

THE MOLECULAR AND PHYSIOPATHOLOGICAL MECHANISMS INVOLVED IN RESPONSE REDUCTION TO CHRONIC DIURETICS THERAPY

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REZUMAT

Acest articol revizuieste aspecte vechi și noi privind modul de acțiune a principalelor tipuri de diuretice utilizate în practica clinică. Clonarea moleculară a transportorilor de Na⁺ distali sensibili la diuretic a permis înțelegerea mecanismului de acțiune a fiecărei clase de diuretic și astăzi este clar definit fiecare tip de proteină de transport pe care acționează specific un anumit tip de diuretic. Un fenomen cunoscut, scăderea eficienței medicației diuretice după un timp de utilizare mai lung sau mai scurt, sau chiar efectul paradoxal de agravare a edemelor la întreruperea bruscă a tratamentului, este explicat la nivel fiziopatologic și molecular. Sunt prezentate pe scurt principalele indicații ale diferitelor clase de medicamente diuretice. Datele prezentate în acest material ajută la înțelegerea faptului că fiecare pacient are o individualitate aparte de răspuns la tratamentul diuretic.

Cuvinte cheie: diuretice, Na⁺-K⁺-2Cl⁻ cotransport, Na⁺-Cl⁻ cotransport, toleranță la diuretic, rezistență la diuretic

ABSTRACT

This article represents an overview on old and novel aspects concerning pharmacokinetics and pharmacodynamics of the main classes of diuretic drugs used in the common medical treatment. The molecular cloning of distal Na⁺ transporters sensitive for diuretics allowed the understanding of the mechanism for the main diuretic classes and at present, each transport protein type for which exists a specific type of diuretic is clearly defined. A common phenomenon, the decrease of diuretic efficiency after a long or short treatment period or even paradoxical effect such as edema aggravation if treatment is suddenly stopped, is explained at molecular and physiological level. We present briefly the main indications of different diuretic classes. The facts presented above in this material helped us to understand that every patient has a different way to respond to diuretic treatment.

Key Words: diuretics, Na⁺-K⁺-2Cl⁻ cotransporter, Na⁺-Cl⁻ cotransporter, diuretic tolerance, diuretic resistance

INTRODUCTION

Diuretics represent a heterogeneous group of drugs regarding their chemical composition as well as their mechanism and share common properties such as renal elimination of sodium and water and thus increase diuresis. All conditions that interfere with normal sodium and water renal excretion, generating their retention in the extracellular compartments and

the increase in arterial tension, represent the main indications of diuretic therapy.¹ The most commonly used diuretics in the clinical practice are loop diuretics, thiazide diuretics, thiazide-like and potassium-sparing diuretics. The use of a certain class of diuretics is mainly conditioned by the type and severity of each disease.

Therefore, in mild and moderate hypertension with active renal function and glomerular filtration rate (GFR) > 40 ml/min and serum creatinine < 180 μmol/l, thiazide diuretics or indapamide are used.^{2,3} Metolazone, a thiazide-like diuretic, could be used for GFR up to 20 ml/min, especially in heart failure.²

In case of chronic renal failure, nephrotic syndrome, severe heart failure, loop diuretics are strongly recommended, as they are considered the most effective and do not decrease the glomerular filtration rate. In case of poor response, they may be associated with high-dose thiazide diuretics.

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In cirrhosis antialdosteronic diuretics are the drugs of choice and may be associated with a thiazide diuretic, or as a last resort, with a loop diuretic, as these patients are extremely sensitive to massive volume depression.

The use of diuretics in clinical practice has demonstrated a diminishing of the diuretic response in time and a need for dosage increase, for changes in route of administration and for combined use of two or three diuretic classes. This phenomenon occurs due to resistance to diuretics induced by pharmacokinetic and pharmacodynamic properties of drugs during a certain disease, which changes with the disease progression as well as because to short and long-term adapting mechanisms to a specific diuretic.^{4,5}

PHARMACOKINETICS AND PHARMACODYNAMICS OF THE MAIN DIURETIC CLASSES USED IN CLINICAL PRACTICE

Loop diuretics are represented by: furosemide, bumetanide, torasemide and ethacrynic acid and they can be administered orally or intravenously. Orally administered furosemide is absorbed in the digestive tract up to 50% and presents individual variation between 10-100%.⁵ In blood, furosemide binds to site I on human serum albumin in proportion of 95-99% and thus restrains its glomerular filtration.⁵⁻⁸ At kidney level, the complex is rapidly cleaved and furosemide is filtered in the glomerulus up to 50%. The remaining of 50% is metabolized in the kidneys through conjugation with glucuronic acid. Oral administered bumetanide and torasemide are almost entirely absorbed from the digestive tract and metabolized in proportion of 50% and 80%, respectively in the liver, while the rest is filtered in the glomeruli.⁵ In liver diseases, an increase of their serum concentration has been registered, while in case of renal failure no accumulation was observed. The plasma half-lives of loop diuretics range from one hour in case of bumetanide to 3-4 hours for torasemide, while for furosemide, it is intermediate.

The edematous disorders do not generate malabsorption of loop diuretics. Absorption is slowed in case of decompensated heart failure but the total absorbed amount is similar with that found in healthy individuals.⁹

Thiazide diuretics are mostly recommended for oral administration due to their fast absorption from the gastrointestinal tract. Some of them are primarily metabolized at hepatic level (bendroflumethiazide, indapamide), while others are excreted in an unchanged

form in the urine (chlorothiazide, chlorthalidone, hydrochlorothiazide). Diuresis commences after 1-2 hours and lasts on an average 6-12 h.³ Metolazone, a thiazide-like diuretic, has a slow absorption rate and prolonged elimination and this explained the treatment effectiveness after 10 days; it is effective even for patients with renal failure and diuretic resistance due to its action on sodium proximal absorption and does not induce reduction of glomerular filtration rate. Indapamide, a thiazide-like diuretic with extended half-life period, when used in small doses generates a vasodilator effects, while in case of large doses it induces a diuretic effect.^{2,9}

Potassium-sparing diuretics have various pharmacokinetics, depending on the drug. Amiloride is excreted by kidneys and its plasma half-life is prolonged in renal diseases. The triamterene pharmacokinetics is complicated: it is converted in an active metabolite in the liver and subsequently it is secreted into the tubular fluid. Renal diseases affect the secretion of this metabolite into the tubular fluid. The amount of active metabolite that reaches the tubular fluid is also diminished in case of liver diseases because in such circumstances the metabolite production in the liver is reduced.⁵

Spirolactone pharmacokinetics is even more complex for it is converted into numerous active metabolites such as canrenone and potassium canrenoate which competitively inhibit the aldosterone binding to mineralocorticoid or type I receptors in many tissues, inclusively in the epithelial cells from distal convoluted tubule and collecting duct.³

All diuretics currently used are efficient only intraluminally except for spironolactone.⁵ Their access in the tubular fluid is performed almost exclusively via secretion in the proximal tract.¹¹ Thiazide and loop diuretics are weak organic anions (OA⁻) and they are up-taken from the peritubular space together with other weak organic anions such as paraaminohippurate (PAH). This transport type is involved in secretion of foreign substances for the organism and shows maximal transport ability.¹⁰ The uptake is inhibited by drugs such as probenecid. Four isoforms of this transport type (OAT⁻ - organic anion transporters) were cloned and were expressed in the kidney.¹¹ OAT-1 was described as transport protein for diuretics.¹²

The peritubular uptake by OAT-1 is a tertiary active process.⁷ (Fig. 1) Na⁺/K⁺ ATP-ase from the basolateral membrane creates a low concentration of intracellular Na and thus allows the associated uptake of α -ketoglutarate (α KG⁻) and maintains its intracellular increased level. This generates also the

strength necessary for the counter transport of $\text{OA}^-/\alpha\text{KG}^-$. Within tubular cells, organic anions and diuretics can be sequestered reversibly in vesicles. OA^- and diuretics are secreted at tubular cell membrane level through voltage-driven OAT and by counter transport with urate or OH^- exchange.^{7,11} A competition has been observed for peritubular uptake as well as for luminal secretion with different substances such as urate that accumulates in uremia. Metabolic acidosis depolarizes the membrane potential from the proximal tubular cells generating a decrease of organic anion secretion.⁷

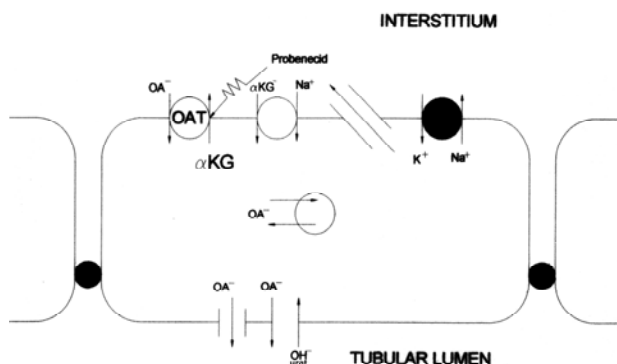


Figure 1. Thiazide and loop diuretics secretion at convoluted proximal tubule level – tertiary active process (Adapted from ⁷). Basolateral Na^+/K^+ ATP-ase determines the low concentration of intracellular Na^+ and facilitates the up-take of α -ketoglutarate (αKG^-), and after up-take of OA^- through $\text{OA}^-/\alpha\text{KG}^-$ countertransporter. OA^- is secreted across the luminal cell membrane by a voltage driven OAT and by a counter transportor in exchange for urate or OH^- . K^+ leaves the cell through the potassium channels recycled at cell surface. OA^- = organic anions. OAT = organic anion transporter.

Once the diuretic reaches the tubular fluid it activates at the primary action site. The relation between the amount of diuretic reaching the site of action (determined on the basis of diuretic excretion rate) and the natriuretic response is represented by a sigmoid curve in case of loop diuretics.

In order to act at the action site level, the diuretic drug must reach a certain concentration. The increase of loop diuretic dose generates simultaneously an increase of diuretic response (the effective dose corresponding to the ascending curve) to a threshold dose and then the sodium excretion becomes insignificant (the maximal or ceiling dose corresponding to the constant curve). In normal subjects, the maximal response to loop diuretics corresponds to excretion of 200–250 mmol Na^+ . The sigmoid-shaped curve is maintained for all loop diuretics, the difference being registered in the position on x axis.

In case of diuretic resistance, frequently encountered in heart failure, renal failure and nephrotic syndrome, the curve moves downwards and to the right. Therefore, in severe heart and renal failure the maximum diuretic response corresponds to

the excretion of 20–30 mmol of Na^+ , this indicating that in order to obtain a diuretic effect, the dose must be repeatedly administered in one day. When Na^+ uptake exceeds 30 mmol, the diuretic effect is blocked and salt and water retention occurs in this category of patients. At the end of dosage period, the diuretic concentration decreases at the action site and increased Na^+ reabsorption ensues as a result of adaptation phenomenon. If, at the end of this period, the patient ingests salt, the salt and water retention is amplified and the initial natriuretic effect may be canceled and it could be clinically interpreted as lack of response to diuretic treatment with maintenance or reinstalling of edema and weight gain in patients.

MECHANISMS INVOLVED IN SODIUM REABSORPTION AT RENAL LEVEL

At glomerular level approximately 20000 mEq Na^+ are filtered every 24h. Of these, approximately 95.5 to 99% is reabsorbed at tubular level from filtered sodium ions.

At *proximal tubule level*, passive reabsorption of sodium is attained according to electrochemical gradient, Na^+ being absorbed together with other ions (for instance Cl^-) and with water in an equimolar ration; in such circumstances isosmotic reabsorption is performed. At the same level, a Na^+/H^+ exchange takes place which requires the presence of carbonic anhydrase and is considered energy consuming. The Na^+/H^+ exchange in the proximal convoluted tubule and Henle's loop is done depending on NHE-2 isomere of the counter transporter NHE-1.¹³ NHE-1 is ubiquitous and its increased expression can be observed in patients with salt-sensitive hypertension.¹⁴ This counter transport determines the increase of Na^+ reabsorption at the renal level and leads to reduction of total and partial sodium excretion and elevation of blood pressure in case of sodium overload. Some researchers had identified a $3\text{Na}^+/\text{H}^+$ exchange pump in the renal proximal tubule regarded as NHE-3 with important role in sodium reabsorption and whose activity is stimulated by AgII .^{4,15}

At *Henle's loop level*, sodium is reabsorbed along the ascending limb in a proportion of 20%. In the medullar portion of ascending limb of Henle, impermeable for water and urea, sodium is absorbed through a passive mechanism according to a certain electrochemical gradient. In the thick ascending limb of Henle (with cortical and medullar segment) active sodium chloride (NaCl) absorption takes place by means of $\text{Na}^+/\text{K}^+/\text{2Cl}^-$ cotransport. The transport proteins belong to a

superfamily consisting of cotransporters of different cations and chlor: $\text{Na}^+\text{-K}^+\text{-2Cl}^-$, $\text{K}^+\text{-Cl}^-$ and $\text{Na}^+\text{-Cl}^-$ coded by genes from SLC12A class.¹⁶ $\text{Na}^+\text{-K}^+\text{-2Cl}^-$ cotransporter is represented by two proteins with resembling structures BSC1 and BSC2, coded by NKCC2 and NKCC1 genes, respectively. Between the two proteins, there are several differences regarding the localization at renal level, ion-binding affinity (K_m values), inhibition extent towards bumetanide (K_i values) and also regarding the role in sodium reabsorption and its regulation at renal level. NKCC2 protein is situated predominantly but not exclusively at the apical membrane level of the tubular cells found in the thick portion of the ascending limb of Henle and also in the macula densa cells.¹⁷ Considering its levels, there are some loci where cations are attached and also the chloride ions. Its localization at this level has been achievable using polyclonal antibodies obtained from transporter cloning. Its functional activity was studied by synthetic messenger RNA injection using complementary from DNA of the cotransporter in the oocyte of *Xenopus laevis*.¹⁷ The oocyte demonstrated increased levels of a Cl^- and K^+ depending on Na^+ intake. The Na^+ intake is specifically inhibited by bumetanide while hydrochlorothiazide and metolazone had no specific effect.

The cotransporter protein $\text{Na}^+\text{-K}^+\text{-2Cl}^-$ performs the transport of a single Na^+ and K^+ ion and two Cl^- ions from the tubular lumen inside the cell. The energy necessary for this transport is provided by Na^+/K^+ ATP-ase from the basolateral membrane that expels two Na^+ ions and introduces into cell one K^+ ion. Cl^- passes through the basolateral membrane due to its increased conductance for Cl^- and thus explains Cl^- free movement through the cell wall. K^+ influx is counterbalanced by K^+ efflux through potassium specific channels depending on ATP from the apical membrane. If they are considered as a whole, these transport processes that occur at apical and basolateral level are different, but they are very well coupled and insure the kidney reabsorption of NaCl , the recycling of K ions at the cell surface and achievement of a positive potential in the lumen which enables Na^+ , Mg^{2+} , Ca^{2+} ion transport in the paracellular spaces.¹⁸ (Fig. 2)

At *the level of distal tubule* 5 to 10 % from the total sodium filtered at glomerular level is absorbed.¹⁸ The main transport type at this level is thiazide sensitive $\text{Na}^+\text{-Cl}^-$ cotransport (TSC). Although insensitive to loop diuretics, this protein known also as NCC, contains 45% of the common amino acids and has a similar overall topology with $\text{Na}^+\text{-K}^+\text{-2Cl}^-$ cotransporter protein from Henle's loop.¹ NCC protein is situated at

the apical membrane of distal convoluted tubule cells. NCC presents two N-linked glycosylation sites. The N-glycosylated sites are essential for a good functioning and expression of cotransporter at membrane surface. $\text{Na}^+\text{-Cl}^-$ cotransport is electroneutral and shows lower affinity for Cl^- than for Na^+ .¹⁸

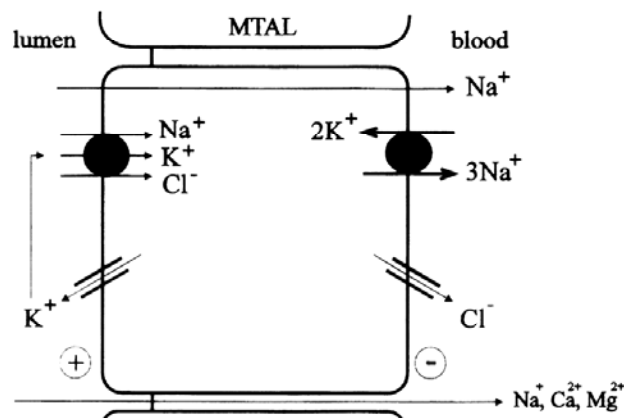


Figure 2. Model of Na^+ reabsorption in Henle's loop (Adapted from ¹⁸). The activity of $\text{Na}^+\text{-K}^+\text{-2Cl}^-$ cotransporter is assured by a voltage-driven basolateral Na^+/K^+ ATP-ase which pump up three Na^+ ions and uptake two K^+ ions. K^+ and Cl^- exit the cells through specific channels place on apical respective basolateral membrane and generates a electropositive lumen that facilitates the absorption of Na^+ ions together with divalent cations through paracellular spaces. MTAL = medullary thick ascending limb.

At *the tubular cell level* from distal nephron antagonist processes of secretion and NaCl reabsorption occur. NaCl secretion in the collecting duct is performed by mean of $\text{Na}^+\text{-K}^+\text{-2Cl}^-$ (NKCC1) cotransport situated on the basolateral membrane. Cl^- gets into the cell by mean of this cotransport and leaves the cell through chlorine channels from the apical membrane.

The H^+ and K^+ luminal secretion via Na^+/H^+ and Na^+/K^+ exchanges is competitive, as if one is less available, the exchange of the other with Na^+ increases.

In the collecting ducts sodium reabsorption is induced through epithelial sodium channels situated apical and by Na^+/K^+ ATP-ase from the basolateral membrane. Ca^{2+} reabsorption is inversely correlated with Na^+ and is achieved through calcium channels with apical position and through $\text{Na}^+/\text{Ca}^{2+}$ exchange pump with basolateral position. The intracellular calcium is bound to calbindine, a protein involved in Ca^{2+} reabsorption. Thiazide diuretics inhibit Na^+ reabsorption and increase the basolateral Ca^{2+} efflux and thus it generates Ca^{2+} reabsorption.¹⁸

The epithelial Na^+ channels (ENaC) were extensively studied using genetic engineering techniques. Polyclonal antibodies show channel proteins at the apical membrane of epithelial cells

in connecting tubules, cortical collecting ducts, outer and inner medullary collecting ducts.¹⁹ ENaC consists of three homologue subunits α ENaC (SCNN1A), β -ENaC (SCNN1B) and γ -ENaC (SCNN1G), joined as a heterotetramer. Each subunit is presented by a protein structure with a large extracellular loop comprised between two transmembrane segments and N- and C terminal ends situated intracellular with role in functional regulation. In order to achieve the Na influx, the presence of only one α subunit is required but the presence of all three correctly assembled components increase the Na⁺ influx. These were studied by injection with ARNm in oocytes of *Xenopus laevis*, where the expression of all three subunits generated a selective Na⁺ current with similar properties to ENaC sodium channels from the native tissue.^{4,20}

Collecting tubule in the presence of vasopressin, become permeable for water increasing water reabsorption towards the hyperosmotic medullar interstice and urine concentration.

Recent studies in the molecular biology showed that this phenomenon occurs through regulatory action of vasopressin on aquaporin-2 channels (AQP-2). A short term as well as a long term regulation is known.⁴ The immediate action of vasopressin determined the migration of AQP2 channels from intracellular cytoplasmic vesicles to the apical surface of the cell membrane.^{4,21} Long term regulation refers to limiting of degradation process of AQP2 water channels.⁴

REGULATION MECHANISMS OF SODIUM RENAL REABSORPTION

At renal tubular level the regulation of water and electrolyte reabsorption involves a multitude of mechanisms like: RAAS, nitric oxide, prostaglandins and atrial natriuretic peptide. These systems co-work in the baroreceptor and tubuloglomerular mechanism to regulate sodium and water reabsorption. NKCC1 gene encodes mRNA and Na⁺-2K⁺-2Cl⁻ cotransporter protein sensitive to bumetanide (BSC2) in most cell types and tissues. At kidney level, it is present in the renin granulation cells and vascular smooth muscle cells of afferent arteriole, in the glomerulus and extraglomerular mesangium which gets in contact with distal convoluted tubule where macula densa cells are found. BSC2 is involved in the tubuloglomerular feedback as well as in rennin release.⁴ Both mechanisms depend on chloride concentration in the thick portion of the ascending limb of Henle. (Fig. 3)

At macula densa level, nitric oxide synthase is present and hence the hypothesis that NKCC2 acts

like a sensor for Cl⁻ ions that reach the macula densa while nitric oxide is regarded a modulator or mediator of the local effects that controls the prostaglandins synthesis.⁴

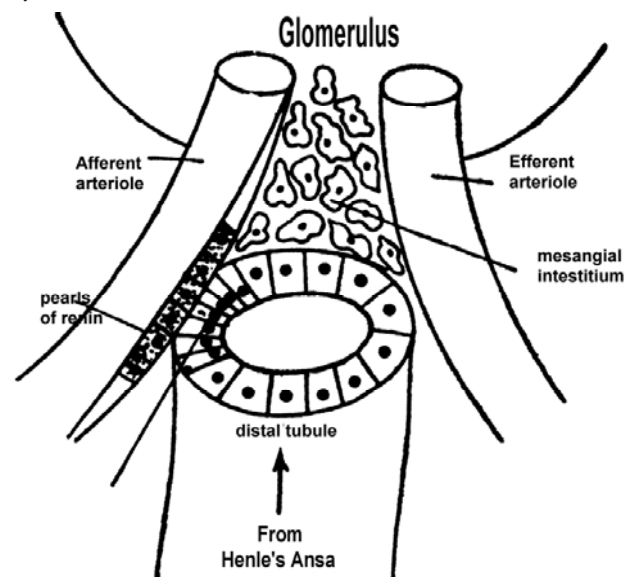


Figure 3. Juxtaglomerular apparatus (Adapted from ¹⁰). BSC1 at macula densa cells level and BSC2 in renin granular cells and vascular smooth cells of the afferent arterioles and in interstitial mesangium co-work in the tubuloglomerular feedback as well as in renin release. Both mechanisms depend of Cl⁻ concentration at macula densa and at mesangial interstitial level.

The Na⁺-K⁺-2Cl⁻ (NKCC2) cotransport from the ascending limb of Henle is influenced by local prostaglandins. PgE2 activates the EP3 receptor which generates the cAMP decrease on Gi path and as a consequence the decrease of NKCC2 gene expression. Moreover, it decreases the drainage force necessary to reabsorb water and the hydroosmotic response to vasopressin, a well known effect in the presence of PgE2.^{4,8} PgE role in NaCl reabsorption may explain the nonsteroid anti-inflammatory effect of increased water and sodium retention, the effect being demonstrated to take place in the thick portion of ascending limb of Henle.

Most researchers have demonstrated that administration of Ag II stimulates BSC-1 expression in rat kidneys and that BSC-1 expression is low in mice that are deficient in angiotensin-converting enzyme.^{22,23} Staahltoft et. al had demonstrated that blockade of Ag II synthesis normalizes both BSC-1 expression and renal sodium expression in rats with cardiac failure, indicating that AgII influences sodium manipulation in the cardiovascular diseases through BSC-1.²⁴

NaCl reabsorption and sodium-potassium exchange in the convoluted distal tubule and collecting tubule is modulated by aldosterone. At present, it is acknowledged that steroid hormones

such as aldosterone and estradiol are involved in NCC protein level regulation.^{4,25} Chronic treatment with aldosterone determines the up-regulation of ENaC activity and selective increase of β ENaC at the apical membrane level.²⁰ Aldosterone stimulates the ENaC activity in two phases: an initial, acute phase that represents “electrically silent” channels resident in the apical membrane activation and a late phase whose mechanism is not understood yet.²⁰

Vasopressin influences the NaCl transport at renal level. Recent studies performed on animal subjects and genetic manipulation methods have underpinned the hypothesis which sustains that vasopressin acts on $G_s\alpha$ protein pathway, increases the cAMP and generates a short term traffic of NKCC2 proteins towards the apical surface of cells and in the mean time increases the NKCC2 mass by intensifying NKCC2 gene expression.⁴ This could explain the resistance to diuretics in case of cardiac failure and cirrhosis where an excess of vasopressin is observed.²¹ Vasopressin increases the traffic to cell surface in β type sodium channels (β -ENaC) without affecting α ENaC and γ -ENaC levels.²⁰

The atrial natriuretic peptide (ANP) stimulates the Na^+ secretion increasing the expression and activity of $Na^+-K^+-2Cl^-$ (NKCC1) cotransport from the basolateral membrane of the collecting tubule cells through an up-regulation phenomenon. Simultaneously, it decreases the absorption through the down-regulation on expression of apical Na^+ channels and Na^+-K^+ pump situated basolaterally.¹⁸

THE MECHANISM OF THE MAIN CLASSES OF DIURETIC DRUGS

The main effect of most diuretics consists in reducing Na^+ reabsorption from the renal tubular cells. This can be attained through action upon Na^+ transport systems at cellular level. Most of these transport systems may be found in other cells of the human organism (red cells, lymphocytes, smooth muscular cells, nervous system, renal tubular cells), with slight differences regarding the transport protein.^{13,14,26,27} This explains the side effects of the diuretic treatment.

Loop diuretics (furosemide, ethacrynic acid, torasemide, bumetanide) inhibit the NKCC2 cotransport by binding at or near to the second Cl^- site using a competitive mechanism. Loop diuretics inhibit simultaneously the NKCC1 cotransport protein. Furosemide inhibits BSC2 and leads to blockage of tubuloglomerular feedback as well as the modulation of renin secretion.⁴ By inhibition of the sodium

chloride reabsorption in Henle's loop, the NaCl amount that reaches the macula densa level is high and it should reduce the glomerular filtration via feedback. Furosemide inhibits also NKCC2 (BSC1) at macula densa level and alters the Cl^- sensing with major role in feedback reaction. Furosemide works also on NKCC1 (BSC2) at juxtaglomerular level reducing Cl^- in the mesangial interstice liquid and so reducing the sensitivity to feedback reaction. Nevertheless, loop diuretics do not exert any action on collecting tubule, it seems that furosemide, besides its primary action on thick ascending limb of Henle, inhibits the vasopressin action.

In acute circumstances, loop diuretics are administered intravenously and generate vasodilatation especially venodilatation. Some of the vasodilatory action is mediated through the release of prostaglandins and endothelial derived relaxing factor (EDRF) and potassium channel activation.^{2,3} The vasodilatory effect is present also in thiazide diuretics, and it is maintained in both classes in chronic administration due to sodium and water loss from the vessel wall.^{2,3}

Thiazide and thiazide-like diuretics inhibit Na^+-Cl^- cotransport (NCC) at distal convoluted tubule level, through competitive binding to or near the Cl^- site. Monroy et al have demonstrated that both sites play an important role in cotransport inhibition by the thiazide.⁴ Thus, both binding sites for thiazide may be occupied or altered by Na^+ and Cl^- in the same manner.

Potassium-sparing diuretics: spironolactone, an anti-aldosteronic diuretic binds to the aldosterone receptor and inhibits sodium reabsorption and implicitly Cl^- .

Amiloride and triamterene inhibit distal sodium reabsorption and potassium excretion, but they prove their effectiveness in the aldosterone absence, having a different action mechanism compared to spironolactone. They induce a hyperpolarization of the epithelial Na^+ channels decreasing their activity.⁴

ADAPTATION TO CHRONIC DIURETIC TREATMENT

The adaptation to a certain diuretic is defined as a physiological process of the organism and it should not be confused with diuretic resistance specific to certain conditions in which drug pharmacokinetics and pharmacodynamic changes occur in terms of disease. Adaptation to a certain diuretic may be on short or long term.

Short-term tolerance relates to diminishing of the diuretic effect after the first drug administration dose.

Hemodynamic regulating mechanisms are involved as well as functional mechanism at cellular and molecular level. The tubuloglomerular feedback, increase of sympathetic nervous system (SNS) activity and angiotensin-renin system are the main hemodynamic regulation mechanism.

Short-term tolerance could be prevented by means of restoration of the lost volume; this indicating that the volume loss per se is a stimulus independent of the responsible effector.

Current researches incriminate the molecular mechanisms such as: traffic to apical membrane surface of the transport molecule, with increase of Na^+ , Cl^- and water reabsorption. AgII at physiological doses stimulates the $3\text{Na}^+/\text{H}^+$ (NHE3) exchange in the proximal convoluted tubule, increasing water and electrolyte reabsorption at this level irrespective of glomerular filtration rate changes. The fast increase of water and sodium absorption is achieved through Ag II stimulation of $3\text{Na}^+/\text{H}^+$ (NHE3) transporter protein traffic from the cytoplasmic level to apical surface of the epithelial cells.

It seems that there are also other adaptative mechanisms, as demonstrated by inhibition of angiotensin-converting-enzyme or the sympathetic nervous system that act separately or concomitantly and do not block the total water and electrolytes reabsorption at this level.

Long-term tolerance develops in case of chronic diuretic administration and the involved mechanisms are:

1. **The substrate stimulation**, which is one of the most important regulation factors of OAT-1 which less diuretic tolerance. This phenomenon may be explained by the increase of transport protein biosynthesis of a certain substance. The furosemide and hydrochlorothiazide stimulate directly the synthesis of OAT-1 and so increase delivery of diuretic in tubular lumen.⁴

2. **The increase of NaCl reabsorption at the primary site of diuretic action**, through increase of the transporter mass at this level. In the experimental studies using shark cells, the activation of the NKCC2 isoform sensitive to loop diuretics has been reported, following a decrease in the concentration of intracellular chloride. Considering that loop diuretics reduce the chloride concentration in the cell from the thick ascending limb of Henle loop, when the diuretic concentration decreases the sodium transporters are rapidly activated. Other researchers demonstrated that chronic perfusion with furosemide increases the NKCC2 mass while the perfusion with hydrochlorothiazide increases the NCC mass. These

data are confirmed in the case of chronic treatment with hydrochlorothiazide, increases [^3H] metolazone binding degree- an indirect indicator of NCC mass - even if NaCl reabsorption is low. Recent studies have demonstrated that amiloride induces, in time, the epithelial Na channel mass at the nephron segment where it activates.⁴

3. **Compensatory up-regulation of Na^+ transporters downstream from the primary site of diuretic action.**

The inhibition of Na^+ reabsorption by loop and thiazide diuretics leads to increase of the distal delivery of NaCl and flow through the terminal nephron. It will generate in time distal structural and functional changes that will conduct to increase of the sodium distal absorption. The increase of distal Na^+ reabsorption is produced by H^+ and K^+ ion exchange, stimulates the activity of the epithelial sodium channel, cellular hypertrophy, and increases the transporter mass (in case of loop diuretics increased the abundance of NCC). An important role is attributed to chronic stimulation of RAAS and high aldosterone level which increased water and Na reabsorption in the distal segment.^{4,7,25} This fact is sustained by the amplification of the diuretic effect by use of angiotensin-converting-enzyme inhibitors (ACEI) or spironolactone. Abdallah et al reported that furosemide infusion increased NCC protein abundance, but this effect was blocked by spironolactone, suggesting that the response was dependent on a rise in plasma aldosterone concentration.²⁸

The mechanism by which chronic exposure to electrolytes determines hypertrophy of the nephron distal segments is unknown but the increase of Na^+ concentration in the renal epithelial cells may exert a mitogenic effect and may promote cellular growth. In chronic treatment with furosemide and hydrochlorothiazide (HCTZ), an increase of ENaC abundance has been observed.^{25,29,30} In fact, perfusion using furosemide generates the increase of all three Na^+ epithelial subunits while HCTZ determines the particular increase of β -ENaC in the cortex and ENaC in the external medulla.

It could be concluded that unabsorbed Na at the primary action site of the diuretic is reabsorbed at distal level and thus it reduces the total diuresis.

THE USE OF DIURETICS IN THE MAIN THERAPEUTIC INDICATIONS

Heart failure

Diuretic drugs are mostly used in systolic heart failure in order to ameliorate the symptoms, to increase

the exercise capacity and to improve the quality of life. In case of edema in isolated diastolic heart failure (caused by lack of relaxation or myocardium infiltration) in which blood pressure (BP) and cardiac output rely on an increased filling pressure, diuretics may be used with extreme precautions.²⁷

Diuretics are indicated for patients with symptomatic cardiac failure (NYHA II-IV) alone or combined with other agents. Thiazide diuretics are recommended in moderate conditions in which renal function is not affected. In more severe cases (NYHA III-IV) and in those with renal failure (serum creatinine > 180 μ mol/l), loop diuretic are the most effective. In severe heart failure, torasemide is better absorbed and produces less fatigability and requires less days of hospitalization.^{3,5,31}

The fact that in chronic heart failure a diuretic tolerance appears generated by the increase of Na⁺ transporter mass at the primary action site as well as to distal level is supported by the phenomena that occur when diuretic treatment is suspended in patients with hemodynamically stable heart failure. In these patients, although ACEI treatment was continued, the discontinuation of the diuretic treatment altered the hemodynamic parameters, heart failure was aggravated and right ventricular systolic and diastolic blood pressures were elevated. These patients gained weight and registered high levels of B-type natriuretic peptide which indicate the release of a large mass of Na⁺ transporters with increase of the ventricular overload and body fluid content.³¹

In case of severely affected patients, chronic diuretic therapy is imperiously required. Chronic diuretic therapy due to its volume constriction determines a chronic stimulation of RAAS. Despite the use of ACEI, aldosterone is maintained at high level and this may cause increased cardiovascular mortality. Spironolactone and eplerenone exhibit strong anti-aldosteronic effects reducing cardiac fibrosis and adrenergic tonus with significant prognostic improvement and reducing the events in patients with cardiac failure. In chronic diuretic treatment, diuretic resistance occurs due to aggravating stage of cardiac failure as well as to adjustment phenomenon to diuretics. Marumo et al found an up-regulation of BSC-1 in rats with heart failure.³²

The decrease of the diuretic response may be determined by nonsteroidal anti-inflammatory drugs, heparin administration (decrease vasodilator prostaglandins) and also by vasodilator drugs (reducing renal blood flow in spite of cardiac output increase). The resistance to loop diuretics may be prevented using

ACEI that increase the diuretic efficiency through a mechanism independent of their capacity to reduce peripheral vascular resistance (PVR) and through increase of administration frequency or intravenous perfusion with an loop diuretic.³

Chronic renal failure (CRF)

In chronic renal failure, diuretic drugs are administered in order to reduce edema, treat hypertension and correct metabolic acidosis and hyperkalemia. Usually, loop diuretics are recommended. Thiazide diuretics could be associated with loop diuretics in mild and moderate CRF and show effectiveness when administered in high doses, generating an efficient diuresis with blood pressure decrease but with an increase of blood urea nitrogen and serum creatinine and occurrence of electrolyte disorders.⁷

Frequently, patients develop diuretic resistance. One of the major factors that limit the response to diuretics in CRF patients may include the basal reduction of fractional Na⁺ reabsorption which situates at the upper limit of the diuretic response with increased NaCl distal reabsorption. The studies performed in this regard have demonstrated the corresponding reduction of transport protein expression for Na⁺ in the proximal tubule and Na⁺/K⁺ ATP-ase but a relative increase per residual nephron of three to fourfold in expression of protein for the BSC-1 and TSC. The diuretic secretion may be inhibited by organic anions – urate and acidosis present in CRF.⁷ In renal failure, the reduction of the renal flux limits the diuretic delivery at the action site and this requires the use of large doses.

Nephrotic syndrome

In the nephrotic syndrome a reduction of the diuretic response is induced. Several mechanisms are involved. Some of them are represented by the increase of distal reabsorption of Na⁺ and this leads to the ineffectiveness of all diuretics, Na⁺ deriving from these being entirely reabsorbed at distal level. A major contribution to this is also brought by RAAS chronic activation (hyperaldosteronism increasing Na⁺ reabsorption in the distal tubules and collecting ducts) and by reduced response to atrial natriuretic peptide. Hypoalbuminemia reduces the overflow of active form of furosemide in kidneys by reduction of the binding form as well as by the increase of diuretic distribution volume and decrease of plasmatic concentration. Hypoalbuminemia favors the furosemide degradation through glucuronidization. On the other hand, the intratubular albumin which is bound to active form of furosemide reduces its access to the action site.⁷ The binding to plasmatic and tubular albumin can be

diminished by the use of bucolome, a nonsteroidal anti-inflammatory agent that amplifies the diuretic effect of furosemide.⁶ The strategies used to improve loop diuretic response are: dose increase, the use of thiazide diuretics to reduce the distal reabsorption and reduce to maximum the albumin excretion (use of IECA with predominant tissues fixation, angiotensin receptor blockers, limitation of the protein intake).

Other drugs, such as warfarin and sulfisoxazole, may release furosemide from plasmatic and/or tubular albumin, but did not show effectiveness in practical use.⁶ In cases of severe hypoalbuminemia, 30 mg of furosemide mixed ex vivo with 25g of albumine could be administered, this generating the increase of active furosemide availability at its primary site of action.^{5,7,33}

Hepatic cirrhosis

In hepatic cirrhosis the excretion rate curve for loop diuretics - natriuresis is shifted downward and to the right. It seems that Na⁺ reabsorption is increased in the proximal and distal tubule but changes at Henle's loop may be also observed. Unless patients have diminished renal function, they deliver normal amounts of diuretic into the urine.⁸

Arterial hypertension

In the experimental studies on spontaneously hypertensive rats (SHR) a markedly up-regulation in BSC-1 expression and a moderate increase of Na⁺-K⁺-ATPase- α 1, NHE-3 and aquaporine-2 channels have been observed.³² Other studies were performed using experimental models of prenatal programmed hypertension and revealed that prenatal programming of hypertension requires transcriptional up-regulation of BSC-1 in ascending limb of Henle and TSC in the distal convoluted tubule.³⁴ This is new proof for involvement of ion transmembrane transport alteration (especially Na⁺) and of aquaporin channels, in the pathogenesis of essential hypertension and support the use of diuretics especially thiazide as first or among first treatment choices in hypertension.³⁵⁻³⁷ In arterial hypertension small doses of thiazide or thiazide-like diuretics are used and they reduce stroke, coronary disease and mortality. In moderate arterial hypertension, thiazide drugs are combined with various antihypertensive drugs such as betablockers, ACEI, angiotensin receptor blockers or potassium sparing diuretics.^{35,37} Loop diuretics are used in severe arterial hypertension with impaired renal function or with marked salt and water retention. Potassium sparing diuretics are associated to prevent hypokalaemia. Spironolactone and eplerenone may prove efficient alone in the arterial hypertension from primary or secondary hyperaldosteronism, in those

with prednisolone therapy and in hypertension with increased RAAS activity.²

CONCLUSIONS

In the last years, great progress has been accomplished in understanding the exact mechanisms for the main classes of diuretic drugs. The effectiveness of diuretic therapy is diminished in time mainly due to some adaptation physiological mechanisms, some factors related to disease pathogenetics or defective management. The physician has to be familiar with these mechanisms in order to adopt the most appropriate decision concerning the drug doses, drug administration interval or combinations with other drugs which interfere with adaptation mechanism.

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