Site and mechanism of the action of diuretics

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Abstract

The mechanism of action of diuretics can be established by studying the molecular mechanism of action, the site of action within the nephron, and the relationship between the pharmacokinetics of the diuretic and its effect. The molecular mechanism of action is known for diuretic agents such as acetazolamide (carbonic anhydrase), theophylline (phosphodiesterase), digitalis glucosides (Na-K-ATPase), spironolactone (aldosterone antagonist) and dopamine (specific receptors?). The "receptor" for the clinically most important diuretics, i.e. loop diuretics, thiazides, and other potassium-sparing diuretics is, however, unknown. It appears from recent studies of the ion transport in the diluting segment that there probably is a sodium-chloride co-transport in this segment and that loop diuretics specifically inhibit the active chloride transport. The main site of diuretic action is well established for the different groups of diuretics: carbonic anhydrase inhibitors act on the proximal tubulus, loop diuretics on the diluting segment, thiazides on the cortical diluting segment/distal tubulus, and potassium-sparing agents on distal tubulus/collection ducts. Moreover, some diuretics have additional tubular sites of action. It is also important to realize that other effects of diuretics, e.g. inhibition of the tubuloglomerular feedback mechanism or renal and extrarenal hemodynamic effects, can modify the tubular diuretic effect. Finally, the renal handling of diuretics is of importance to the diuretic effect by determining the concentration of the drug at the "receptor" site(s). It is emphasized that knowledge of the different aspects of the mechanisms of action of diuretics is a prerequisite for rational use of diuretics, clinically as well as experimentally.

Key words: Diuretics, kidney, pharmacokinetics, mechanism of action.

<table>
<thead>
<tr>
<th>Diuretic</th>
<th>Na⁺</th>
<th>K⁺</th>
<th>Ca²⁺</th>
<th>Mg²⁺</th>
<th>Zn²⁺</th>
<th>Cl⁻</th>
<th>HCO₃⁻</th>
<th>PO₄³⁻</th>
<th>EF Na¹ (%)</th>
<th>Acid/base effect (Blood)</th>
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<tr>
<td>Carbonic anhydrase inhibitors</td>
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<td>+</td>
<td>5 - 8</td>
<td>Acidosis</td>
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<td>Thiazides and related substances</td>
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<td>5 - 10</td>
<td>Alcalosis</td>
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<td>Loop diuretics</td>
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<td>Spironolactone</td>
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<td>3 - 5</td>
<td>(Acidosis)</td>
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<tr>
<td>Amiloride (Triamterene)</td>
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<td>3 - 5</td>
<td>(Acidosis)</td>
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¹ EF = fractional sodium excretion (excreted sodium in % of filtered load).

Table 1. Urinary excretion of electrolytes and acid/base effects of different diuretics. [++, +, (+) = degree of increase; 0 = no effect; -- = decrease].

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Diuretics differ in their maximal efficiency, in their electrolyte excretion pattern and in their acid/base effects (Table 1). These differences reflect differences in the mechanism and site of action of the diuretics and constitute the necessary background for the rational treatment of edemas. Moreover, this knowledge is a prerequisite for the frequent use of diuretics as tools in studies of renal physiology. The different aspects of the mechanism of action of diuretics have been reviewed extensively (Goldberg 1973, Burg 1976, Eknayan et al. 1976, Seely & Dirks 1977), including studies of structure-activity relationships (Koechel 1981). The mechanism of action of diuretics can be determined by studying a) the molecular mechanism of action, b) their site of action within the nephron, and c) the relationship between pharmacokinetics and diuresis. The purpose of this paper is to give a brief summary of our current knowledge about the former two aspects of the action of different diuretics. It should be pointed out here that the renal handling of diuretics (cf. Figure 1) is of importance to the diuretic effect by determining the concentration of the drug at the "receptor" site. The determinants of access of diuretics to their site of action has been reviewed elsewhere (Odlind 1983). Pharmacokinetic aspects and metabolic effects of diuretics are discussed elsewhere in this volume by other authors.

**Molecular mode of action**

Diuretics can reduce the reabsorption of electrolytes a) by affecting the membrane permeability of ions in the tubules, b) by a direct effect on a specific ion transport system, or c) by interfering with the energy supply to an ion transport system. The molecular mode ("receptor") of diuretic action is established for diuretic agents such as acetazolamide (carbonic anhydrase), theophylline (phosphodiesterase), digitalis glycosides (Na-K-ATPase), spironolactone (aldosterone antagonism), and dopamine (specific receptors). However, as will be discussed below, the "receptor" for most of the clinically important diuretics (thiazides, loop diuretics, amiloride) is still unknown.

**Site of action**

The tubular site of action of the different diuretics has been rather well established in experimental studies, using for instance isolated perfused tubules (Burg 1976), micropuncture (Seely & Dirks 1977), free water clearance (Eknayan et al. 1976), and other techniques. It is important to realize the limitations of the respective techniques in such studies. Due to the functional integration of different nephron segments, registered changes in the ion reabsorption may be secondary to direct effects on segments other than those under observation. Moreover, the net diuresis of drugs with effects on proximal tubules is blunted by a compensatory increase of the reabsorption in more distal parts of the nephron. Such pitfalls can be avoided by studying the direct effects of diuretics on different isolated perfused tubular segments (Burg 1976). However, this technique does not take into account the effect of diuretics on for instance tubulo-glomerular feedback mechanisms or renal hemodynamics. It is not possible to study the site of action of diuretics directly in the human kidney but an indirect way is to study the effects of diuretics on the

CARBONIC ANHYDRASE INHIBITORS

Acetazolamide exerts its diuretic effect by inhibition of renal carbonic anhydrase (Maren 1977); it is probably the anion form of the sulphonamide, SO$_2$NH$^-$ that binds to a zinc atom at the active site of the enzyme. Inhibition of carbonic anhydrase reduces the active secretion of hydrogen over the luminal membrane of proximal tubular cells (Figure 2), causing a reduced reabsorption of filtered bicarbonate in these cells. The diuretic effect is thus due to an increased bicarbonate and sodium excretion (Table 1). The acute diuretic efficiency is small, due to a compensatory increase of the electrolyte reabsorption in the distal parts of the nephron, and due to an activation of the tubulo-glomerular feedback mechanism, leading to a marked reduction in GFR (Persson & Wright 1982). Moreover, after repeated administration metabolic acidosis develops within a few days with a subsequent further decrease of the diuretic effect. According to Pushchett (1981), there is a relationship between the renal handling of phosphate and carbonic anhydrase activity. Thus, it is considered that a phosphaturic effect reflects a proximal tubular effect of sulphonamide diuretics. Acetazolamide also inhibits carbonic anhydrase in the distal tubules, an effect which reduces hydrogen ion secretion and increases the potassium secretion in this segment.

![Figure 2. The site of action of diuretics. CAi = carbonic anhydrase inhibitors, LOOP = loop diuretics, THIA = thiazides and related diuretics, ALD antag = aldosterone antagonists, e.g. spironolactone, AMIL = amilorid. The shaded areas indicate the main site of diuretic action and the striped areas denote possible or established additional sites.](image)

In 1973, Burg and coworkers published their studies (Burg 1976) of the electrical properties of isolated perfused segments of thick ascending limb of the loop of Henle (diluting segment) from rabbits. They demonstrated an active chloride transport in this segment; a finding which has been verified by others (Rocha & Kokko 1973). Moreover, they showed that furosemide very effectively inhibits the active chloride transport in the diluting segment, when it is added to the perfusate; i.e. when it acts from the inside of the nephron. However, when even a ten-fold higher concentration of furosemide was added to the bath – acting from the outside of the nephron – it had little effect. A diuretic action from the luminal (urine) side of the nephron has been confirmed in vivo for e.g. furosemide and piretanide in experimental (Odlind 1978) and human (Odlind & Beermann 1980, Odlind et al. 1983) studies. The diuretic effect is dependent on active tubular secretion and thus on the

LOOP DIURETICS

The loop diuretics, e.g. furosemide, ethacrynic acid and bumetanide, are very effective diuretic agents; the fractional excretion of sodium chloride may reach 25% (Table 1). Some new loop diuretics, e.g. piretanide (Odlind et al. 1983), azosemide (Brater 1979), muzolemine (Canton et al. 1981), ozolinone (isomer; Greven et al. 1980) and indacrinone (isomer; Tobert et al. 1981) have been developed.
tubular fluid concentration of the drug. It has recently become clear that loop diuretics inhibit the active chloride transport in a number of other cells and epithelial membranes (Kanaky 1980, Candia et al. 1981).

**Site of action**

An action of loop diuretics on the *diluting segment* (Figure 2) is well documented from a number of other studies, utilizing for instance micropuncture techniques (Seely & Dirks 1977) and determinations of the local metabolic rate in the kidney (Lie et al. 1974). It has also been demonstrated that loop diuretics reduce both the ability to concentrate the urine during hydropenia (an effect of the medullary diluting segment) and to dilute the urine after a water load (an effect of the whole diluting segment; Eknoyan et al. 1976). The cortico-medullary osmolality gradient is abolished by the loop diuretics, which tend to make the urine iso-osmotic with blood.

*A proximal tubular effect* has been demonstrated for furosemide, provided that the extracellular volume is maintained by supplementation of the urine volume loss (Burke et al. 1972). This effect has, at least partly, been attributed to the inhibition of carbonic anhydrase (cf. above). However, since chronic administration of loop diuretics usually leads to metabolic alkalosis, it is likely that the proximal tubular effects of furosemide only give a minor contribution to the final diuresis. The same conclusion would be expected for other loop diuretics, particularly for ethacrynic acid, the proximal tubular effects of which are ambiguous (Puschett 1981).

Furosemide increases the fractional sodium reabsorption in the *distal tubules*, due to an increased delivery to this segment, secondary to the inhibition of the sodium chloride reabsorption in the diluting segment (Giebisch 1976). Loop diuretics may also have a *direct* effect on the ion transport in the early distal tubules (Stoner et al. 1974).

**Molecular mode of action**

The precise molecular mechanism of the diuretic action of loop diuretics is not known. The concept of a "receptor" for loop diuretics is supported by the finding of a stereo-selectivity in the diuretic effect of ozolinone (Greven et al. 1980). Table II summarizes some of the theories proposed for the molecular mode of action of loop diuretics based on studies especially with furosemide. It is unlikely that loop diuretics act by competitive anion inhibition; some of the new loop diuretics are in fact bases. Most loop diuretics cause only a weak

| 1. Competitive anion (chloride) inhibition |
| 2. Carbonic anhydrase                      |
| 3. Na-K ATPase                            |
| 4. Cl-HCO₃ ATPase                         |
| 5. Adenylate cyclase                       |
| 6. Intermediary metabolism                |
| 7. Prostaglandin 15-OH-dehydrogenase      |

Table II. Theories for the molecular mode of action of loop diuretics.

An inhibition of carbonic anhydrase *in vitro*. An inhibition of sodium, potassium-dependent-ATPase has been reported, especially for ethacrynic acid (Klahr 1976). This seems incompatible with the active chloride transport being the primary effect. However, a coupling (co-transport) between ouabain-sensitive Na-K-ATPase – dependent sodium and the ATPase-independent chloride transport has been proposed (Epstein et al. 1980, Eveloff et al. 1981) and suggests the possibility of separate mechanisms of sodium-chloride transport inhibition in the diluting segment. The inhibition of Cl-HCO₃-ATPase has only recently been demonstrated in the mammalian kidney (Gassner & Komnick 1982). Inhibition of adenylate cyclase has been demonstrated for several diuretics (Dousa 1976). This is of special interest, since it has been proposed that ethacrynic acid exerts its diuretic effect by inhibition of ADH (Abramow 1974). Interestingly, it has recently been suggested that ADH can stimulate the active chloride transport in the
medullary diluting segment (Hall & Varney 1980). It is also well known that furosemide can increase the free-water clearance in SIADH syndrome (Decaux et al. 1981). It is very difficult to interpret the numerous in vitro studies of the effects of loop diuretics on the renal intermediary metabolism (Klahr 1976); very high doses have been used in many studies and the relationship with diuresis is often unclear. Interaction between loop diuretics and the prostaglandin system has been studied extensively (Gerber 1983). It is unlikely that the tubular actions of loop diuretics are mediated by prostaglandins (Data et al. 1978, Gerber 1983).

**Inhibition of the tubulo-glomerular feedback mechanism**

An important prerequisite for the diuretic effect of loop diuretics is an effective inhibition of the tubulo-glomerular feedback (TGF) mechanism, which has been demonstrated for these diuretics (Wright & Schnermann 1974, Odlin et al. 1983). The importance of this action can be illustrated by the poor diuretic effect of ouabain. According to Kil and co-workers (Lie et al. 1974) ouabain is as effective as the loop diuretics for the inhibition of the sodium chloride transport in the diluting segment; the difference in diuresis probably being due to the inability of ouabain to inhibit the TGF with a great resulting reduction of the GFR. The effect of furosemide on the GFR is probably related to the degree of extracellular volume contraction. Thus, GFR can be maintained by substituting the urine volume lost caused by the diuretic.

**Renal hemodynamic effects**

It is generally thought that loop diuretics increase the total renal blood flow. A redistribution of the cortical blood flow, from superficial to deeper (zone 2 and 3) zones, has been demonstrated (Birch et al. 1967). This effect is probably of minor importance to the diuretic effect (Data et al. 1978, Greven et al. 1980), and may even be secondary to the diuretic effect (Gerber & Nies 1980). It has been postulated that an increase in the medullary blood flow could contribute to the diuretic effect of loop diuretics (Stowe & Hook 1970). However, in an experimental study of the rat, furosemide markedly decreased the medullary blood flow in all areas (Odlin et al. 1982). It has been suggested that the renal hemodynamic effects of loop diuretics are mediated by prostaglandins (Gerber 1983), although opposing opinions exist and an alternative explanation is that this effect is obtained by direct vasodilatation (Duchin et al. 1977).

Loop diuretics stimulate the renin release in several ways (Hummerich et al. 1981, Gerber 1983). Partly, this may be a direct effect of the loop diuretics, involving macula densa and probably mediated by prostaglandins. Moreover, the renin release may be secondary to extracellular volume contraction which, during long-term treatment with diuretics, will lead to secondary hyperaldosteronism.

**Extrarenal hemodynamic effects**

Loop diuretics have been shown to increase the capacitance of the vascular bed, which will reduce the venous return to the heart and therefore also reduce the left atrial pressure (Dikshit et al. 1973). Such an effect is desirable in the treatment of pulmonary edemas and has been demonstrated immediately after intravenous administration of loop diuretics. This effect may be unrelated to the diuretic effect (Dikshit et al. 1973), although opposing opinions exist (Hesse et al. 1975, Mukherjee et al. 1981). It has been proposed that the peripheral vascular effects of loop diuretics are caused by substances (prostaglandins?) liberated from the kidney (Bourland et al. 1977).

**Other effects of loop diuretics**

Furosemide may reduce the intracranial pressure (Thilmann & Zeumer 1974), probably by reducing the production of cerebrospinal fluid (Vogh & Langham 1981). Loop diuretics can also increase the cerebral blood flow (Rovere & Screenim 1974) and it has been
proposed that loop diuretics may have a protective action against cerebral damage after trauma (Miller et al. 1980). The mechanism behind this is unclear.

Furosemide has also been shown to reduce the gastric acid secretion (Ayalon et al. 1980) and to increase the biliary secretion.

**THIAZIDES**

The most important qualitative difference between loop diuretics and thiazides is the hypocalciuric effect of the latter agents (Constanzo & Windhager 1978). This effect probably contributes to the prophylactic effect of thiazides on renal stone formation. The hypocalciuric effect is accompanied by rising total and ionized serum calcium levels, which persists during long-term treatment. It is not known whether this action — or the calciuric effect of loop diuretics — affects bone mineralization. The mechanism behind the hypocalciuric effect of the thiazides is not clear and may comprise ECV contraction, stimulation of the PTH release, enhancement of the PTH effects and a direct effect on calcium reabsorption in the distal tubules.

The thiazides differ with respect to pharmacokinetic properties, such as potency and plasma half-life, and their in vitro carbonic anhydrase inhibition. The latter explains the varying degree of bicarbonate excretion brought about by the thiazides, as a result of an action on the proximal tubular cells (Figure 2). The similarity of the saluretic effects of thiazides suggests, however, that these drugs have another and more important common mechanism of diuretic action.

**Site of action**

It has been shown that thiazides reduce the free water production (CH₂O) in hydrated individuals but free water reabsorption (TCH₂O) during hydropenia is not affected (Eknoyan et al. 1976). This suggests that thiazides act predominantly on the cortical diluting segment (Figure 2); an action which may help to explain the therapeutic effect of thiazides in diabetes insipidus. Micropuncture studies have clearly shown an effect of thiazides on the distal tubulus (Figure 2); (Seely & Dirks 1977). Unfortunately, there are no data available on the effect of thiazides on isolated perfused tubules.

**Mechanism of action**

Despite their extensive clinical use for 25 years, very little is known about the molecular action of thiazides (Klahr 1976). Apparently they do not give specific effects on the sodium or chloride transport in experimental models. The potency of different thiazides is rather well correlated to their lipid solubility and to their accumulation in subcellular fractions from isolated tubules (Duggan 1966). It is unlikely that their main saluretic effect is due to the inhibition of carbonic anhydrase, since this varies greatly for the different thiazides in vitro and since chloruresis is the dominating anion effect. It has been shown that thiazides inhibit gluicosis in the medulla (Baer & Beyer 1972), but this has probably nothing to do with the diuretic effect (Bowman et al. 1973) which is supposed to be a cortical effect. Thiazides can block the renal uptake of non-strified fatty acids (Barac-Nieto & Cohen 1968) and thereby reduce the oxidative metabolism. The diuretic effect of thiazides appears to be independent of the prostaglandin system (Fanelli et al. 1980). It has been proposed that the antihypertensive effect of thiazides could be due to a direct vascular effect to lower the peripheral resistance (Jones & Nanra 1979), perhaps by increasing the prostacyclin production in the vessel wall (Webster et al. 1980). However, most of the evidence suggests that the antihypertensive effect is secondary to the natriuretic effect (Wilson & Fries 1959, Bennet et al. 1977) and to the extracellular volume contraction (van Brummelen & Schalekamp 1980). (This is discussed further by Hjermndahl in this volume.)

**Other renal effects**

Thiazides usually reduce the glomerular filtra-
tion. Experimental studies show only a small inhibitory effect of thiazides on the tubulo-glomerular feedback mechanism. Renal blood flow is usually decreased.

**Metolazone**

It has been suggested that this diuretic has several properties which differ from those of the "ordinary" thiazides. For instance, the sodium chloride reabsorption in the proximal tubules is inhibited despite the fact that metolazone does not reduce carbonic anhydrase activity (Fernandez & Puschett 1973). Furthermore, it has been suggested that metolazone decreases the GFR less than other thiazides do (Fernandez & Puschett 1973). Finally, it has been shown that metolazone is an active diuretic agent also in patients with severely reduced renal function (Dargie et al. 1972), while it is usually thought that thiazides have little or no diuretic effect in uremia (Reubi & Cottier 1961). This may, however, be a question of dose. Very high doses of metolazone may be needed to get an effect in uremic patients (Dargie et al. 1972). It remains to be established if these clinical effects of metolazone are unique among the thiazides.

**POTASSIUM-SPARING DIURETICS**

Loop diuretics and thiazides reduce serum potassium levels by several mechanisms. One important factor is an increased sodium/potassium exchange in the distal tubules secondary to the diuretic-induced increase in the sodium load to this segment. Moreover, during long-term treatment, secondary hyperaldosteronism and hypochloremic alkalosis will contribute to hypokalemia. A magnesium deficiency may aggravate the symptoms of hypokalemia.

**Aldosterone antagonists**

Spironolactone is a weak diuretic; the maximal excretion of sodium is about 3–5% of the filtered load (Table 1). The substance may reduce the excretion of magnesium, but it has little effect on the excretion of calcium, on GFR or on renal blood flow. Spironolactone gives metabolic acidosis (Hulter et al. 1981).

**Site and mechanism of action**

Spironolactone exerts its effects (renal as well as extrarenal) by a competitive inhibition of aldosterone on the receptor level (Corvol et al. 1981). This is probably brought about by an inability of the antagonist-receptor complex to be translocated into the nucleus. This stops the production of an aldosterone-dependent protein, which is responsible for the electrolyte effects. Spironolactone acts on the distal tubules and collecting ducts (Figure 2) where specific binding sites for aldosterone have been demonstrated (Farman et al. 1982). It has also been shown that spironolactone can inhibit the biosynthesis of aldosterone *in vitro* but this is probably of little importance for the diuretic effect *in vivo* (Corvol et al. 1981). The metabolism of spironolactone is extremely complex and a large number of metabolites has been identified (Karim 1978), several of which contribute to the net effect of spironolactone.

**Amiloride**

Amiloride is also a weak diuretic; maximal sodium excretion is 3–5% of the filtered load (Table 1). It gives metabolic acidosis (Hulter et al. 1980). Its clinical importance is due to its potassium-sparing effect, which is independent of aldosterone.

**Site and mechanism of action**

Amiloride affects the electrolyte transport in the distal tubules and collecting ducts (Figure 2). In micropuncture studies it has been shown, that this effect is predominantly exerted from the luminal side of the nephron (Stoner et al. 1974). The exact mechanism of action is unknown. In experimental studies on frog skin and toad bladder it has been shown that amiloride reduces the sodium permeability and transepithelial potential difference (Barratt 1976). It is, however, unknown if it is this mechanism that gives the potassium-sparing
effect. An alternative possibility is a direct effect of amiloride on the potassium secretion (Giebisch 1976). Recently, a specific binding of an amiloride analogue has been demonstrated in homogenates from renal cells. This binding was competitively displaced by amiloride and may reflect the presence of a receptor/acceptor for amiloride (Cuthbert & Edwardson 1981). It has been shown that amiloride inhibits the enzymatic properties of kallikrein (Margoliu & Chas 1980), an enzyme primarily located to the luminal membrane in the distal tubules. During long-term treatment with amiloride there may be an "escape" from the potassium-sparing effect which, however, is maintained if the negative potassium balance persists (Hohenegger 1973).

URICOSURIC DIURETICS

Several diuretics with uricosuric properties have been developed (Emmerson 1980). The diuretic effects of tienilic acid appear to be comparable to that of thiazides (Maass & Snow 1979). Clinical use of this drug has been stopped in most countries, due to reports of severe liver damage. Indacrinone is an interesting new diuretic, the stereoisomers of which have very different properties (Tobert et al. 1981). The effect of the + isomer is similar to that of loop diuretics while the + isomer is uricosuric and its diuretic effects are more like those of a thiazide.

REFERENCES


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