Review

The renal urodilatin system: clinical implications

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Abstract

A renal natriuretic peptide and the ‘renal urodilatin system’ were identified after the observation that immunoassayable ANP in urine may not be identical to the circulating cardiac hormone ANP, which is a peptide of 28 amino acids. Urodilatin (INN: Ularitide) is a natriuretic peptide isolated from human urine and belongs to the family of A-type natriuretic peptides. Urodilatin is differentially processed to a peptide of 32 amino acids from the same precursor as ANP. It is synthesized in kidney tubular cells and secreted luminally. After secretion from epithelial cells of the distal and/or connecting tubules, Urodilatin interacts downstream at distal segments of the nephron with luminally located receptors whereby it regulates Na⁺ and water reabsorption. Thus, the physiological function of the renal Urodilatin system can be described as a paracrine intrarenal regulator for Na⁺ and water homeostasis, considering Urodilatin as a real diuretic-natriuretic regulatory peptide. However, the regulation upon which the Urodilatin secretion depends is still not clear. Since Urodilatin has been discovered, a great number of pharmacological and clinical investigations have been carried out using Urodilatin as a drug for several indications. So far, clinical phase I and II studies for acute renal failure, congestive heart failure, and bronchial asthma have been performed. © 2001 Elsevier Science B.V. All rights reserved.

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1. Introduction and general considerations

1.1. Discovery of natriuretic peptides

Peptide hormones of the natriuretic peptide family have been biochemically characterized since 1983 (see reviews [1–4]). They were identified as regulatory diuretic–nat-riuretic substances responsible for salt and water homeostasis [5,6] and as hormones lowering blood pressure [7–11]. Later, a number of homologous peptides with manifold functional implications were described [12–14]. The natriuretic hormone cardiодilatin/atrial natriuretic peptide (CDD/ANP) or A-type natriuretic peptide is synthesized in the heart atrial myoendocrine cells as a precursor molecule of 126 amino acid residues (Fig. 1), ANP-1-126 (CDD/ANP-1-126), and stored in specific granules of atrial myoendocrine cells [4,11,15]. The prohormone is processed into ANP-99-126 [16] while released into the circulation via exocytosis [15,17,18]. The other members of the natriuretic peptide family were identified from the brain as brain natriuretic peptide (BNP or B-type natriuretic peptide) [13], and CNP (C-type natriuretic peptide) [14] (Fig. 2). The natriuretic peptides share common features and exhibit a specific tissue distribution of gene expression as well as functional and pharmacological characteristics.

1.2. Localization und biochemistry of a-type natriuretic peptides

The different natriuretic peptides ANP, BNP, and CNP have been detected in many organs showing expression by different techniques such as immunocytochemistry, detection of specific transcripts (mRNA determination or in situ hybridization), and by immunoassays of tissue extracts (for review, see [15]). Many endocrine organs, the brain,
and other systems show the widespread distribution of this peptide family. ANP (or A-type natriuretic peptide) [6–8], BNP [13], and CNP [14] exhibit a similar, homologous primary structure of their processed functional form (Fig. 2). Less homology is seen between the precursors (Fig. 1). In this review, special reference has to be made to the renal localization of natriuretic peptides (see below), which was shown in a series of immunocytochemical publications [15–23]. In summary, all natriuretic peptides share common but also specific features, exhibiting tissue-specific distribution of gene expression which results in a diversity of functional and pharmacological characteristics.

1.3. Receptors for and metabolism of natriuretic peptides

Natriuretic peptides mediate their activity by intracellular generation of cyclic guanosine 3', 5'-monophosphate (cGMP) [24,25]. Type I membrane proteins have been identified as receptors of natriuretic peptides which are characterized by an intracellular domain of functional guanylyl cyclase [26–30]. cDNA cloning resulted in the discovery of three types of natriuretic peptide receptors (NPR): NPR-A, B and C (see reviews, [28–30]). Only NPR-A and NPR-B exhibit the intracellular guanylyl cyclase catalytic domain, whereas the third cloned receptor, NPR-C, contains no guanylyl cyclase domain. The interaction with the NPR-A and G-protein-coupled receptors is depicted in Fig. 3.

The inactivation of natriuretic peptides probably occurs via two pathways, namely the binding to receptors and enzymatic degradation [31–37] resulting in a half-life in the range of less than a few minutes. In this respect, the physiological role of NPR-C has been described as a clearance receptor. Besides binding of circulating natriuretic peptides to NPR-C, enzymatic degradation takes place in lung, liver, and kidney [31–33]. The main enzyme responsible for this degradation is the metalloendoprotease E.C.3.4.24.11 [34,36]. The localization of this protease in the brush border of the kidney proximal tubule and in the lung shows the importance of these organs in the metabolism of natriuretic peptides. E.C.3.4.24.11 opens the loop of CDD/ANP-99-126 between positions Cys-105 and Phe-106, inhibiting the receptor affinity and inactivating the regulatory function of the peptide.

2. Urodilatin, a renal natriuretic peptide

2.1. Biochemistry and molecular biology of Urodilatin

Urodilatin is a non-glycosylated peptide of 32 amino acid residues [12] considered as a naturally occurring human paracrine mediator [4,15,37,38]. In 1988, Urodilatin was isolated from human urine [12]. The peptide being synthesized in kidney tubules (Fig. 4a) belongs to the family of A-type natriuretic peptides, representing a differentially processed molecular form [4] (Fig. 6). Urodilatin is N-terminally extended by 4 amino acids, comparable to the circulating atrial natriuretic peptide, ANP-99-126 [16]. Therefore, Urodilatin and ANP-99-126 are derived from the same gene and a common precursor peptide, ANP-1-126 (Fig. 6).

Urodilatin applied by intravenous injection, like other natriuretic peptides, is inactivated by binding to the natriuretic peptide clearance receptor (NPR-C) and by enzymatic degradation through the neutral endopeptidase (NEP), E.C.3.4.24.11 [39]. In comparison to ANP-99-126, Urodilatin is characterized by a higher stability against this enzymatic degradation by neutral endoproteases [34], which explains why the renal effects are more effective when exogenous infusions of Urodilatin and ANP-99-126 are compared [40,41].
2.2. Receptors of Urodilatin

As mentioned above, Urodilatin exerts its function by stimulation of intracellular guanylyl cyclase at the intracellular domain of the natriuretic peptide receptor A (NPR-A), the enzyme that catalyzes the conversion of guanosine triphosphate (GTP) to 3',5'-cyclic guanosine monophosphate (cGMP) [42,43] (Fig. 3). This receptor (NPR-A) is located in kidney tubules (Fig. 4b) and also in the vascular smooth muscle of the kidney [4,44–50] as shown by different techniques of molecular biology, immunocytochemistry, and autoradiography. In the signaling, cGMP-dependent protein kinase is activated which in turn inhibits Na\(^+\) reabsorption via an amiloride-sensitive channel. Furthermore, in kidney arterial smooth muscle, a relaxation occurs via a change of intracellular Ca\(^{2+}\) concentration [42,43,45] (see also Figs. 3 and 5).

Recently it was shown that natriuretic peptides may also regulate renal electrogenic transport processes via cGMP-dependent and cGMP-independent pathways based on the presence of CNP-activated NPR-B which exists in at least two splice variants [51]. The significance of this ‘CNP
Fig. 3. Cellular mechanism of natriuretic peptides and Urodilatin in concert with G-protein-coupled receptors (such as β₂-adrenergic receptor in bronchial smooth muscle), the signaling of natriuretic peptide receptor finally results in a lowered intracellular calcium concentration which reduces the smooth muscular tonus to relaxation.

natriuretic system of the proximal tubular epithelium may be explained by regulation of the Na⁺ transport by influencing the potassium conductance [51].

2.3. Systemic function of the intrarenal paracrine system

The integration of the intrarenal paracrine system using Urodilatin as a diuretic regulator is considered to be of great importance in systemic regulation, namely the control of renal Na⁺ and water excretion (Fig. 7). Thus, basically the homeostasis of body fluid and electrolytes depends on this paracrine Urodilatin system [52–56]. The relation of renal Urodilatin excretion and different experiments in electrolyte homeostasis and imbalance have been investigated in animal studies and preclinical trials. Long-term Na⁺ diets in healthy volunteers revealed a significant correlation between natriuresis and Urodilatin excretion: increased Na⁺ load was found by our group [52] to induce an elevated Urodilatin excretion and an enhanced Na⁺ excretion, as also shown by others [57]. Similarly, acute volume load by saline infusion [54], balloon dilatation of the left atrium [53], and water immersion [58] result in stimulated Urodilatin secretion and increased Na⁺ excretion. Furthermore, circadian rhythm of urinary Na⁺ excretion is correlated with Urodilatin excretion [59]. In some of these experiments in which a natriuretic response is observed, circulating ANP-99-126 plasma levels are not changed at all [60–62]. Thus Urodilatin, the renal natriuretic peptide, is, by means of its paracrine interaction, an essential physiological factor responsible for Na⁺ and water regulation.

3. Pharmacology of Urodilatin

3.1. General pharmacological actions of Urodilatin

Systemic administration of the peptide in normal rats [63], dogs with cardiomyopathy [64], and healthy volunteers [65] shows the pharmacological effects on renal, cardiovascular, or pulmonary parameters of Urodilatin. Enhanced diuresis, natriuresis, and a variable drop in blood pressure have been observed. These effects are related to the predominant vasodilation in renal, pulmonary, and coronary arterial vessels and to electrolyte transport in renal tubules (Fig. 8). The lack of response or little
sensitivity of vasorelaxation of other arterial vessels such as mesenteric or peripheral arteries by Urodilatin results in a redistribution of the blood stream in the body.

Bolus injections of Urodilatin in healthy volunteers are followed by a natriuresis and diuresis which are double that of exogenously applied ANP-99-126 in the same dose ranges. In contrast, the hypotensive effect of Urodilatin is apparent only at higher doses than with ANP-99-126 [63–68].

Pulmonary parameters are distinctly influenced by Urodilatin, i.e. a bronchodilation (see below), as well as a decrease of pulmonary arterial pressure and of pulmonary wedge pressure are found after bolus injection of the peptide [69]. Urodilatin applied as a bolus in patients suffering from congestive heart failure produces a moderate hypotension and a subsequent reflex tachycardia. In spite of these changes, Urodilatin is of benefit to the patients as it significantly ameliorates cardiac index and stroke volume [70].

3.2. Toxicology of Urodilatin

Toxicology and pharmacological effects in animal models have so far shown no incompatibility reactions during or after local and systemic application of Urodilatin. When doses used in preclinical acute and long-
3.3. Impact of specific pharmacological actions of Urodilatin

The Urodilatin system plays an important role in smooth muscle dilatation, electrolyte-body fluid homeostasis, and immune defense. To further understand the multi-functional role of Urodilatin, in particular as a drug candidate for potential clinical indications, the pharmacological effects of Urodilatin and other natriuretic peptides are summarized in this following section (see Fig. 8).

Smooth muscle relaxation by Urodilatin results from a stimulation of intracellular guanylyl cyclase via the in-
tracellular domain of the NPR-A (Fig. 3). Further down-
stream, the generation of cGMP then leads to the activation
of cGMP-dependent protein kinase and subsequently to a
decrease of intracellular Ca\(^{2+}\) concentration thereby relax-
ing smooth muscle. This effect is in concert with several
GPCRs (G protein-coupled receptors) such as \(\beta_2\)-receptors
(see Fig. 3). The resulting vasorelaxant activity of
Urodilatin and synthetic human ANP-99-126 in physiolog-
ical and pharmacological concentrations was determined
on precontracted vascular strips and demonstrated an
equipotent dose-dependent relaxation [36].

The airway-relaxing effects of Urodilatin were shown in
vitro [71], using tracheal muscle strips and in in vivo
experiments in rodents [72]. Catecholamine inhibition is
detected in studies demonstrating that ANP-99-126 de-
creases sympathetic nervous activity by stimulation of
vagal afferences [73], and thereby contributing to the
diminishment of the smooth muscular tonus of the vascular
system.

\(\text{Na}^+\) homeostasis is triggered by several mechanisms
such as the antagonism of renin and the inhibition of
angiotensin. ANP-99-126 and BNP decrease renin secre-
tion from the macula densa, directly inhibit aldosterone
secretion from the zona glomerulosa, and attenuate the
stimulatory effect of angiotensin II on aldosterone release
[73,74]. The interaction with aldosterone was seen in
studies with healthy volunteers. These demonstrated that
aldosterone levels decrease significantly in a dose-depen-
dent manner following Urodilatin infusion [75,76].

The diuretic effect of Urodilatin is certainly the primary
physiological role of this regulatory peptide. As shown in trials with healthy volunteers, Urodilatin induces a strong diuresis and natriuresis under different Na$^+$ diets [65]. Furthermore, Urodilatin administered in bolus injections in healthy volunteers results in a natriuresis and diuresis which are stronger than those induced by ANP-99-126 [69].

The impact of natriuretic peptides modulating the immune defense is derived from the observation that ANP-99-126 inhibits macrophage activity. This action is suggested to be mediated by the NPR-A expressed in macrophages and the subsequent increase of intracellular cGMP levels which in turn reduces e.g. the activation of NF-$\kappa$B [69].

In patients with congestive heart failure NYHA III–IV, intravenously administered Urodilatin increased cardiac index and decreased pulmonary artery pressure, pulmonary capillary wedge pressure, pulmonary and systemic vascular resistance [70,78,79]. In a randomized, double-blind, placebo-controlled study, infusion of Urodilatin (15 ng/kg/min) for 10 hrs significantly decreased systolic blood pressure and central venous pressure. Furthermore, urine flow as well as urinary Na$^+$ excretion were significantly increased. These effects are accompanied by an increase in plasma and urinary cGMP levels. No neurohumoral activation or adverse side effects were observed [78].

The beneficial effects of Urodilatin in patients suffering from cardiac failure have been confirmed by other natriuretic peptides. Infusion of ANP-99-126 or brain natriuretic peptide (BNP) in patients with congestive heart failure improved left ventricular function by vasodilation and noticeable natriuretic action [79].

5.2. Urodilatin in renal insufficiency

Acute renal failure (ARF) is a frequent post-operative
complication after major surgical interventions due to hemodynamic, ischemic or vasoconstrictive mechanisms [80–82]. The vasoconstrictive effect of cyclosporine A on arterial circulation [81] was suggested to be a particular reason for ARF in the post-operative phase after organ transplantation. Therefore, the predominant vasoconstrictive effect of natriuretic peptides on vascular smooth muscle in the kidney was postulated to intervene with incipient renal failure in major surgery. Experimental studies showed that natriuretic peptides such as Urodilatin may exert a beneficial effect in ARF [83,84].

In the past, Urodilatin was used for prevention and therapy of acute renal failure following e.g. organ [85,86] and bone marrow transplantation [87] in pilot studies. Overall, Urodilatin significantly improved the deteriorated renal function as demonstrated by the decrease in serum creatine and blood urea nitrogen and the reduction of hemodialysis/hemofiltration.

A pivotal phase II trial assessing the effects of Urodilatin in patients with oliguric ARF following cardiac surgery, heart or liver transplantation [86] did not reveal a significant difference in patient outcome (avoidance of hemodialysis) in the Urodilatin-treated groups versus placebo. These results are in contrast not only with our previous results but also with a study which was performed by Allgren and coworkers [88]. In this trial, ANP administration revealed a significant improvement of dialysis-free survival in a subgroup analysis in patients with oliguric acute tubular necrosis. The reason for the differences observed in these trials, is probably the difference in criteria for inclusion of patients. In the studies without successful treatment, the patient collective contained mostly multimorbid cases and patients with serious clinical conditions. These results with the different trials indicate that an earlier intervention at the onset of oliguria/anuria would have been more adequate to demonstrate the beneficial effect of Urodilatin. As the time interval of incipient oliguria/anuria was too long, the renal deterioration possibly due to peri- or post-operative renal hypoperfusion could already have entered into an irreversible tubular necrosis. These considerations favor an approach by prophylactic treatment with Urodilatin in risk patients for ARF.

5.3. Urodilatin in bronchoconstriction and acute asthma

Urodilatin was shown to exhibit airway-relaxing effects in vitro using tracheal muscle strips [89]. In vivo experiments in rodents confirmed these results [90] and later in patients with mild asthma [91]. The bronchodilatory activity by Urodilatin is based on its stimulation of intracellular cyclic guanosine monophosphate (cGMP) as an alternative pathway to β2-agonists such as albuterol, the current first-line bronchodilator, which interacts with smooth bronchial muscle by an increase of intracellular concentration of cyclic adenosine monophosphate (cAMP) inducing a bronchodilation.

A randomized, double-blind, placebo-controlled clinical phase II study with cross-over design, using intravenous infusions of Urodilatin at various doses (Fig. 9) showed beneficial effects in patients suffering from bronchial asthma [92]. Urodilatin increases forced expiratory volume in 1 s (FEV1.0), maximal vital capacity (VCmax), peak expiratory flow (PEF), maximal expiratory flow at 75% (MEF75), at 50% (MEF50) and at 25% (MEF25) of forced vital capacity (FVC) at infusion doses of 10, 30 or 60 ng/kg/min. Optimal effects were observed at Urodilatin doses of 30 and 60 ng/kg/min. A further objective of this study was to show whether the bronchodilatory effects of intravenously administered Urodilatin compared with inhaled albuterol or whether a combination of the two drugs can improve the efficacy of the treatment. Urodilatin monotherapy shows a bronchodilation which is comparable to that induced by a standard dose of albuterol. Urodilatin infusion combined with albuterol inhalation results in a significantly stronger bronchodilatory effect compared to that obtained by monotherapy of either drug [91] (Fig. 9). The pharmacological effects of Urodilatin, such as diuresis, drop in central venous pressure, and pulmonary resistance, are considered to be of additional benefit for patients suffering from cor pulmonale. We conclude from this study [92] that the use of Urodilatin combined with a β2-agonist in the treatment of acute asthma exacerbation

Fig. 9. Summary of the results of a clinical phase II study using Urodilatin for the treatment of mild asthma exacerbation: This study showed the advantage of combined Urodilatin and albuterol treatment (see upper trace). The FEV1 values in combined albuterol (200 μg single inhalation) and Urodilatin (infusion 30 ng/kg/min infused for 60 min) is compared to treatment with albuterol alone, Urodilatin alone, and control. The combined application shows the highest efficacy comparable to maximum bronchodilation by 1250 mg albuterol. The placebo treatment of control shows no changes in the FEV1 values (modified from [92,93] with permission from Fluge et al., Eur J Med Res 4:411–415. Copyright 1999 Holapfer Verlag, München and Forssmann et al., Prog Respir Res, Vol. 31, Basel: Karger, 2001, pp. 81–84. Copyright 2001 S. Karger AG, Basel).
will result in a significant improvement in the outcome of these patients [92,93].

Urodilatin infusion may also result in an improvement of pulmonary function in patients with bronchial asthma and other obstructive pulmonary diseases when given periodically. Urodilatin treatment can be of advantage for patients with cardiovascular risk and in patients when the intervention via the cAMP-mediated mechanism of bronchodilation is not effective, due to receptor desensitization and downregulation. Then, the alternative cGMP-mediated bronchodilatory mechanism induced by Urodilatin can be used for more beneficial treatment [93].

6. Further potential clinical applications of natriuretic peptides

6.1. Hepatorenal syndrome

The clinical course of patients with decompensated Laennec’s cirrhosis is frequently complicated by progressive impairment of renal Na⁺ handling, leading to formation of ascites and peripheral edema [94–96]. Since the natriuretic peptides are shown to participate in the regulation of volume homeostasis [97,98], there are theoretical considerations suggesting a potential role for ANP-99-126 in the pathogenesis of the impaired Na⁺ homeostasis in cirrhosis. Infusions of ANP-99-126 to overcome the Na⁺-retaining status in these patients, however, led to various results, and in particular blunted natriuretic effects combined with hypotensive episodes were observed [99]. As shown earlier, Urodilatin is known to exhibit stronger natriuretic effects and less hypotensive side effects compared with ANP-99-126 [65,100]. Therefore, the therapeutic effect of Urodilatin administered in patients suffering from hepatorenal syndrome combined with ascites was assessed and Urodilatin was found to be effective in increasing Na⁺ and urine output in these patients [101].

6.2. Immune defense

Natriuretic peptides have been shown to be involved in a number of further physiological and pharmacological mechanisms which may be of future clinical interest. The implication of ANP-99-126 and macrophage functions in the sense of suppression of macrophage activation by autoregulation is under discussion [77]. Thereby, the NPR-A of macrophages inhibit by cGMP increase the activation of NF-κB and AP-1 which in turn reduces the TNF-α transcription and release of TNF-α [101].

6.3. Modulation and innate defense mechanisms

Recent results indicate that BNP exhibits an anti-microbial effect on specific germs [102], the significance of which is still under investigation.

6.4. Erectile dysfunction

The induction of penile erection depends on a complex system of signal transduction pathways resulting in cyclic nucleoside monophosphate changes of corpus cavernosus smooth muscle. In particular, cAMP and cGMP generated by the corresponding adenylyl and guanylyl cycles as well as the modulation of the activity of the cyclic nucleoside monophosphate degrading phosphodiesterases play a pivotal role in the modulation of the smooth muscle tonus of corpus cavernosus. Thus, the enzymes involved represent important target molecules for the development of drugs for the treatment of erectile dysfunction [103]. Guanylyl cyclase B (NPR-B), a receptor for the C-type natriuretic peptide (CNP) is expressed in human corpus cavernosus [104]. CNP induces an increase in intracellular cGMP levels as well as the relaxation of penile smooth muscle strips showing the role of CNP and its receptor in penile erection and its possible future use as a therapeutical approach in erectile dysfunction.

7. Summary of the status quo of the clinical development of natriuretic peptides

7.1. Urodilatin

Urodilatin (INN: Ularitide) is currently developed for the treatment of acute heart failure [105].

7.2. BNP

BNP (INN: Nesiritide) is currently developed for the treatment of acute heart failure in the USA. The peptide is planned to be administered during the first 24 h of hospitalization for AHF to normalize hemodynamic disturbances [106]. The therapeutic benefit of BNP has been substantiated in phase II and phase III trials in patients suffering from AHF [107–109]. The pharmacological concept for BNP is suggested to be an alternative to nitrates in the treatment of AHF. Food and Drug Administration approval is expected for the year 2001.

7.3. ANP

ANP (INN: Carperitide) is registered for the market in Japan for the therapy of AHF. Studies in patients with AHF revealed beneficial hemodynamic effects such as decrease in PCWP, systemic vascular resistance, and an increase in natriuresis and diuresis. Administration of ANP-99-126 in patients suffering from AHF results in a significant improvement of left ventricular function by reduction of pre- and afterload [110–113].

In conclusion, natriuretic peptides demonstrate a pharmacological profile which is desirable for the treatment of patients suffering from AHF. Clinical studies of ANP-99-
126, BNP, and Urodilatin as well as approval of ANP-99-126 and the expected FDA approval for BNP support the potential of these human peptides to have a significant impact as an ‘add-on’ therapy for the treatment of patients admitted to the hospital for AHF.

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