BHARATHIDASAN UNIVERSITY

Tiruchirappalli- 620024, Tamil Nadu,
India

Programme: M.Sc., Biomedical Science

Course Title : Pharmacology and Toxicology
Course Code : BM35C7

Unit-III
Pharmacology of Urinary System- Part 2

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Physiology of Urine Formation

- Urine formation starts from **Glomerular Filtration**
- Normally, about **180 L** of fluid is filtered everyday
- All soluble constituents of **blood minus the plasma proteins and lipids,** are filtered at the glomerulus.
- More than 99% of the glomerular filtrate is reabsorbed in the tubules; about **1.5 L** urine is produced in 24 hours.

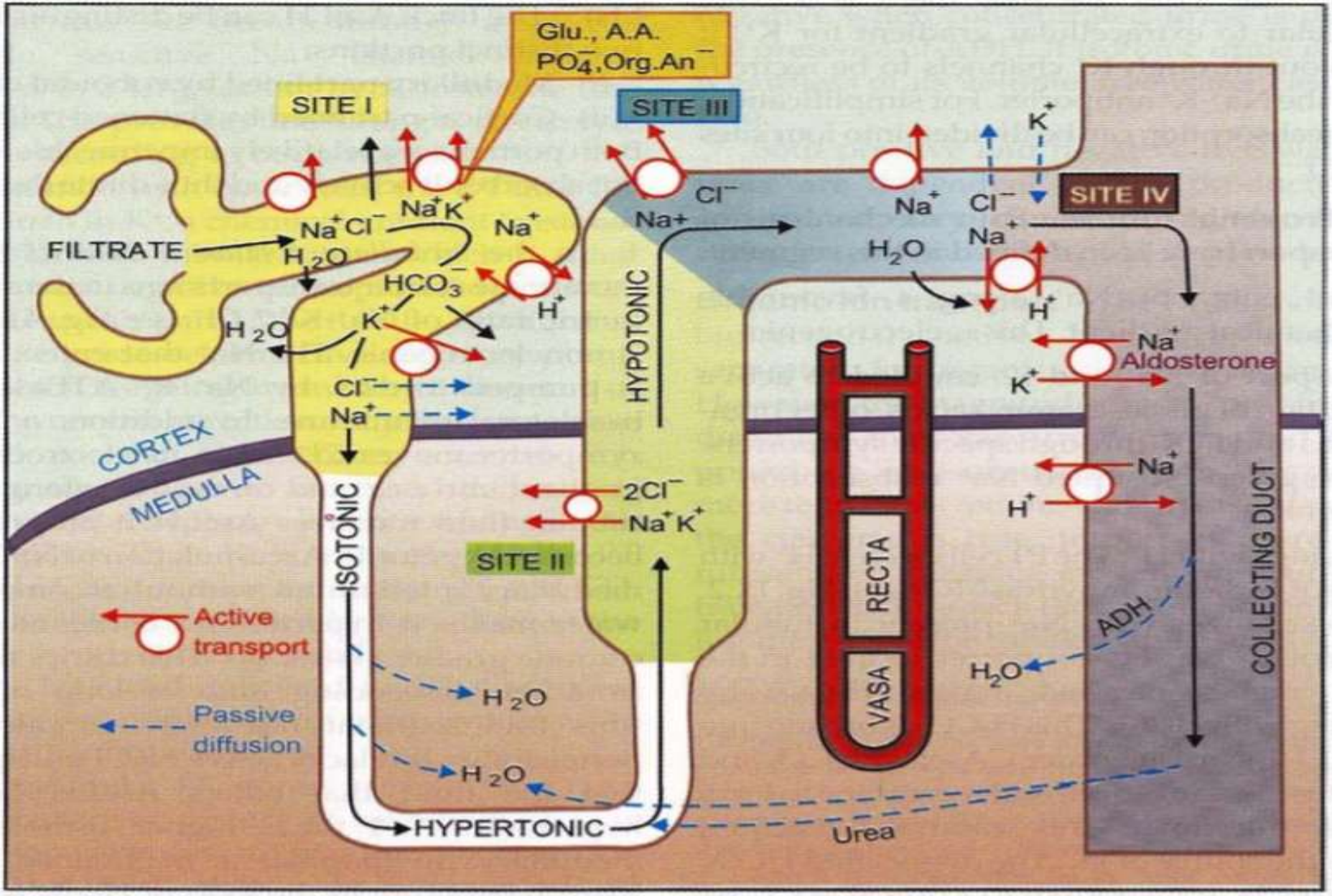


Fig. IX.1: Diagrammatic representation of nephron showing the four sites of solute reabsorption. The thick ascending limb of loop of Henle is impermeable to water; Glu.—Glucose; A.A.—Amino acid; Org. An.—Organic anions.

Site I: Proximal tubule

Four mechanisms of Na+ transport have been defined in this segment.

(a) Direct entry of Na+ along a electrochemical gradient.

(b) Transport of Na+ and K+ coupled to active reabsorption of glucose, amino acids, other organic anions and P04

(c) Exchange with H+: The PT cells secrete H+ with the help of carbonic anhydrase (CAse),H+ ion exchanges with Na+ present in tubular fluid through Na+-H+ antiporter located in the luminal membrane and forms H2C03 by combining with HCO3

(d) The disproportionately large HCO3 acetate, pot, amino acid and other anion reabsorption create passive driving forces **for Cl- to diffuse through the paracellular pathway**

- This takes Na+ and water along to maintain electrical neutrality and isotonicity
- Reabsorption in PT is isotonic.
- Major part of **filtered K+** is reabsorbed in the PT.
- Thus, an isotonic tubular fluid with major changes in composition enters the thin descending limb of loop of Henle.

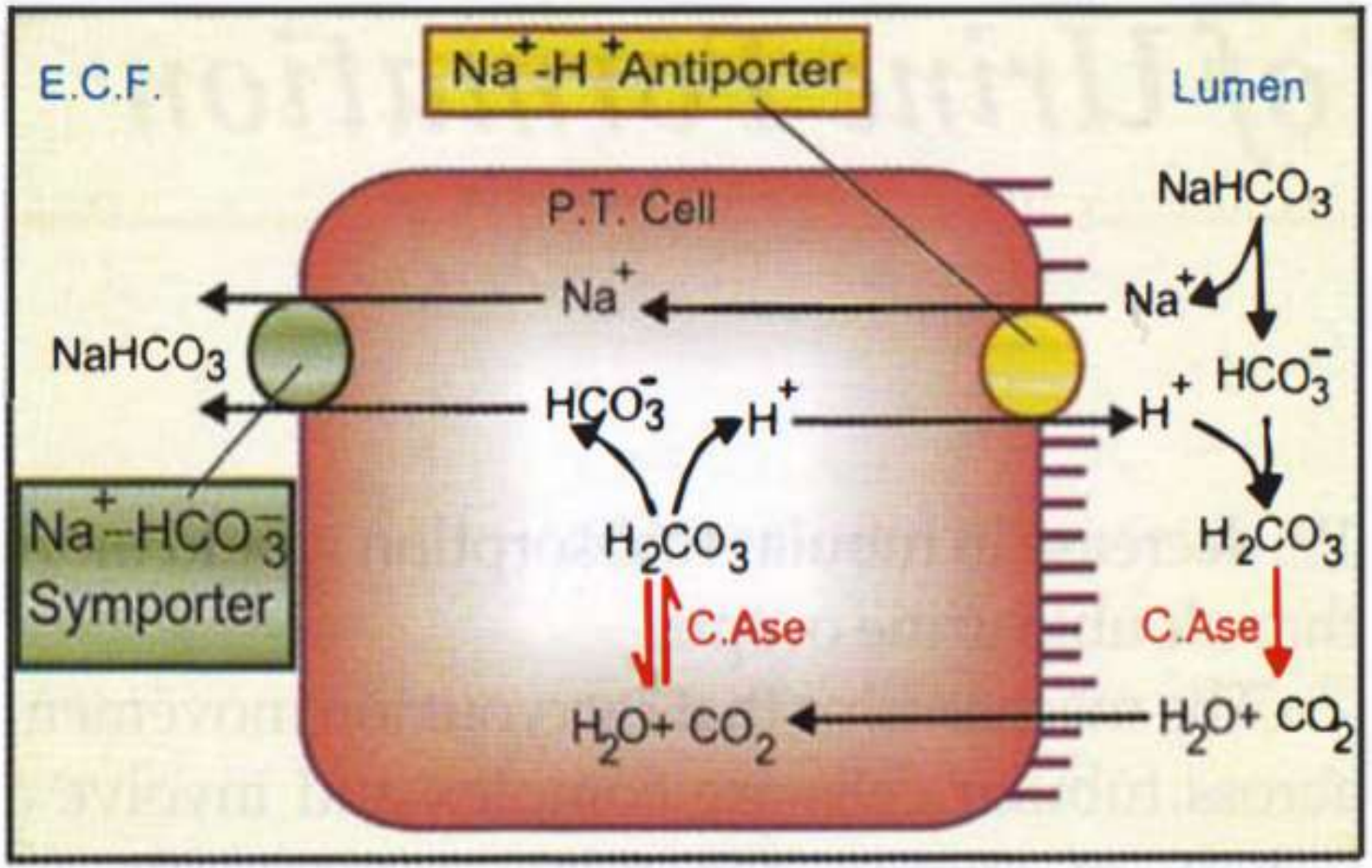


Fig. IX.2: The carbonic anhydrase (C.Ase) mediated bicarbonate absorption in proximal tubule (P.T.)

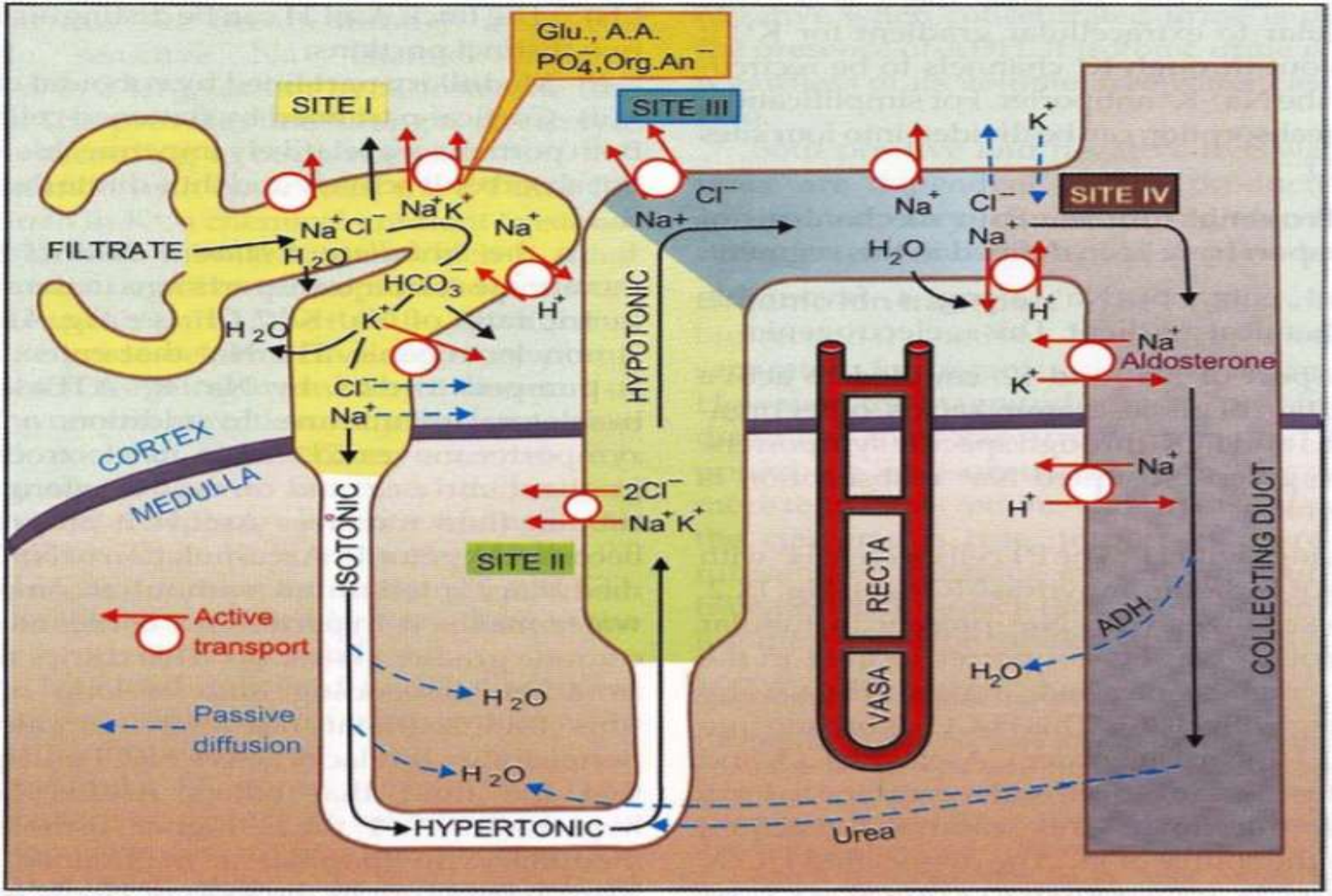
Acetazolamide

- It is a sulfonamide derivative which noncompetitively but reversibly inhibits CAse in PT cells resulting in slowing of hydration of CO2 decreased availability of H+ to exchange with luminal Na+ through the Na+-H+ antiporter.
- Inhibition of brush border CAse retards dehydration of H2C03 in the tubular fluid so that less CO2 diffuses back into the cells.
- The net effect is inhibition of HCO; reabsorption in PT
- Secretion of H+ in DT and CD is also inhibited.

Site II: Ascending limb of loop of Henle (Asc LH)

- The thick AscLH can be distinguished into two distinct portions:
- (i) Medullary part lined by cuboidal cells.
- (ii) Cortical part lined by flattened cells.

- In the medullary portion a distinct luminal membrane carrier transports ions in the stoichiometric ratio of Na+-K+-2 Cl
- The Na+ that enters the cell is pumped to e.c.f. by Na+ K+ ATPase at the basolateral membrane.
- In addition, a Na+-Cl symporter moves cl down its electrochemical gradient into e.c.f. and carries Na+ along



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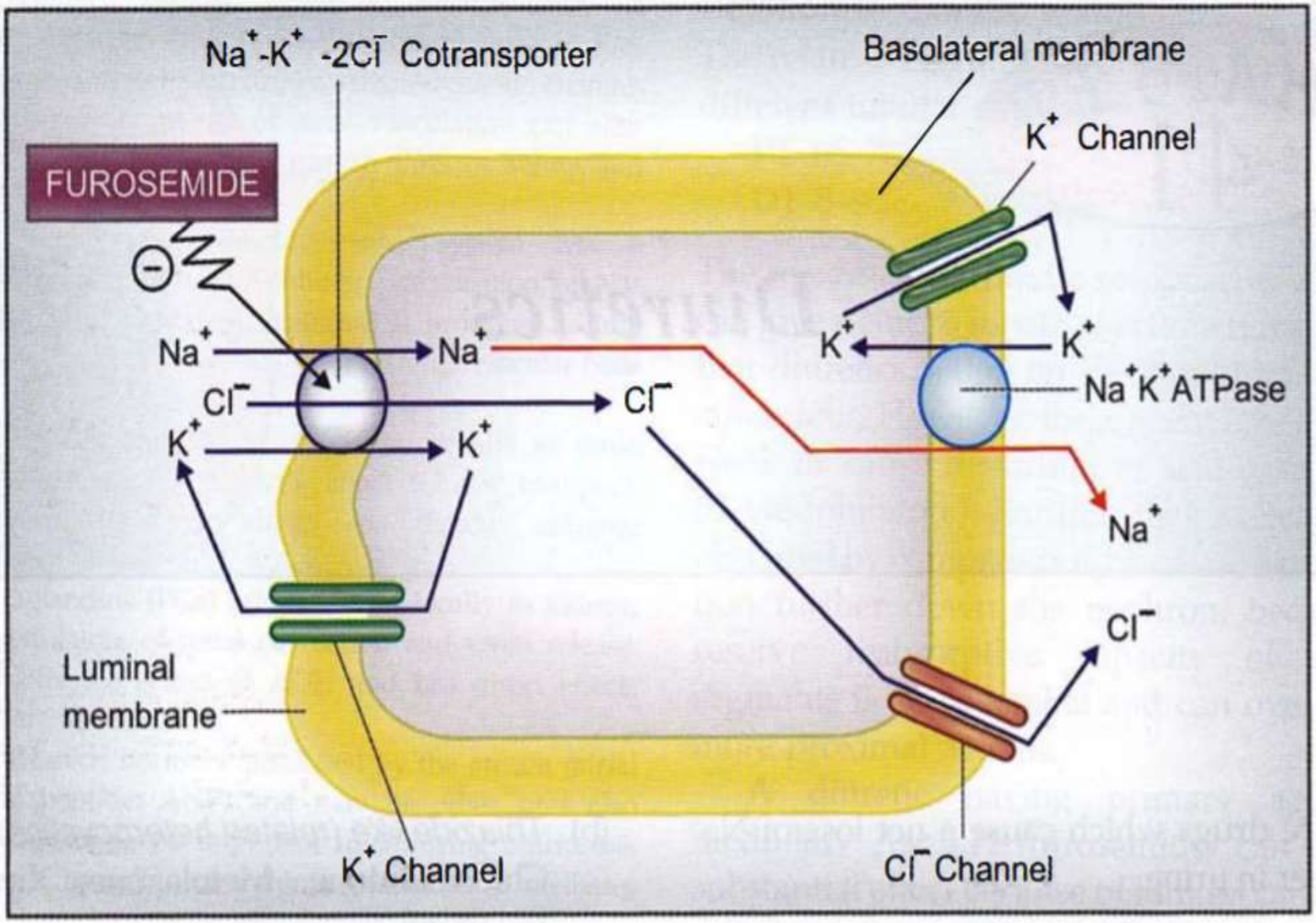


Fig. 41.1: Mechanism of salt reabsorption in the thick ascending limb of loop of Henle (AscLH) cell, and site of action of furosemide on the Na^+-K^+-$2Cl^-$ cotransporter

Molecular mechanism of action

- A glycoprotein with 12 membrane spanning domains has been found to function as the Na+-K+-2Cl- cotransporter in many epithelia performing secretory / absorbing function, including AscLH.
- Luminal membrane of thick AscLH-furosemide attaches to the Cl- binding site of this protein to inhibit its transport function.
- The secretory form is expressed on the basolateral membrane of most glandular and epithelial cells

Pharmacokinetics Furosemide

- Orally bioavailability is about 60%.
- In severe CHF oral bioavailability may be markedly reduced.
- Lipid-solubility is low, and it is highly bound to plasma proteins.
- It is partly conjugated with glucuronic acid and mainly excreted unchanged by glomerular filtration as well as tubular secretion.
- Some excretion in bile and directly in intestine also occurs.
- Plasma tlh averages 1-2 hour but is prolonged in patients with pulmonary edema, renal and hepatic insufficiency.
- Dose Usually 20-80 mg once daily in the morning

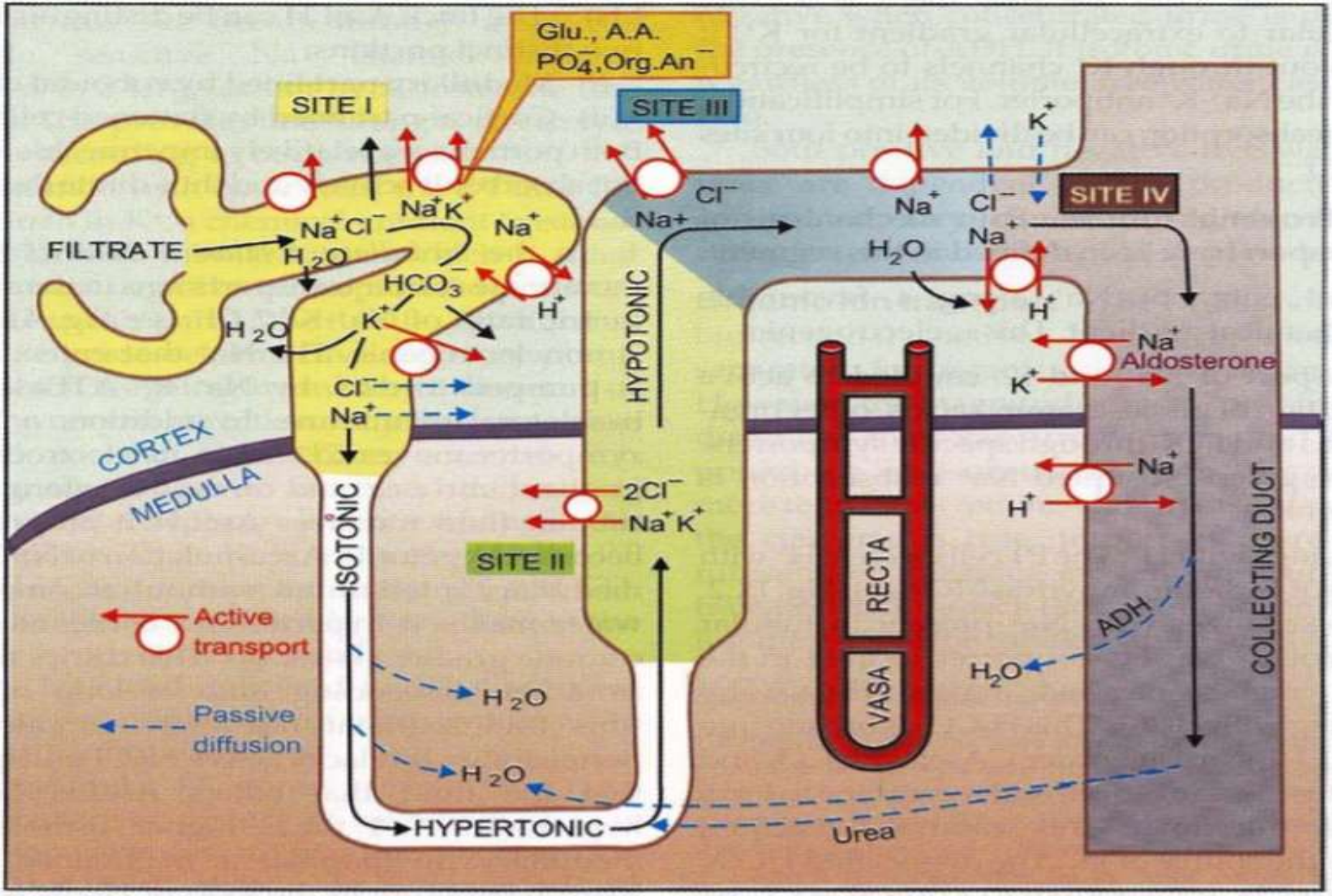
Use of high ceiling diuretics

- 1. Edema
- 2. Acute pulmonary edema
- 3. Cerebral edema
- 4. Hypertension
- 5. Along with blood transfusion in severe
- anaemia, to prevent vascular overload.
- 6. Hypercalcaemia and renal calcium stones:

- Fluid traverses AscLH it progressively becomes **hypotonic.**
- Accumulation of NaCl in the medullary interstitium without accompanying water makes **it hypertonic: Corticomedullary osmotic gradient**
- A higher osmolarity of medullary tip is maintained by the hairpin structure of the loop of Henle acting as passive counter current multiplier and the arrangement of blood vessels as vasa recti with shunt that prevents washing away of the osmotic gradient by progressively reducing blood flow to the inner medulla.

Site III: Cortical diluting segment of loop of Henle

- This segment, also impermeable to water, continues to absorb salt, but here it is through a Na+ -Cl- symporter. Tubular fluid gets further diluted.



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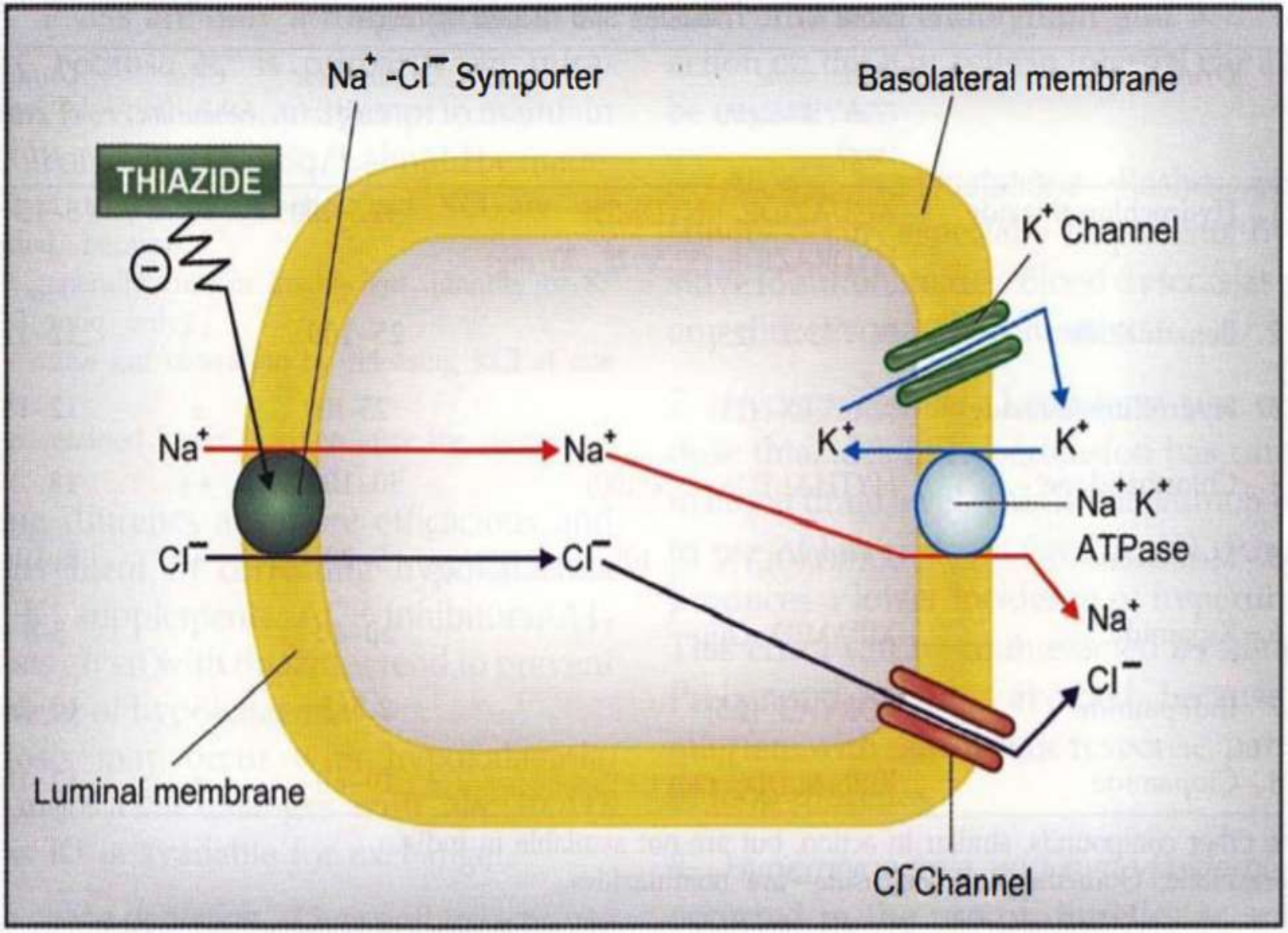


Fig. 41.2: Mechanism of salt reabsorption in early distal tubular cell and site of action of thiazide diuretics on Na^+Cl^- symporter

- These are medium efficacy diuretics with primary site of action in the cortical diluting segment or the early DT (Site III).
- Here they inhibit Na+-Cl- symport at the luminal membrane.

Table 41.1: Thiazides and related diuretics

Drug	*Trade name (Tab. strength) (mg)*	*Daily dose (mg)*	*CAse inhibition*	*Duration of action (Hr)*
1. Hydrochlorothiazide	AQUAZIDE, HYDRIDE HYDRAZIDE (12.5, 25, 50 mg)	12.5–100	+	5–15
2. Benzthiazide	FOVANE (25)	25–100	++	12–18
3. Hydroflumethiazide	NACLEX (25)	25–100	±	12–18
4. Chlorthalidone	HYTHALTON (50,100)	50–100	++	48
5. Metolazone	XAROXOLYN (5, 10)	5–20	+	8–10
6. Xipamide	XIPAMID (20)	20–40	+	5–8
7. Indapamide	LORVAS (2.5)	2.5–5	–	12–24
8. Clopamide	BRINALDIX (20)	10–60	±	12–18

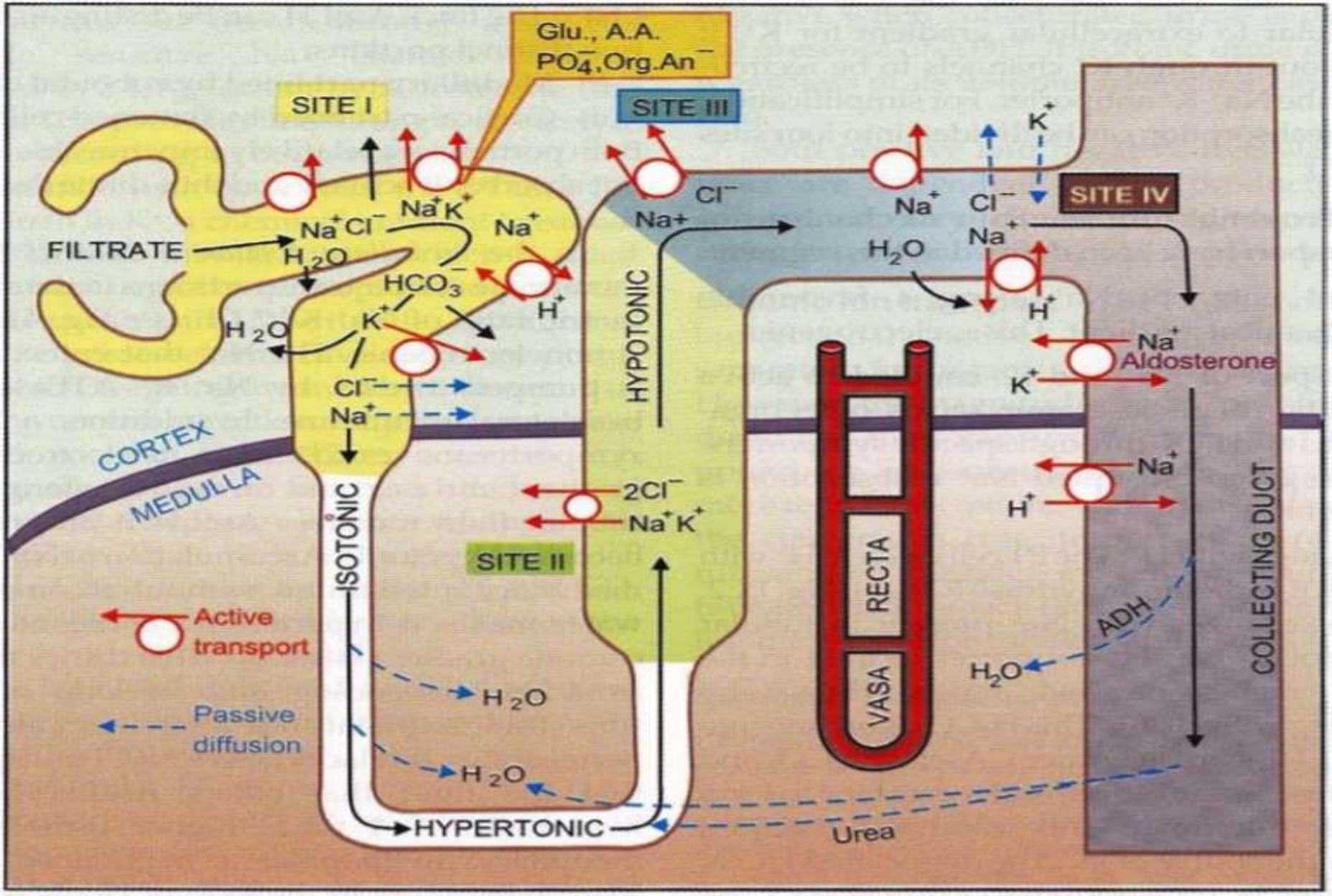
Some other compounds, similar in action, but are not available in India.
Chlorexolone, Quinethazone, Mefruside—are nonthiazides.

Site IV: Distal tubule (DT) and collecting duct (CD)

In the late DT and CD, Na+ is again actively reabsorbed

The cation-anion balance being maintained partly by passive Cl- diffusion and partly by secretion of K+ and H+.

Absorption of Na+ at this site occurs through a specific **amiloride sensitive Na+ channel** and is controlled to a large extent by aldosterone



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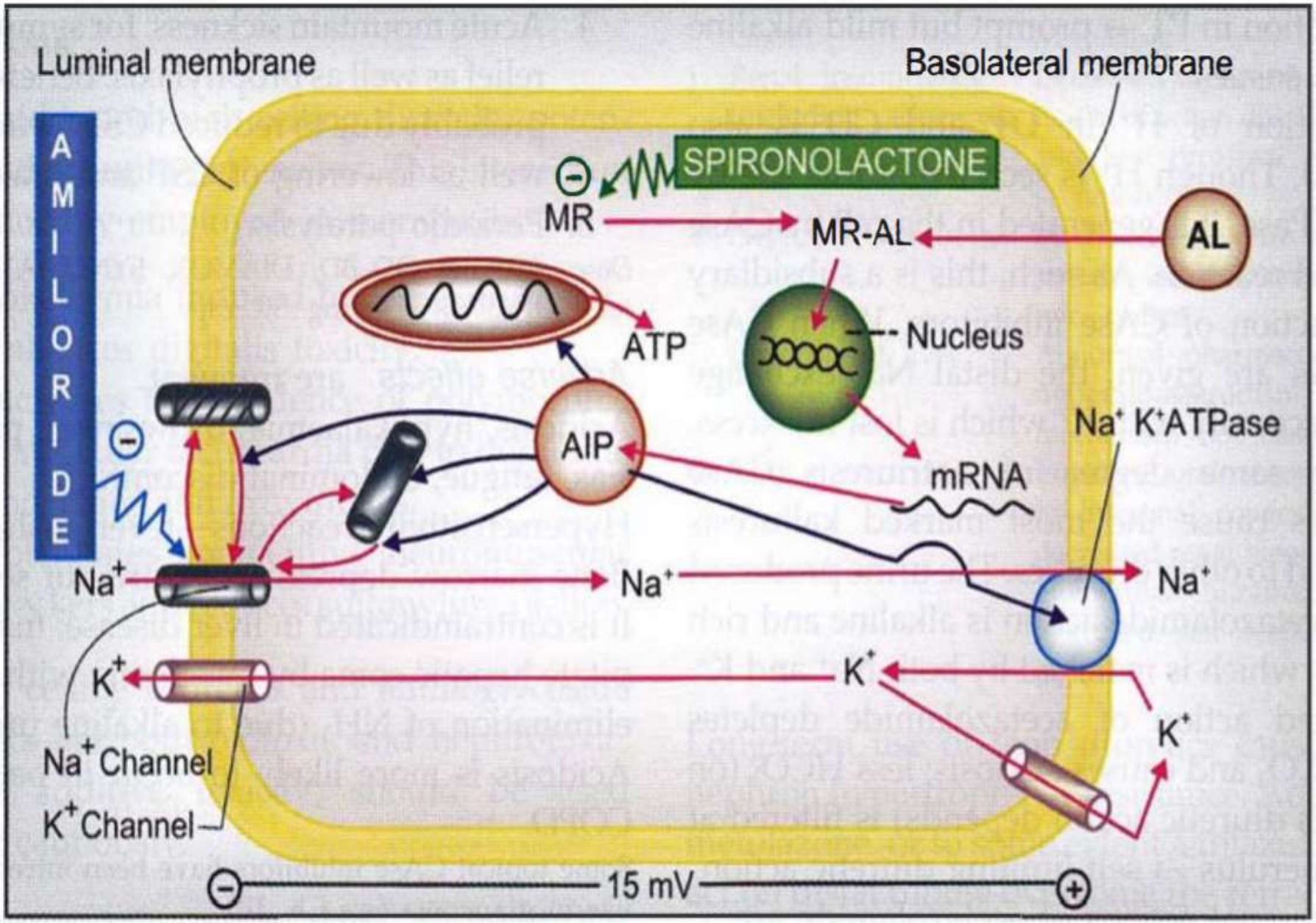

Luminal membrane
Basolateral membrane
AMILORIDE
SPIRONOLACTONE
MR
MR-AL
AL
ATP
Nucleus
AIP
mRNA
Na+ K+ATPase
Na+
Na+
Na+
K+
K+
K+
Na+ Channel
K+ Channel
15 mV

POTASSIUM SPARING DIURETICS

- These are either aldosterone antagonist or
- directly inhibit Na+ channels in DT and CD cells
- to indirectly conserve K+.

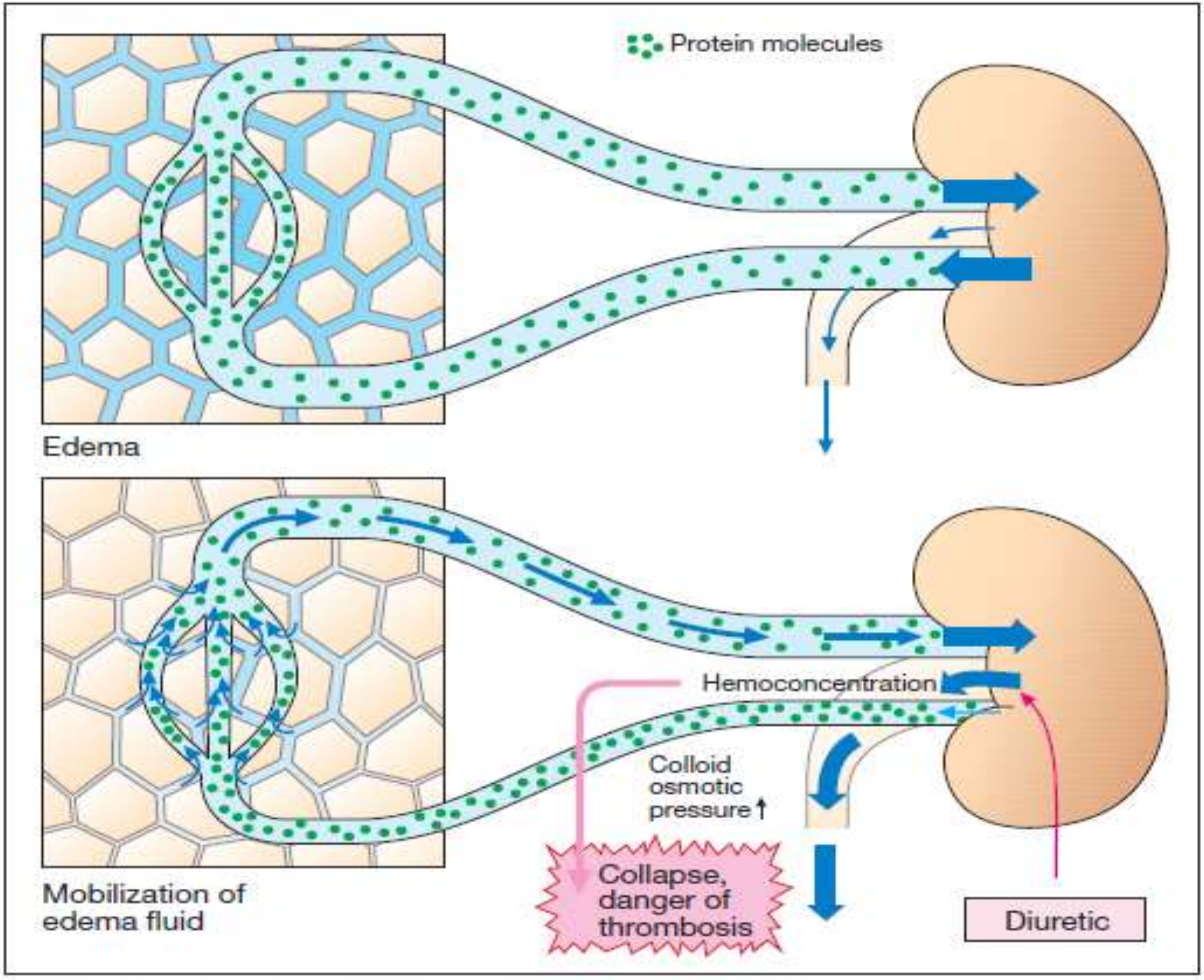
Potassium Sparing Diuretics

- MOA- late distal tubule/collecting duct
- Aldosterone (AL) penetrates the cell from the interstitial side and combines with the mineralocorticoid receptor (MR).
- The complex translocates to the nucleus-promotes gene mediated mRNA synthesis.
- The mRNA then directs synthesis of aldosterone induced proteins (AlPs).
- The AlPs include Na+K+ ATPase and amiloride sensitive Na+ channels.
- AlPs also activate Na+ channel, translocate Na+ channels from cytosolic site to luminal membrane and Na+K+ATPase to basolateral membrane, increase ATP production by mitochondria.
- All these changes promote Na+ reabsorption-more K+ and H+ is secreted indirectly.

- Spironolactone binds to MR, prevents AL action and produces opposite effects.
- Amiloride approaches the Na+ channel from the luminal side and blocks it-reducing the lumen negative transepithelial potential difference which governs K+ and H+ secretion

*Mobilization of edemas **(A)***

- Accumulation of fluid- in extracellular (interstitial) space.
- Increased **renal excretion of Na+ and H2O** causes a reduction in plasma volume with **hemoconcentration.**
- As a result, **plasma protein concentration rises along with oncotic pressure**.
- As the latter operates to attract water, fluid will shift from interstitium into the capillary bed.
- ***The fluid content of tissues thus falls and the edemas recede.***
- The decrease in plasma volume and interstitial volume means a **diminution of the extracellular fluid volume (EFV).**
- Depending on the condition, use is made of: thiazides, loop diuretics, aldosterone antagonists, and osmotic diuretics.



A. Mechanism of edema fluid mobilization by diuretics

Antihypertensive therapy

- *Diuretics* - first choice for lowering elevated blood pressure.
- Even at low dosage, they **decrease peripheral resistance** (without significantly reducing EFV) and thereby normalize blood pressure.

Therapy of congestive heart failure.

- By lowering peripheral resistance
- Diuretics aid the **heart in ejecting blood** (reduction in afterload); **cardiac output and exercise tolerance are increased.**
- Due to the increased excretion of fluid, **EFV and venous return decrease** (reduction in preload).
- Symptoms of venous congestion, such as ankle edema and hepatic enlargement, subside
- **Thiazides (possibly combined with K+-sparing diuretics) and loop diuretics.**

Prophylaxis of renal failure

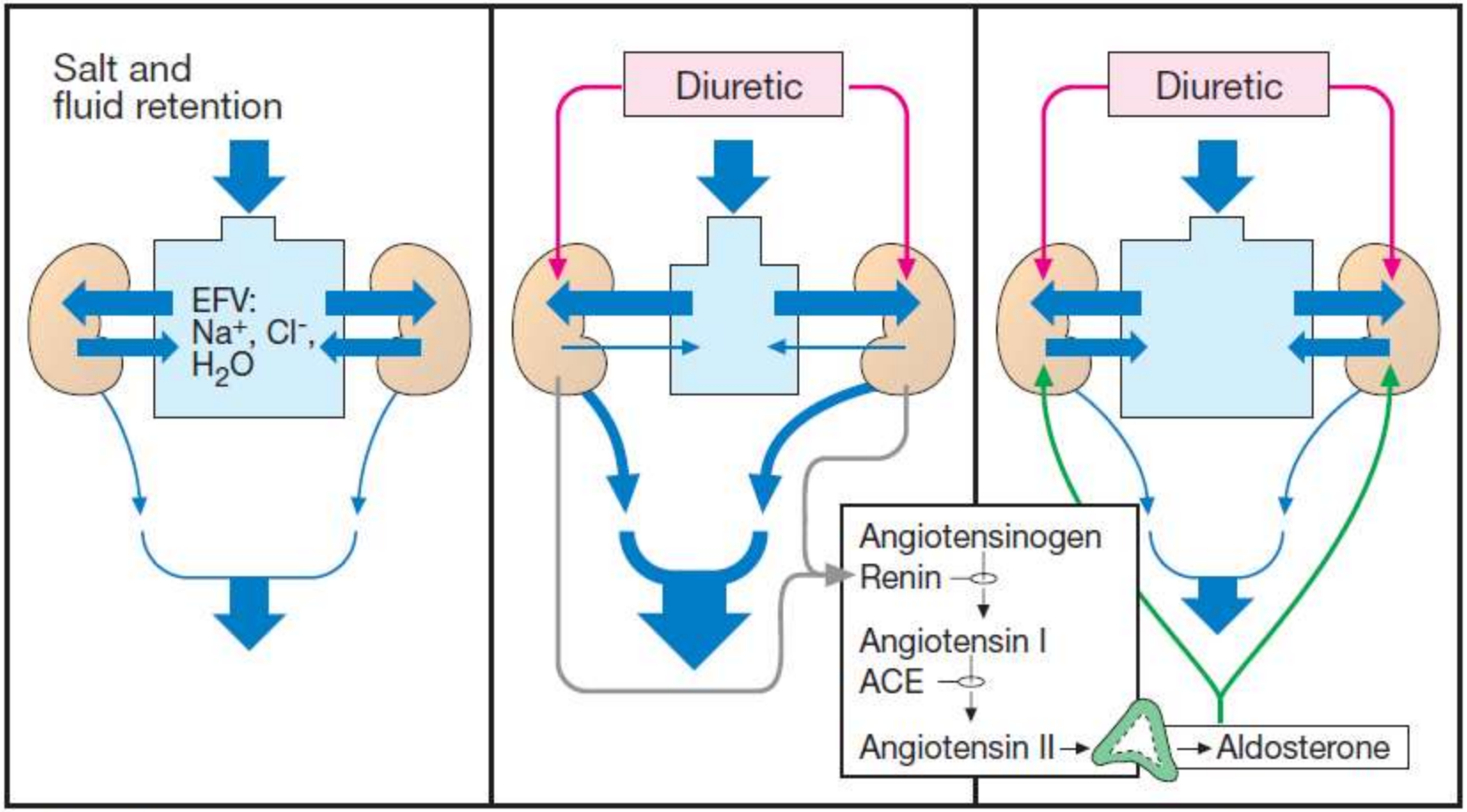
- *In circulatory* failure (shock), e.g., secondary to massive hemorrhage, renal production of urine may cease (anuria).
- By means of diuretics an attempt is made to maintain urinary flow.
- **OSMOTIC OR LOOP DIURETICS**

Massive use- **Adverse effects**

- **The decrease in blood volume** can lead to **hypotension and *collapse;***
- ***Blood viscosity*** rises due to the **increase in erythro- and thrombocyte concentration,** bringing an increased risk of intravascular **coagulation or *thrombosis***

Counter-regulatory responses

- When depletion of NaCl and water (EFV reduction) occurs as a result of diuretic therapy, the body can initiate- Response
- Activation of the renin-angiotensin- aldosterone system
- Because of the diminished blood volume, renal blood flow is jeopardized.
- Release of the hormone, **Renin** (Kidney) which enzymatically catalyzes the formation of **angiotensin I.**
- Angiotensin I is converted to **angiotensin II** by the action of **angiotensin-converting enzyme (ACE).**
- Angiotensin II stimulates release of **aldosterone.**
- The mineralocorticoid promotes renal reabsorption of NaCl and water and thus counteracts the effect of diuretics.



B. Possible counter-regulatory responses during long-term diuretic therapy

High efficacy diuretics

(Inhibitors of Na+-K+- 2Cl cotransport)

Sulphamoyl derivatives Furosemide, Bumetanide, Torasemide

Medium efficacy diuretics

(Inhibitors of Na+ Cl symport)

Benzothiadiazines (thiazides), Hydrochlorothiazide, Benzthiazide, Hydroflumethiazide, Clopamide

Thiazide like (related heterocyclics)
Chlorthalidone, Metolazone, Indapamide.

Weak or adjunctive diuretics

Carbonic anhydrase inhibitors Acetazolamide

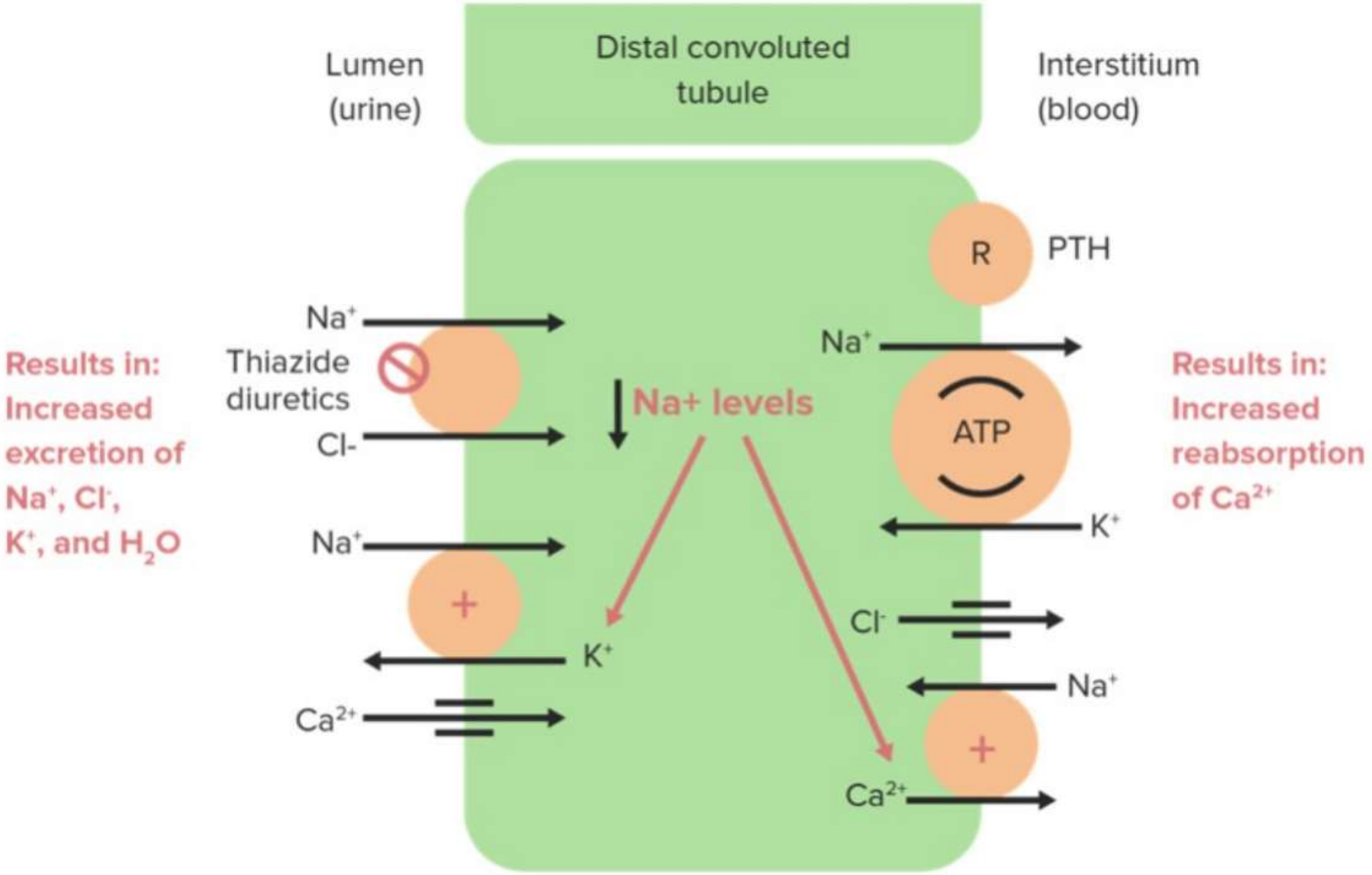
Potassium sparing diuretics

Aldosterone antagonist: Spironolactone

Inhibitors of renal epithelial Na+ channel:
Triamterene, Amiloride.

Osmotic diuretics

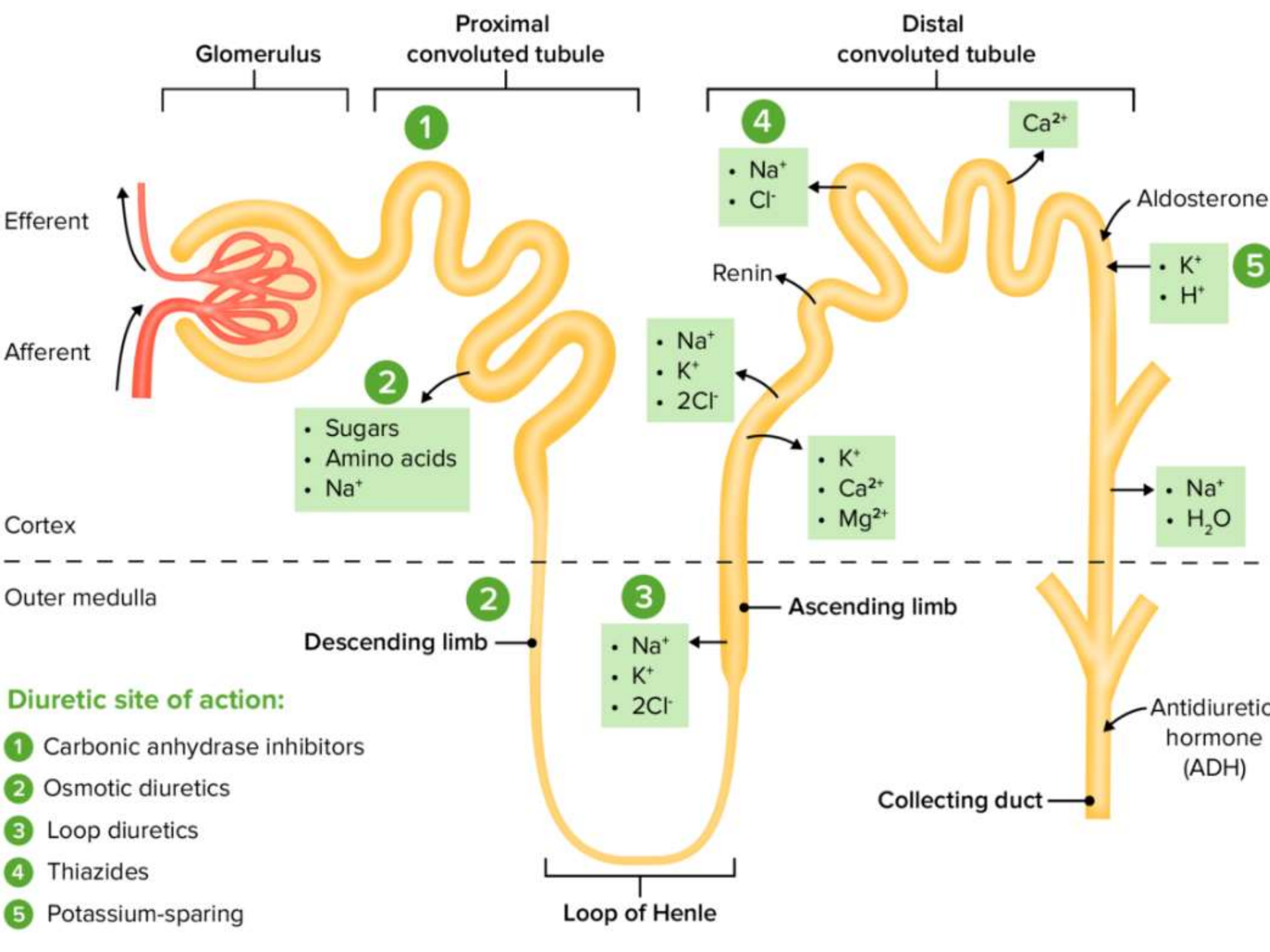
Mannitol, Isosorbide, Glycerol


Lumen
(urine)
Distal convoluted
tubule
Interstitium
(blood)
R
PTH
Na+
Thiazide
diuretics
Cl-
Na+ levels
Na+
ATP
K+
Na+
+
K+
Cl-
Na+
Ca2+
+
Ca2+
Results in:
Increased
excretion of
Na+, Cl-,
K+, and H2O
Results in:
Increased
reabsorption
of Ca2+

Mechanism of action (MOA)

- **Primary MOA:** ↓ reabsorption of NaCl through the inhibition of the Na^+/Cl^- cotransporter in the distal convoluted tubule (DCT):
  - With this channel blocked → ↓ Na^+ reabsorption
  - Water always follows Na^+:
    - Water stays with Na^+ in the tubules (rather than being reabsorbed).
    - Diuresis results from the osmotic effect of Na^+.
  - Diuresis → ↓ plasma volume → ↓ BP:
    - Initially causes a transient ↑ in the RAAS and sympathetic tone to compensate for ↓ BP and cardiac output.
    - Transient ↑ in the RAAS explains why there is a synergistic effect between thiazides and ACEis/ARBs.
  - There is also a modest ↑ in vasodilation via an unclear mechanism.
- **Thiazide use results in:**
  - ↑ Excretion of Na^+, Cl^-, K^+, and water
  - ↑ Reabsorption of Ca^{2+}:
    - ↑ Na^+ excretion → ↓ cellular Na^+ levels →
    - ↑ Compensatory exchange of Ca^{2+} for Na^+ (via the basolateral Na^+/Ca^{2+} exchanger)

- **Development of hypokalemia:**
  - ↓ Na^+ reabsorption in the DCT →
  - ↑ Na^+ delivered to the collecting ducts (CDs) →
  - Stimulates ↑ aldosterone release
  - Aldosterone stimulates Na^+/K^+ exchanger to ↑ Na^+ reabsorption and excrete K^+ → hypokalemia
- **Development of a metabolic alkalosis:**
  - ↑ K^+ excretion in the CD →
  - Aldosterone-stimulated ↑ in K^+/H^+ exchanger →
  - Reabsorbs some of the extra K^+ in the tubule in exchange for H^+ (which is excreted) → metabolic alkalosis via H^+ loss



Diuretic site of action:

1. Carbonic anhydrase inhibitors
2. Osmotic diuretics
3. Loop diuretics
4. Thiazides
5. Potassium-sparing

Antidiuretics

- Reduce urine volume, Diabetes insipidus
- **Antidiuretic hormone** (ADH, Vasopressin),
- Desmopressin, Lypressin, Terlipressin
- **Thiazide diuretics:** Amiloride.
- **Miscellaneous:** Indomethacin, Chlorpropamide, Carbamazepine.

ANTIDIURETIC HORMONE

- Nonapeptide - Secreted by posterior pituitary (neurohypophysis) along with oxytocin
- Synthesized in the hypothalamic nerve cell bodies as a large precursor peptide along with its binding protein 'neurophysin',
- Transported down the axons to nerve endings
- Osmoreceptors in hypothalamus
- Volume receptors in left atrium, ventricles and pulmonary veins
- Regulate the rate of ADH release governed by body hydration.
- Impulses from baroreceptors and higher centres also impinge on the nuclei synthesizing ADH and affect its release.
- Physiological stimuli for ADH release
  - Rise in plasma osmolarity
  - Contraction of e.c.f. volume.

Mechanism of action

V2 subtype of ADH receptors CD cell membrane. Activation of these receptors

- increases cAMP formation intracellularly
- activation of cAMP dependent protein kinase A
- phosphorylation of relevant proteins
- promote exocytosis 'aquaporin-2' water channel containing vesicles (WCVs) through the apical membrane more aqueous channels get inserted

- Rate of endocytosis & degradation of WCVs reduced.
- The water permeability of CD cells is increased in proportion to the population of aquaporin-2 channels in the apical membrane at any given time.
- Continued V2 receptor stimulation (during chronic water deprivation) in addition upregulates aquaporin-2 synthesis through cAMP response element of the gene encoding aquaporin-2.

- Increases urea permeability of terminal part of CDs by stimulating a **vasopressin regulated urea transporter (VRUT or UT-I)**
- Augments medullary hypertonicity.
- AVP on **AscLH** have been demonstrated which further reinforce medullary **hypertonicity**
- By activating the **Na+K+2Cl- cotransporter**

- The VI receptors also participate in the renal response to ADH.
- While **VIa receptor activation constricts vasa recta to diminish blood flow to inner medulla** which will help in maintaining high osmolarity
- Thus contribute to antidiuresis.

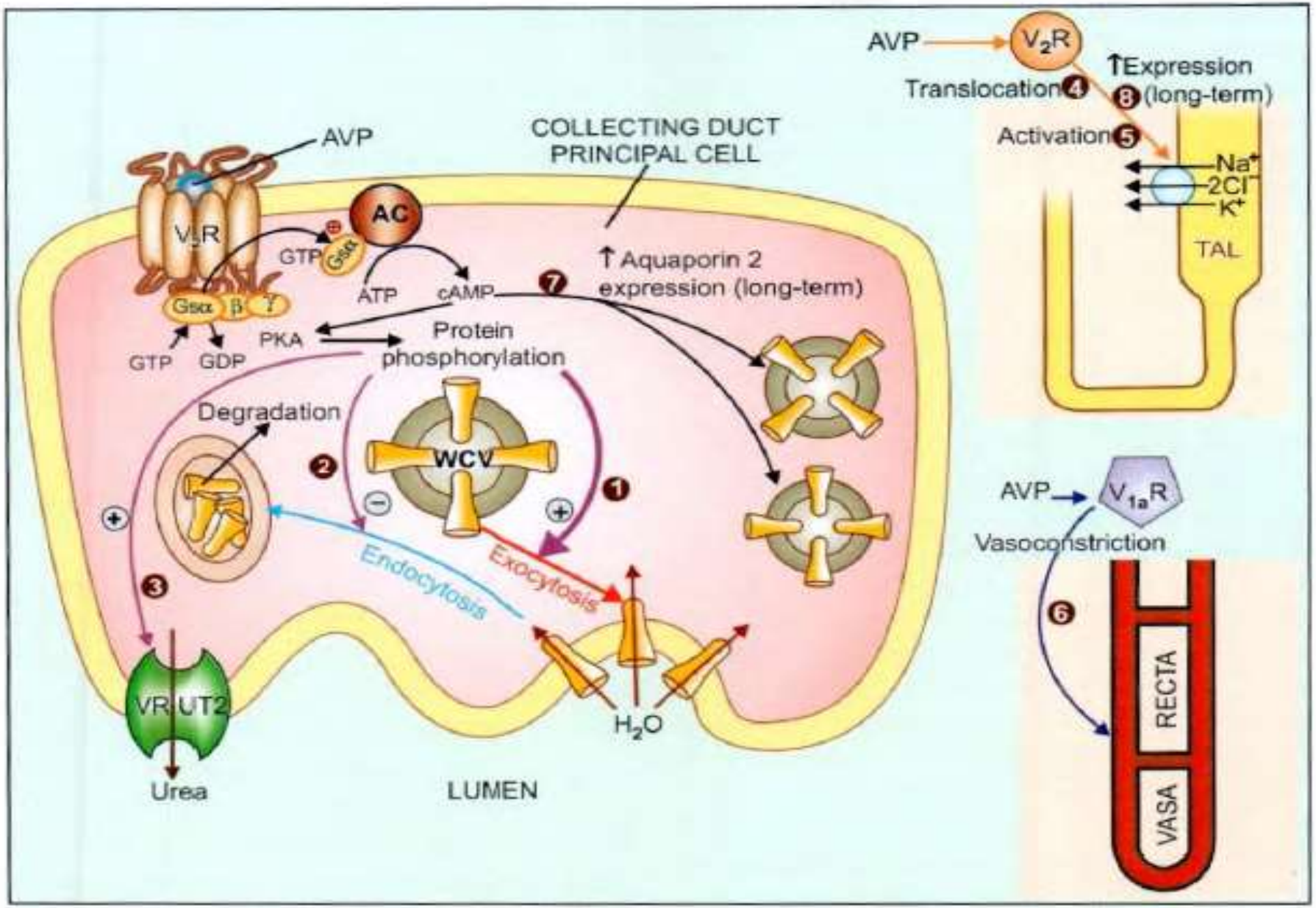


Fig. 43.1: Mechanisms of rapid and long-term anti-aquaretic action of vasopressin

- *Uterus is contracted by AVP acting on oxytocin* receptors.
- *CNS - Exogenously administered AVP does not* penetrate blood-brain barrier.
- **AVP may** be involved in regulation of body temperature
- AVP induces platelet aggregation
- Hepatic glycogenolysis.
- It releases coagulation factor VIII and von Willebrand's factor from vascular endothelium by acting on V2 receptors

Pharmacoklnetics AVP

- **Inactive orally** because it is destroyed by trypsin.
- It can be administered by **any parenteral route or by intranasal application.**
- The peptide chain of **AVP is rapidly cleaved enzymatically liver and kidney;**
- Plasma **t½ is short ~ 25 min.**
- Action of aqueous vasopressin lasts **3-4 hours**

A. Based on V 2 actions (Desmopressin)

- *Diabetes insipidus*
- *Bedwetting in children and nocturia in adults*
- *Renal concentration test*
- *Haemophilia, von Willebrand's disease*

B. Based on V1 actions

- *Bleeding esophageal varices*
- Often stop bleeding by constricting mesenteric blood vessels and reducing blood flow through the liver to the varices,
- allowing clot formation.
- Terlipressin stops bleeding in ~80%

Acknowledgement

- The Presentation is being used for educational and non commercial Purposes
- Thanks are due to all the original contributors and entities whose pictures were used in the creation of this presentation.