hypertensive rats will affect blood pressure, heart rate, and renal hemodynamics and excretion.

**Surgical preparation**

Experiments were performed under thiopental sodium anaesthesia (Samarth, Lodhimajra, Baddi, India), 100 mg kg\(^{-1}\) i.p. which provided stable anaesthesia for at least four hours, with additional small doses when needed. On the day of the experiment all the rats first underwent right-side nephrectomy. Subsequently they were placed on the servo-controlled heated surgery table to maintain rectal temperature at about 37 °C. A polyethylene tube was placed in the trachea to ensure free airways. The jugular vein was cannulated for infusion of fluids: during surgery, to maintain plasma volume, fluid losses were compensated by infusion of 3% bovine albumin in Ringer’s solution at 3 ml h\(^{-1}\). With surgery completed, this infusion was replaced by 0.9% NaCl maintained until the end of the experiment.

For measurement of mean arterial pressure (MAP) and heart rate (HR), a Teflon catheter was introduced into the left femoral artery and connected with a Stoelting blood pressure meter (Stoelting, Wood Dale, IL, USA).

The left kidney was exposed from a subcostal flank incision and immobilized in a plastic holder; the ureter was cannulated for timed urine collection to measure urine flow (V), urine osmolality (U\(_{\text{osm}}\)) and sodium concentration (U\(_{\text{Na}}\)), for further calculation of sodium and total solute excretion (U\(_{\text{NaV}}\), U\(_{\text{osmV}}\)). In one group of rats urine concentration of sodium and attendant ions (U\(_{2\text{Na}}\)) and of estimated concentration of urea (U\(_{\text{eUr}}\)) were calculated.

Renal artery blood flow was measured with a cuff probe connected with a Transonic flowmeter (Type T106, Transonic System Inc. Ithaca, NY, USA). Blood perfusion of the renal medulla (MBF) was measured as laser-Doppler flux using Periflux 4001 system (Perimed, Jarfalla, Sweden). A 32-gauge stainless steel cannula, connected with an infusion pump, was inserted into the kidney, with the tip located in the outer medulla layer.

**Protocols**

Similar procedures and basic protocol were used with two rat hypertension models. With the surgery completed, about one hour was allowed for equilibration of the haemodynamic and urine excretion parameters measured.

**Intramedullary BQ788 infusion in SHR and DOCA-salt groups**
During a 30-min control period an intramedullary infusion of 0.9% NaCl (BQ788 solvent) was given at volume rate of 0.5 ml h\(^{-1}\) for control haemodynamic measurements and urine collection. Subsequently, for two 30-min experimental periods the solvent infusion was replaced by BQ788 solution given at 0.67 mg kg\(^{-1}\) h\(^{-1}\). This dosage was established in preliminary dose-response studies and found to reproducibly alter all the parameters measured, including mean arterial pressure (MAP). However, it was found that a slightly lower dose (0.21 mg kg\(^{-1}\) h\(^{-1}\)) induced similar changes but without altering MAP; therefore an additional group was created and analyzed separately. After two experimental (BQ788) periods the solvent infusion was restored for the final 30 minutes of recovery measurements, the blocker was replaced by solvent infusion and recovery measurements and urine collections were made over a 30-min period. Control monitoring of all haemodynamic parameters (MAP, HR, RBF, MBF) was conducted throughout the experiment.

In parallel time control experiments BQ788 solvent was infused throughout the experiment with measurements performed as usual.

**Intramedullary GR103697 infusion in SHR and DOCA-salt groups**

The solvent for GR103697 was dimethyl sulfoxide (DMSO, P.P.H. “STANLAB” Sp. J. Lublin, Poland), and this was the reason why the protocols for the study of D3 receptor blocker were not exactly the same as those used for BQ788. DMSO was reported to alter per se some functional features, especially the microvascular tone [3, 27]. Therefore in control (C) 30-min measurement and urine collection period 0.9% saline was infused into the medulla at 0.5 ml per hour. Subsequently, this infusion was replaced for two 30-min periods of GR103691, given at 0.2 mg kg\(^{-1}\) h\(^{-1}\) or by its solvent (3% DMSO), at 0.5 ml/h. Thereafter, either infusion was followed by a 30-min isotonic saline in the recovery period (R).

At the end of each experiment, the kidney was removed, weighed and the rats were killed with an overdose of thiopental. The location of the tip of the laser-Doppler probe and of the infusion needle in the medulla was checked at the kidney cross section.

**Analytical procedures**

Urine volume was determined gravimetrically. The freezing point depression method (Osmomat® 030 M, Gonotec, Berlin, Germany) was used to measure urine osmolality. Urinary sodium concentration was determined by flame photometry (BWB-XP, BWB Technologies Ltd, Newbury, United Kingdom). A commercially available kit was used to assess the urinary
levels of nitric oxide metabolites (Nitrate/Nitrite Colorimetric Assay Kit, cat: 780,001, Cayman Chemical, Michigan, United States).

**Statistical analysis**

All values are expressed as means ± SEM. Significance of changes within one group over time was first evaluated by repeated measures analysis of variance (ANOVA) followed by modified Student’s t test for dependent variables, using Bonferroni correction for multiple comparison. Differences between profiles for GR103697 and solvent infusion studies (DMSO) were first analysed by repeated measures multivariate ANOVA, followed by Duncan’s test (STATISTICA 10.0, StatSoft Polska Inc.). The values exceeding the 95% probability limits (P < 0.05) were considered statistically significant.

**Results**

**Effects of intramedullary BQ788 in SHR**

The basic data for intramedullary blockade of ETB receptors in SHR are shown in Fig. 1. Baseline MAP was slightly below 190 mmHg and modestly increased (about 6 mmHg) after BQ 788, with partial recovery after cessation of blocker infusion. Simultaneously, there was a modest and delayed decrease in heart rate from 358±12 to 346±8 beats min⁻¹ (data not shown). A change pattern inverse to that was seen for RBF and MBF which decreased about 1.3 ml min⁻¹g⁻¹ KW (-18%) and 45 PU (-24%), respectively, again, with a partial post-infusion recovery.
Fig. 1 Effects of intramedullary blockade of ETB receptors with BQ788 on mean arterial pressure, renal haemodynamics and excretion parameters in spontaneously hypertensive rats (SHR).

MAP, mean arterial pressure; RBF, renal total blood flow; MBF, renal medullary perfusion; V, excretion of water; U_{NaV}, total sodium excretion; U_{osmV} total solute excretion.

Means ± SEM, black symbols on the curves (n = 7-8) represent the values in BQ788 infusion experiments and blank symbols (n = 5-10) the values in time-control studies.

*Significantly different from control at \( P < 0.05 \) or less; † significantly different from the second period of blocker infusion at \( P < 0.05 \) or less.

The changes in renal excretion parameters were not uniform: while \( V \) and \( U_{NaV} \) were not significantly affected by ETB blockade, total solute excretion (\( U_{osmV} \)) increased significantly. A puzzling increase in \( V \) and \( U_{NaV} \) (but not in \( U_{osmV} \)) was seen in the post-infusion period.

Fig. 1 presents the responses to the BQ788 dose of 0.67 mg kg\(^{-1}\)h\(^{-1}\) which was found to affect all the parameters measured, including a significant increase in MAP. Since MAP changes per
se are likely to affect renal haemodynamics and excretion, we additionally assessed the effects of a lower dose (0.21 mg kg\(^{-1}\)h\(^{-1}\)) which had been found to affect renal haemodynamics and excretion without significantly altering MAP. A comparison of the response to the high and low BQ788 dose are shown in Fig. 2. It is seen that in the absence of MAP increase after the low dose, the decreases in RBF and MBF were similar as with the high dose, as was also the case with the increase in U_{osm}V. Neither high nor low dose caused significant changes in V and U_{Na}V.

Fig. 2 A comparison of the effects of the high dose (0.67 mg kg\(^{-1}\)h\(^{-1}\), striped columns, n=8) and low dose (0.21 mg kg\(^{-1}\)h\(^{-1}\), blank columns, n=5) of intramedullary BQ788 infusion on MAP, renal haemodynamics (RBF, MBF) and renal excretion parameters (V, U_{Na}V, U_{osm}V) in SHR. Denotations as in Fig. 1. The values (mean Δ ± SEM ) are increases from control to the second period of blocker infusion. *Significantly different from control at \(P < 0.05\) or less.

The pattern of the observed changes in renal excretion was in some aspects surprising. First, BQ788 infusion significantly increased total solute excretion (U_{osm}V) without changing urine flow or sodium excretion (V, U_{Na}V). Another puzzling finding was that after withdrawal of the receptor blockade there were significant increases in V and U_{Na}V but not U_{osm}V (Fig. 1). These phenomena are later presented and analysed in more detail in Table 1 (see Discussion).

Fig. 3 (upper panel) shows that ETB blockade with BQ788 did not alter nitrate/nitrite excretion
(\(U_{\text{NOx}}V\)) in SHR while a significant increase occurred in the recovery period, concurrent with the rise in V and \(U_{\text{Na}}V\) (see Fig. 1).

**Fig. 3** Effect of intramedullary blockade of ETB receptors with BQ 788 in SHR and DOCA-salt hypertensive rats \((n = 5)\) on nitrite/nitrate excretion \((U_{\text{NOx}}V)\).

† significantly different from the second period of blocker infusion at \(P < 0.05\) or less;

# significant difference between profiles at \(P < 0.026\) (multivariable ANOVA).

**Effects of Intramedullary BQ788 in DOCA-salt hypertensive rats**

The basic data for intramedullary blockade of ET B receptors in DOCA-salt rats are shown in Fig. 4. Baseline MAP was slightly below 150 mmHg and did not change after BQ788. There was a significant but very modest decrease in heart rate from 337±9 to 306±8 beats min\(^{-1}\) (not shown in the figure). Mean RBF and MBF decreased after the blockade (25±2.5% and 26±6% respectively); a partial recovery was seen for the latter only.

All parameters of renal excretion, V, \(U_{\text{Na}}V\) and \(U_{\text{osm}}V\), progressively and significantly increased after BQ788; there was no recovery after cessation of blocker infusion. In time-
control experiments, during the infusion of BQ788 solvent (isotonic saline) all the parameters measured were stable.

**Fig. 4** Effects of intramedullary blockade of ETB receptors with BQ788 on mean arterial pressure, renal haemodynamics and excretion parameters in DOCA-salt hypertensive rats.

Denotations as in Fig. 1. Means ± SEM, black symbols on the curves (n = 5-7) represent the values in BQ788 infusion experiments and blank symbols (n = 5) the values in time-control studies. *Significantly different from control at \( P < 0.05 \) or less; †significantly different from the second period of blocker infusion at \( P < 0.05 \) or less.

Fig. 3 (lower panel) shows that the blockade did not significantly alter \( U_{\text{NaV}} \), however, in the recovery period a significant increase was seen. This resembled the pattern seen in SHR (upper panel), however, unlike in SHR, it was not associated with any recovery increase in \( V \) or \( U_{\text{NaV}} \) (see Fig. 4).
Effects of intramedullary GR103691 in SHR

In SHR intramedullary dopamine D3 receptor blockade with GR103691 decreased MAP modestly (5.1±2%) but significantly. Simultaneously mean RBF decreased 8.2±2% whereas MBF changed in the opposite direction: it increased substantially (16.7±7%), which was followed by a partial recovery after cessation of blocker infusion yet within the first 30 min MBF was still significantly below the pre-infusion control level.

In the assessment of the GR103697-induced changes in renal excretion one has to consider that during time control infusion of the blocker solvent (DMSO) the curves for V, U\textsubscript{Na}V and U\textsubscript{osm}V at least tended to increase throughout experiment (Fig. 5).

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**Fig. 5** Effects of intramedullary blockade of D3 receptors with GR103697 on mean arterial pressure, renal haemodynamics and excretion parameters in SHR

Denotations as in Fig. 1. Means ± SEM, black symbols on the curves (n = 5) represent the values in GR103697 infusion experiments and blank symbols (n = 5) the values in DMSO.
As shown by the multivariable ANOVA, the three excretion curves significantly differed from the respective curves for GR103697 infusion (at the degree of freedom of 3, F values were 3.92, 8.58 and 6.62, with P values 0.017, 0.00037 and 0.0013, respectively). Moreover, during intramedullary DMSO infusion the total solute excretion (UosmV), but not V or UNaV, increased significantly. Another puzzling finding of DMSO experiments was a significant increase in V, UNaV and UosmV after cessation of the solvent infusion; no such “recovery” was observed after cessation of GR103697 infusion.

Effects of intramedullary GR103691 in DOCA-salt rats

In DOCA-salt rats intramedullary blockade of D3 receptors caused a rapid pronounced (20±4%) increase in MAP, with a significant but only partial recovery within 30 min from withdrawal of blocker infusion (Fig. 6). Simultaneously, there was a 40±9% decrease in RBF and 25±5% decrease in MBF, again, followed by a partial recovery. Given the decreasing renal perfusion (RBF) concurrent with increasing renal perfusion pressure (known to be almost equal to MAP), one can calculate that the renal vascular resistance (RVR) increased substantially from control of 10.4±0.6 to 15.4±2.0 mmHg ml min⁻¹ in the second GR103697 infusion period (P < 0.03). After administration of GR103691 the mean values of all parameters of renal excretion (V, UNaV, UosmV) increased rapidly; remarkably, the initial changes were highly variable, then, in the second 30-min period of infusion they stabilized and became significant. In time control experiments, during infusion of the blocker solvent (DMSO), all the haemodynamic and excretion parameters were stable, unlike the situation observed in SHR (see above).
Fig. 6 Effects of intramedullary blockade of D3 receptors with GR103697 on mean arterial pressure, renal haemodynamics and excretion parameters in DOCA-salt hypertensive rats

Denotation as in Fig. 1. Means ± SEM, black symbols on the curves (n = 5) represent the values in GR103697 infusion experiments and blank symbols (n = 5) the values in DMSO (GR103697 solvent). *Significantly different from control at $P < 0.05$ or less; †significantly different from the second period of blocker infusion control at $P < 0.05$ or less.
Discussion

ETB receptor blockade

Effects on MAP and renal haemodynamics in SHR and DOCA-salt hypertensive rats

After ETB blockade blood pressure increased in SHR whereas it remained stable in DOCA-salt rats. For comparison, in an early study intravenous administration of Ro 46-8443, a non-peptide ETB antagonist, increased blood pressure in either hypertension model, evidently due to elimination of vasodilator action of NO [8]. We found that in both models the prominent effect of the blockade was a simultaneous decrease in total renal (i.e. mostly cortical) and in medullary blood flow. The observation of such a parallel change does not support the view that while ETA receptors control renal cortical perfusion, ETB activity is important for regulation of blood flow through the renal medulla [21]. In anaesthetized rabbits BQ788 decreased RBF but did not change MBF [12]. The observation that in our SHR renal haemodynamics (both MBF and RBF) decreased also in the absence of a fall in MAP, strongly suggests that the action of the blocker was confined to the kidney and the fall in renal haemodynamics did not simple reflect systemic vasoconstriction. The pattern of changes suggested that BQ788 leaked to some extent from the outer medulla to the cortex but not in any significant amount to systemic circulation. The decrease in renal perfusion after BQ788 indicates tonic vasodilator action of ETB located in the renal vascular endothelium, in accordance with the vast evidence from experimental studies using diverse experimental models [10]. Evidently, this action prevailed over any direct vasoconstrictor influence of ETB located on the vascular smooth muscle of renal vessels [21]. However, since ETB have also an established role as clearance receptors [13, 26] another mechanism of post-blockade vasoconstriction effect could be the release of additional endothelin from ETB and increased activation of ETA receptors.

The effect of ETB blockade on the renal haemodynamics appeared roughly similar in SHR and DOCA-salt rats, even though more pronounced action could be expected in the latter in which the intrarenal synthesis of ET-1 is known to be elevated [17] and ETB were shown to be upregulated [15, 26].

The renal microvascular dilation related to endogenous ET-1 is mostly ascribed to the ETB-mediated release from the endothelium of nitric oxide (NO) [21]. Therefore, the blockade of ETB receptors should reduce the local NO bioavailability and probably also the excretion of NO metabolites. However, their excretion (U\textsubscript{NOx}V) did not decrease after BQ788, similarly in SHR and DOCA-salt rats (Fig. 3). Possibly, the post-blockade renal vasoconstriction was due
to elimination of some other endogenous ETB-dependent mediator of vasodilation; the role of prostanoids has long been here considered [7]. A puzzling observation was the “recovery” increase in $U_{NOx}V$ in SHR and DOCA-salt rats after withdrawal of the blocker infusion. This was not simply related to the concurrent rise in diuresis because $V$ increased in SHR but not in DOCA-salt rats; the reason for the final “recovery” $U_{NOx}V$ increase is unclear.

To summarise, evidence from most studies with normotensive, salt-loaded and hypertensive rats indicates that endogenous endothelin exerts a tonic vasodilatory influence on the renal microcirculation that is mediated by ETB receptors [reviewed in 10, 21]. This conclusion has come from in vitro and in vivo studies using diverse experimental preparations and cannot always be regarded as direct. We supplement this evidence in a more direct way, by selective inhibition of ETB in two models of hypertension, wherein the ETB antagonist was delivered to the renal interstitium.

It will be noticed that our demonstration of a decrease in renal haemodynamics after intramedullary blockade of ETB receptors in SHR and DOCA-salt rats is in contrast to no effect of such blockade accomplished also using intramedullary delivery but using another ETB antagonist, A192621, as reported by Guo & Yang [14] in normotensive volume-expanded rats. However, given the limited evidence of the in vivo selectivity of the blocker used by Guo & Yang [14] and earlier Wessale et al., [32], and the absence of time-control experiments in their study, their results should be interpreted with caution.

**Effects on renal excretion in SHR and DOCA-salt rats**

In SHR there is sound evidence that endothelin, generated in abundance in the collecting duct (CD), inhibits local reabsorption of sodium and vasopressin-dependent transport of water via stimulation of ETB receptors, the subtype prevailing in this tubule segment [11, 21]. Consequently, intramedullary BQ788 administration would be expected to reduce $V$ and $U_{Na}V$. Antidiuresis but not clear antinatriuresis was reported from the already quoted study after intramedullary administration of A192621 (another ETB antagonist) to volume expanded Sprague-Dawley rats [14]. Nor did renal artery infusion of BQ788 alter $U_{Na}V$ in normotensive rats [36]. In our study with ETB-R blockade neither $V$ nor $U_{Na}V$ changed significantly in SHR whereas in DOCA-salt rats a modest increase was seen (see below).

Surprisingly, in our SHR the absence of change in $V$ and $U_{Na}V$ was in contrast to a clear rise in urine osmolality ($U_{osm}$) and total solute excretion ($U_{osm}V$). (Fig. 1). It is reminded that a rise in $U_{osm}$ was also observed after A192621 by Guo & Yang [14]: this was perhaps the first signal
that *in vivo* elimination of renal medullary ETB activity might differently affect renal handling of sodium and attendant anions compared to the tubular transport of water and urea. An unusual pattern of the response of urine concentration and non-electrolyte solute excretion in our SHR is a novel finding and deserves a detailed analysis. In Table 1 the data for urine osmolality (*U*_osm), urine concentration of sodium plus attendant anions (*U*_2Na), and estimated (not measured) urine concentration of urea (*U*_eUr) is shown aside the excretion rate parameters (*U*_osm*V*, *U*_2Na*V*, *U*_eUr*V*). *U*_eUr is calculated as *U*_osm - *U*_2Na.

**Table 1** Effects of BQ788 treatment and its withdrawal on the parameters describing renal excretion and urine concentration in SHR.

<table>
<thead>
<tr>
<th></th>
<th>C</th>
<th>BQ 788</th>
<th>Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>V</em> µl·min⁻¹·g⁻¹·KW</td>
<td>11.8 ± 1.8</td>
<td>11.5 ± 1.1</td>
<td>18.0 ± 1.2*</td>
</tr>
<tr>
<td><em>U</em>_osm mmol·L⁻¹</td>
<td>829 ± 91</td>
<td>1102 ± 109*</td>
<td>732 ± 55*</td>
</tr>
<tr>
<td><em>U</em>_osm<em>V</em> µmol·min⁻¹·g⁻¹·KW</td>
<td>9.5 ± 0.8</td>
<td>15.8 ± 2.8*</td>
<td>16.0 ± 2.3</td>
</tr>
<tr>
<td><em>U</em>_2Na mmol·L⁻¹</td>
<td>198 ± 16</td>
<td>156 ± 16*</td>
<td>222 ± 14*</td>
</tr>
<tr>
<td><em>U</em>_2Na<em>V</em> µmol·min⁻¹·g⁻¹·KW</td>
<td>2.4 ± 0.4</td>
<td>1.8 ± 0.3</td>
<td>3.9 ± 0.4*</td>
</tr>
<tr>
<td><em>U</em>_eUr mmol·L⁻¹</td>
<td>631 ± 94</td>
<td>946 ± 108*</td>
<td>510 ± 61*</td>
</tr>
<tr>
<td><em>U</em>_eUr<em>V</em> µmol·min⁻¹·g⁻¹·KW</td>
<td>7.3 ± 0.7</td>
<td>12.8 ± 1.8*</td>
<td>10.2 ± 0.8</td>
</tr>
</tbody>
</table>

*V* – urine flow (water excretion); *U*_osm – urine osmolality; *U*_osm*V* – total solute excretion; *U*_2Na – urine concentration of sodium and attendant anions; *U*_2Na*V* – excretion of sodium and attendant anions; *U*_eUr – estimated urinary urea concentration; *U*_eUr*V* – estimated urinary urea excretion; C, BQ788, Recovery – data for the control, the second period of blocker infusion (see Fig. 1) and after cessation of blocker infusion. Mean values +/- standard error. *Significantly different from previous period, n = 7-8
In SHR after BQ788 application urine flow (V) did not change, in contrast to a distinct rise in total solute excretion (U_{osm}V), which was the result of a rise in U_{osm}, resembling the response reported in the earlier study [14]. U_{osm} increase was exclusively due to rising concentration of urea (U_{eUr}) since the electrolyte component (U_{2Na}) actually decreased; also U_{2Na}V tended to decrease. Evidently, the kidney was excreting the same volume of urine with more concentrated non-electrolyte solutes (which suggested reduced tubular backflux of urea) and with less concentrated electrolytes (suggesting their increased reabsorption). The BQ788-induced increase in U_{osm} and urea (U_{eUr}) might also suggest a primary increase in reabsorption of water. Indeed, ETB activity appeared to decrease the water-retaining vasopressin (AVP) action in the collecting duct, in agreement with the early reports suggesting involvement of endothelin system in the control of such changes [11, 14, 20, 25]. However, in the absence in our study of a decrease in V (see Table 1), a more probable explanation is decreased reabsorption of solutes, especially urea.

Increased reabsorption of electrolytes after ETB blockade suggested by a decrease in U_{2Na} is in agreement with the sound evidence that endogenous ET-1 in the collecting duct inhibits local Na^{+} reabsorption, mostly due to ETB-mediated inhibition of the transport through the epithelial Na^{+} channel (ENaC) and that depending on Na^{+}/K^{+} ATP-ase [10, 21]. Therefore, elimination of this effect with BQ788 should result in increasing electrolyte reabsorption (decreasing U_{2Na}), as observed here. Admittedly, U_{2Na}V only tended to decrease, probably the effect was obscured by simultaneous changes in water and urea transport.

After withdrawal of ETB blockade (recovery period) a major increase in urine flow (about 1.6x) and U_{2Na}V (2.2x) was seen; this was unexpected because earlier application of the blocker did not decrease these parameters. During the recovery period U_{osm}V remained at the level elevated after BQ788 administration (no recovery), arithmetically this stability was the result both of an increase in V (1.6x) and a decrease in U_{osm} (1.5x), especially in the non-electrolyte (urea) concentration (1.9x). This pattern of changes was compatible with a defect of urine concentration, and suggested a decrease in AVP effect on osmotic water permeability in the medullary collecting duct. Up to date no sound data is available on the possible effect of endogenous tubular endothelin on the transport of urea and the possible effects of ETB blockade.

Simultaneously there was a major (about 2.2x) increase in the excretion of sodium-plus-anion solutes (U_{2Na}V) which resulted from the already described increase in V (1.6x) and also in U_{2Na} (1.4x); this indicated a decrease in electrolyte reabsorption and possibly reflected a recovery after withdrawal of ETB blockade.
On the whole, our data on the effects of intramedullary blockade of ETB receptors are the first to indicate that in SHR endogenous intrarenal endothelin induces urine excretion and concentration changes that are much more complex than originally conceived, depending evidently on the interference with the mechanisms governing the tubular transport of sodium, water (such as AVP-dependent changes in osmotic water permeability), and of transtubular flux of urea.

In DOCA-salt rats ETB blockade induced almost parallel modest increases in V, U\textsubscript{Na}\textsuperscript{V} and U\textsubscript{osm}\textsuperscript{V} despite some decrease in renal haemodynamics (Fig. 4), which might suggest that ETB receptor activity tonically promoted reabsorption. This was unexpected: as established in several studies [16, 30, 31], inhibition of ETB should oppose the natriuretic effect of ET-1 under conditions of body salt surfeit, especially in DOCA-salt rats [26] and result in antidiuresis and antinatriuresis. There is no straightforward explanation for the discrepancy of our results and those reported from other groups. It can be speculated that, for some unknown reason, intramedullary route of the antagonist administration results in inhibition of the tubular salt transport. It is noteworthy that in the study using intramedullary infusion of another ETB antagonist (A192621) in volume-expanded Sprague-Dawley rats V and U\textsubscript{Na}\textsuperscript{V} only tended to decrease [14].

**D3 receptor blockade**

**D3 blockade in SHR**

After D3 blockade MAP decreased modestly, which suggested that there was some basal tonic vasopressor action of D3 receptors. Of greatest interest was the differential response to GR103697 of the renal haemodynamics: a modest fall in total renal blood flow (RBF) concurrent with a more substantial increase in medullary perfusion (MBF). Since RBF reflects mostly perfusion of the renal cortex, in most circumstances the changes are parallel: reduced cortical perfusion results in smaller inflow to the deep nephron efferent arterioles and the *vasa recta*, and leads to a decrease in MBF. Indeed, in the present study such parallel changes in RBF and MBF were seen after ETB blockade in SHR and both after ETB or D3 blockade in DOCA-salt rats.

A first obvious explanation of opposite changes in RBF and MBF would be that renal microcirculation as a whole was under tonic vasopressor influence of D3 receptors. Since the blocker reached primarily the medulla, the result was an increase in medullary perfusion; however, the cortical perfusion would then be expected either to show a smaller increase, dependent on the leakage of the blocker to the cortex or to remain unchanged: this was not the
case. It is not very likely that the decrease in RBF was due to falling arterial pressure because after withdrawal of the blockade RBF at least tended to recover whereas the pressure did not.

Alternatively, selective increase in MBF after blockade of D3 receptors could result from elimination of their tonic vasoconstrictor influence confined to the medulla. Such interpretation would be compatible with the early indirect evidence that activation of D3 receptors induced postglomerular vasoconstriction in the rat [22], however, given the concurrent decrease in cortical perfusion this conclusion would be valid only if such constriction were confined to deep nephrons.

In general, our results do not support the prevailing opinion that while dopamine synthesis is increased in SHR, the functional role of D3 receptors in this hypertension model is marginal [34, 37]. Our finding that D3 receptor activity seems to selectively depress medullary perfusion is of interest in the context of the still debated view that medullary hypoperfusion is a factor in the pathogenesis of arterial hypertension [9, 23, 28].

Our results regarding D3-R blockade on renal excretion suggested no effect but were inconclusive because in time control experiments the GR103697 solvent (DMSO) per se affected water, sodium and total solute excretion. The observation that the D3 blocker appeared to oppose the action of its solvent (DMSO) does not allow for valuable interpretation. DMSO is a widely used solvent of many active agents used in functional studies and was reported to affect some functional parameters, also in the kidney [3, 27].

**D3 blockade in DOCA-salt rats**

The most spectacular effect of intramedullary D3 receptor blockade in DOCA-salt hypertensive rats was a rapid and pronounced decrease in renal haemodynamics. This was concurrent with a distinct increase in MAP, hence renal vascular resistance increased significantly (+50%). This observation suggests very strongly that in our DOCA-salt rats the renal and systemic resistance vessels were under substantial tonic vasodilator action of dopamine-activated D3 receptors. The results of the blockade suggest that in the absence of this influence the baseline blood pressure could be expected to be about 25 mmHg higher than actually observed. The response to the blockade was apparently in disagreement with the evidence that in DOCA-salt hypertensive rats i.e. under conditions of volume expansion and low renal nerve activity D3 receptor activity results in vasoconstriction [reviewed in 1, 34, 37]. However, a review of the pertinent literature shows that D3 effect differed between normotensive (volume expanded or not) and hypertensive animals, depended on the model and
the exact model variant of hypertension, as well as on the duration of the blockade and time of observation (early or distant effects). Our findings provide a straightforward evidence that in our variant of relatively mild DOCA-salt hypertension the early effect of D3-R blockade was as described, and indicated tonic vasodilator action of these receptors, both on the renal and systemic resistance vessels.

Since GR103697 was delivered mostly to the renal medulla, the mechanism of a decrease in RBF and MBF coincident with an increase in MAP should be sought in the kidney. Renal vasoconstriction after D3 blockade in DOCA-salt rats could be related to increasing activity of the RAS which is known to be low in this model [5] perhaps in part due to the activity of D3 receptors. Their activation is known to inhibit renin release and there is also evidence that D3 inhibits the intrarenal expression of angiotensin type 1 (AT1) receptors, at least in some tubule segments [1, 34, 37]. Therefore elimination of the usual D3 receptor dependent RAS inhibition could result in vasoconstrictor effects of angiotensin II on renal and systemic resistance microvessels.

Since activation of D3 receptors, probably in synergism with D1 subtype [35] induces diuresis and natriuresis via inhibition of renal tubular transport [1, 34, 37], the expected change after GR103697 would be a fall in V and $U_{Na}V$. This decrease could be even enhanced due to decreasing renal perfusion, both total and medullary. However, in our study any such effects were most probably superseded by very significant arterial pressure elevation leading to the phenomenon of pressure diuresis and natriuresis. Therefore the ultimate result was a uniform increase in renal excretion. It is generally accepted that in DOCA-salt hypertensive rats activated D3 receptors induce blood pressure decrease via inhibition of tubular salt transport and the resultant natriuresis, which in the long run helps attenuate volume expansion. The present rapid MAP increase after GR103697 was obviously caused by another mechanism: most probably it was triggered by abolishment of the unexpected tonic vasodilator influence of D3 receptor activity. Evidently, the increase in renal excretion after D3 receptor blockade was just a secondary effect of pressure elevation.

Concluding remarks

ETB receptor blockade. In SHR and DOCA-salt rats selective ETB receptor blockade caused a parallel decrease in RBF and MBF: this is a direct in vivo support for the evidence that activation of this receptor species causes intrarenal vasodilation, however, the finding does not support the view that the cortical circulation is controlled by endothelin-A receptors. No effect of ETB blockade on urinary excretion of NO metabolites speaks against the common evidence that tonic ETB-mediated vasodilator effect of endothelin in the kidney depends on the release.
of NO.
In spite of a decrease in renal cortical and medullary blood flow, in SHR the ETB receptor blockade did not affect sodium and water excretion, however, it was followed by a clear rise in urine osmolality and total solute excretion. Detailed analysis of changes in the concentration and excretion of main urine components: water, sodium and attendant ions, and non-electrolyte solutes (mostly urea) showed for the first time that the tonic influence of ETB activity on the mentioned parameters is more complex than originally conceived: it decreased reabsorption of electrolytes but apparently increased transtubular backflux of urea (Table 1).

In experiments with DOCA-salt mildly hypertensive rats ETB blockade moderately but clearly raised water, sodium and total solute excretion (decreased reabsorption), despite some decrease in renal haemodynamics. This indicates that tonic effect of ETB receptors was an increase of water and solute reabsorption. This in vivo finding was contrary to the ample evidence from studies using diverse experimental models that ETB activity inhibits water and salt reabsorption; the obvious discrepancy deserves additional studies.

**D3 receptor blockade.** In SHR the most striking finding was that the blockade caused a distinct increase in medullary perfusion despite a decrease (admittedly modest) in blood flow through the cortex, which suggests tonic vasoconstrictor influence of D3 receptors in the medulla. This argues for the role of D3 receptors in control of medullary circulation and against the prevailing opinion that the functional role of D3 receptors in SHR is marginal.

In DOCA-salt hypertensive rats intramedullary D3 receptor blockade caused a rapid and pronounced decrease in renal haemodynamics concurrent with a distinct increase in arterial pressure. Evidently, under baseline conditions the renal and systemic resistance vessels were under substantial tonic vasodilator action of dopamine-activated D3 receptors. This finding apparently contradicts the evidence that in this model, under conditions of volume expansion and low renal nerve activity, D3 receptor activity should result in vasoconstriction. The blockade-induced increase in renal excretion was secondary to a major blood pressure elevation.
Declarations

Ethical Approval
The procedures used in experimental rats were approved by the extramural Second Local Ethical Committee for Animal Experimentation, Warsaw (WAW2/121/2021) and conform with EU legislation regarding ethical aspects of animal experimentation.

Competing interests
All authors declare that there are no competing financial, personal or professional interests.

Authors’ contributions
All experiments and measurements were performed in the laboratories of the M. Mossakowski Medical Research Institute, Polish Academy of Sciences, Warsaw. B. B. and J. S. conceived and designed the work. B. B. performed experiments and acquired the data, prepared the data in a graphical version. I. B. performed all analytical procedures, performed and interpreted the statistical analysis. J. S. participated in data interpretation and prepared the final manuscript version. All authors have approved the final version of the manuscript, and agree to be accountable for all aspects of the work.

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Availability of data and materials
The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.
References


https://doi.org/10.1124/pr.115.011833


https://doi.org/10.1097/00004872-200110000-00013


https://doi.org/10.1248/bpb.21.800


https://doi.org/10.3109/10641969709080809

https://doi.org/10.1073/pnas.91.11.4892

https://doi.org/10.1152/ajprenal.1993.265.5.F670


https://doi.org/10.1152/ajpregu.00321.2002


https://doi.org/10.1080/10641963.2021.1890762


37. Zeng Ch, Jose PA (2011) Dopamine receptors: important antihypertensive counterbalance against hypertensive factors. Hypertension 57:11-17.

https://doi.org/10.1159/000323135