*Ionophores*

Ionophores are small hydrophobic lipid-soluble molecules that dissolve in lipid bilayers and increase their permeability to specific inorganic ions. Ionophores shield the charge of the ion to be transported, enabling it to penetrate the hydrophobic interior of the lipid bilayer. There are two classes of ionophores – *mobile ion carriers* (which move within in the bilayer) and *channel formers* (which span the lipid bilayer).

**Valinomycin** (isolated from *Streptomyces fulvissimus*) is an example of a mobile ion carrier. It transports $K^+$ down its electrochemical gradient. Similarly, ionophore *momensin* acts as a carrier for $Na^+$ ions. **Gramicidin A** (produced by the bacterium *Bacillus brevis*) is a 15-residue peptide with alternating D- and L-amino acids. In membranes it forms a channel. The channel permits the passage of water and univalent cations, but not anions.

is the most common example of primary active transport. Virtually every animal cell maintains lower $Na^+$ concentration and high $K^+$ concentration than they are found in its surrounding. This imbalance is established and maintained by an active transport system in the plasma membrane, involves the carrier protein $Na^+$-$K^+$ ATPase coupled with breakdown of ATP (discovered by Jens Skou in 1957).

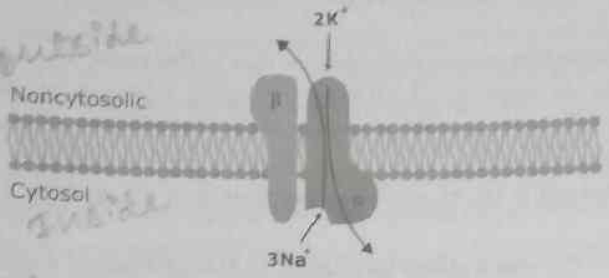
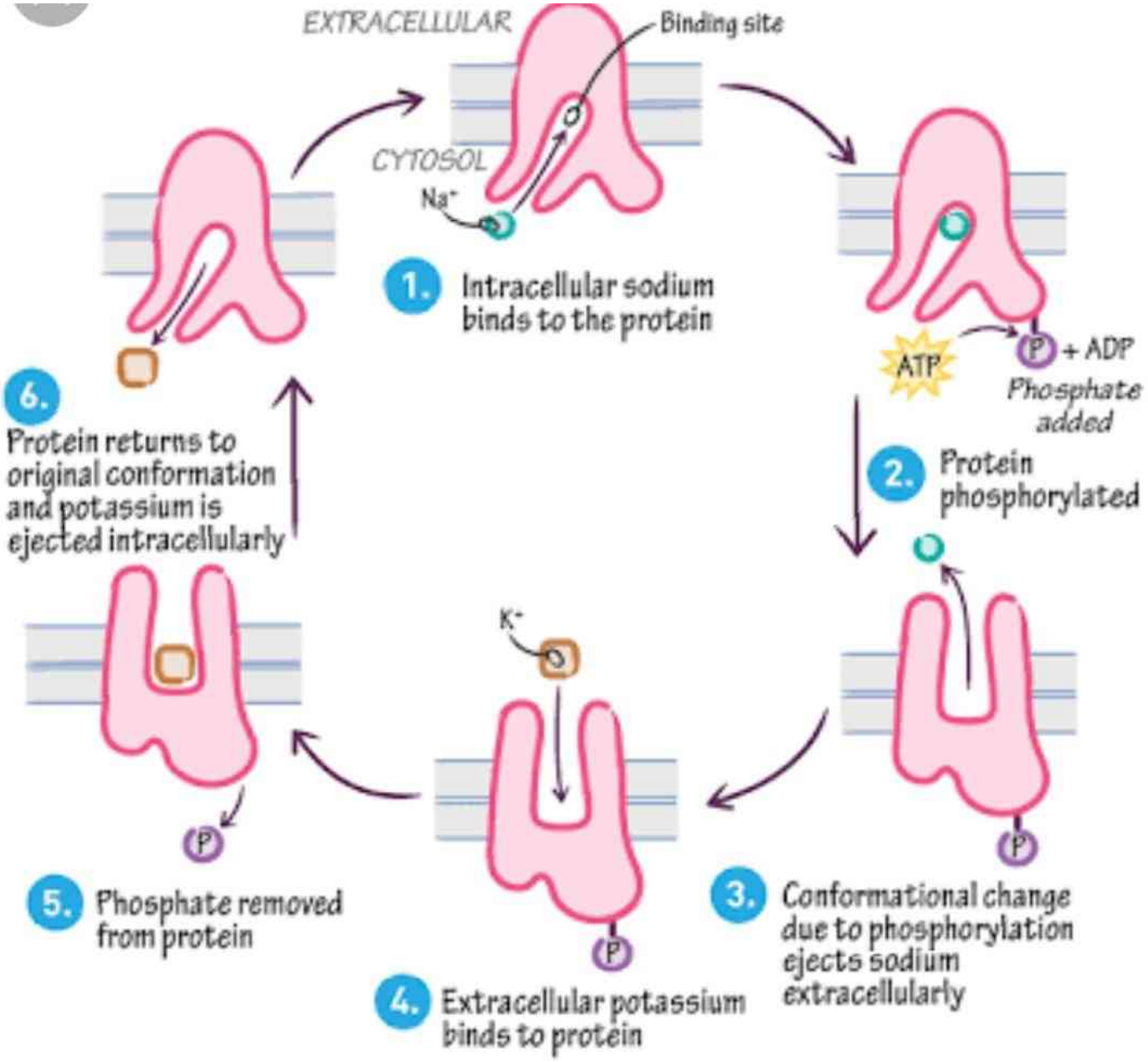


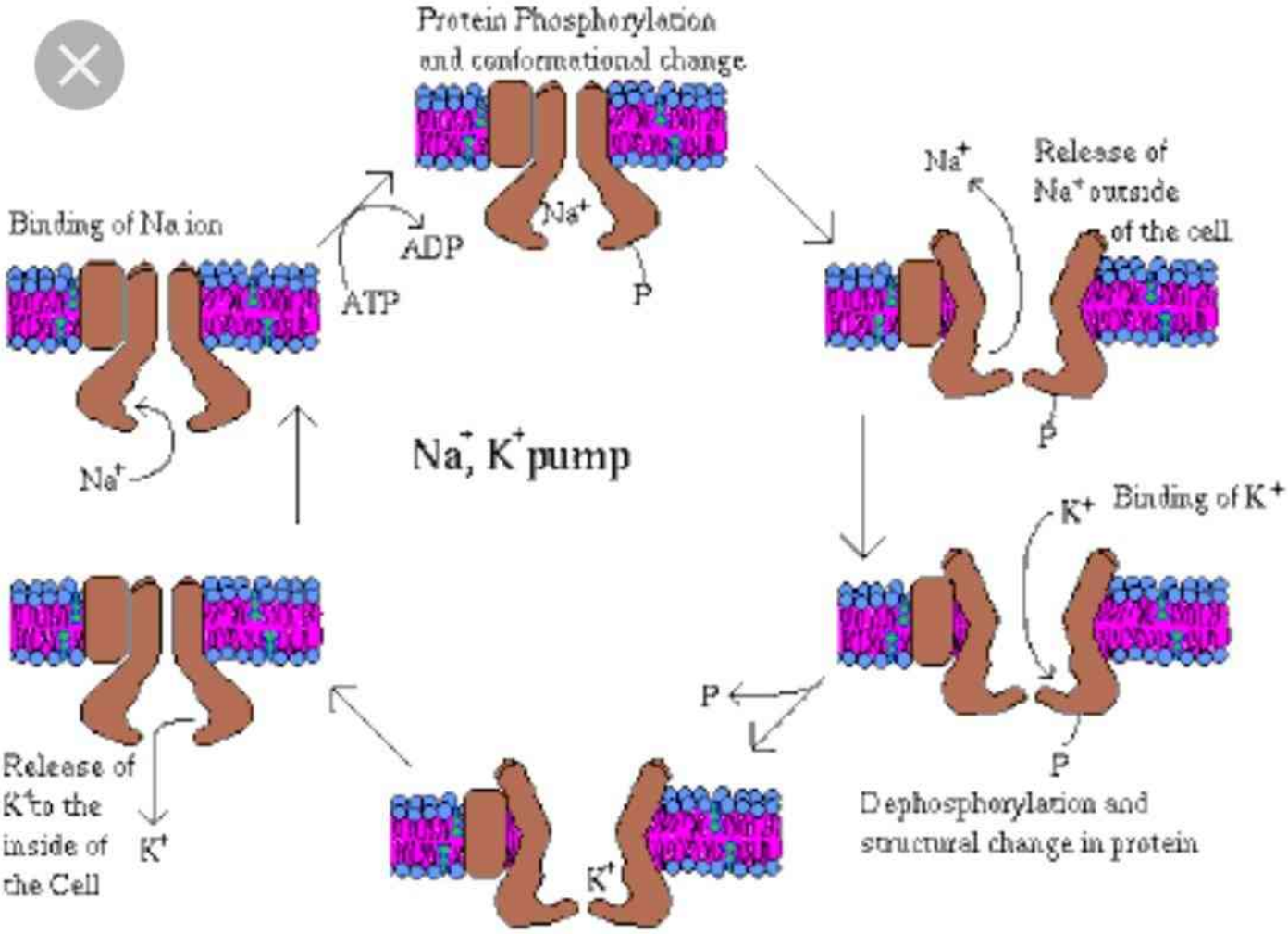

**Figure 3.13**
Structure of $Na^+$-$K^+$ ATPase. The $Na^+$-$K^+$ ATPase is primarily responsible for setting and maintaining the intracellular concentrations of $Na^+$-$K^+$ and for generating the transmembrane electrical potential, which it does by moving $3Na^+$ out of the cell for every $2K^+$ it moves in.

$Na^+$ and ATP binding ➡ Phosphorylation of aspartate ➡ Conformational change ➡ $Na^+$ release ➡ $K^+$ binding ➡ Dephosphorylation and conformational change ➡ $K^+$ release

For each molecule of ATP hydrolyzed, two $K^+$ move inward and three $Na^+$ ions move outward. It is an example of *electrogenic* pump because it contributes to the charge separation across the membrane. It is a heterodimer composed of an α-subunit and a glycoprotein β-subunit. The α-subunit is responsible for ATP hydrolysis and ion pumping while the β-subunit is important for the correct assembly of the ATPase in the endoplasmic reticulum. The α-subunit contains 10 transmembrane segments. Certain steroids like *Digitalis* and ouabain, which are known as *cardiotonic steroids* (also called cardiac glycosides) are potent inhibitors of the $Na^+$-$K^+$ pump. These compounds inhibit the dephosphorylation of $Na^+$-$K^+$ pump when applied on the extracellular face of the membrane. These cardiotonic steroids are used in the treatment of congestive heart diseases because they increase the force of contraction of heart muscle by inhibiting the $Na^+$-$K^+$ pump. It happens as the level of $Na^+$ increases inside the cell, this slows the $Na^+$-$Ca^{2+}$ exchanger, leading to increase in concentration of $Ca^{2+}$ inside, which ultimately increases the force of contraction of the heart muscle. *Palytoxin* (toxic non-peptide substances) also inhibits the function of $Na^+$-$K^+$ pump. It converts $Na^+$-$K^+$ pump into a non-specific ion channel, which allows the passive transport of both $Na^+$ and $K^+$ ions, thereby destroying the ion gradient.

The active transport of calcium across the plasma membrane is driven by a $Ca^{2+}$ pump that is structurally related to the $Na^+$-$K^+$ pump and is similarly powered by ATP hydrolysis. The $Ca^{2+}$ pump transports calcium out of the cell so intracellular calcium concentrations are extremely low.


EXTRACELLULAR
Binding site
CYTOSOL
Na+
1. Intracellular sodium binds to the protein
ATP
P + ADP
Phosphate added
2. Protein phosphorylated
3. Conformational change due to phosphorylation ejects sodium extracellularly
K+
4. Extracellular potassium binds to protein
P
5. Phosphate removed from protein
6. Protein returns to original conformation and potassium is ejected intracellularly




- The Top is the Outter membrane.
- The Bottom is the inner membrane (inside of the Cell)

Sodium-glucose transporter (SGLT)

or opposite directions. A common example of secondary active transport is the symport of $Na^+$ and glucose. The transmembrane protein *$Na^+$-glucose transporter* (also known as $Na^+$-glucose co-transporters or symporters) allows $Na^+$ and glucose to enter the cell together.

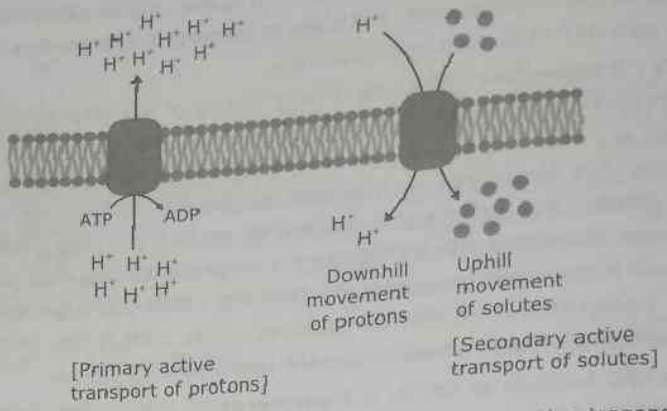



**Figure 3.15** Comparison of primary and secondary active transport.

$Na^+$-glucose symporters are a family of glucose transporter present on the apical surface (facing the lumen) of the epithelium cell of the small intestine and actively transports glucose molecules into the cell from the gut. The $Na^+$ flows down their concentration gradient while the glucose molecules are transported against their concentration gradient into the cell. Later, the $Na^+$ is pumped back out of the cell by the $Na^+$-$K^+$ ATPase and thus maintaining the inward $Na^+$ gradient. The GLUT confined to the basolateral (basal and lateral) surfaces of the cell allows the same molecules to leave the cell by facilitated diffusion into the extracellular fluid on the other side of the epithelium.

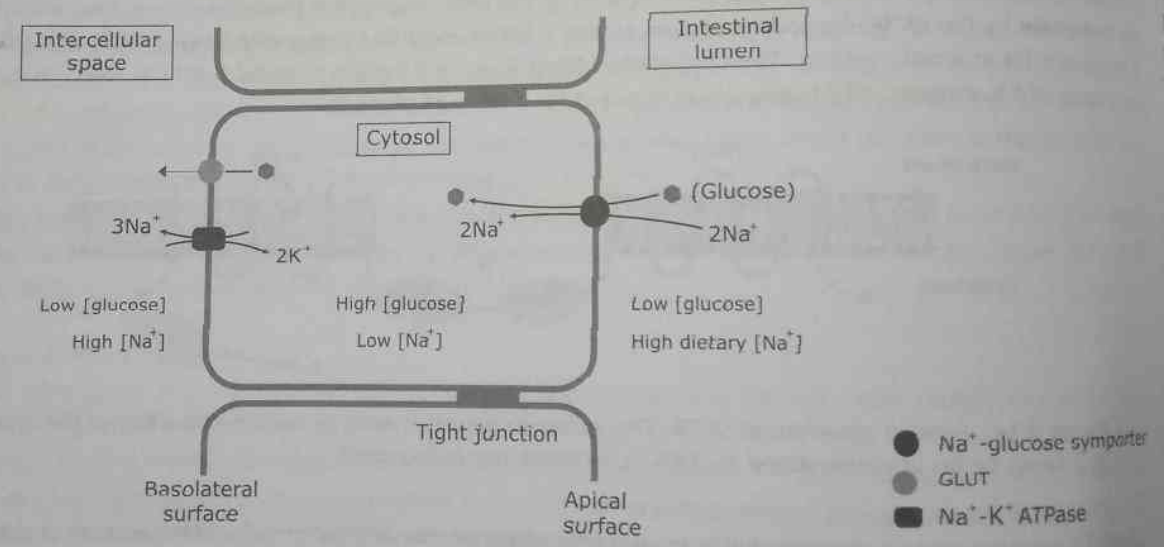
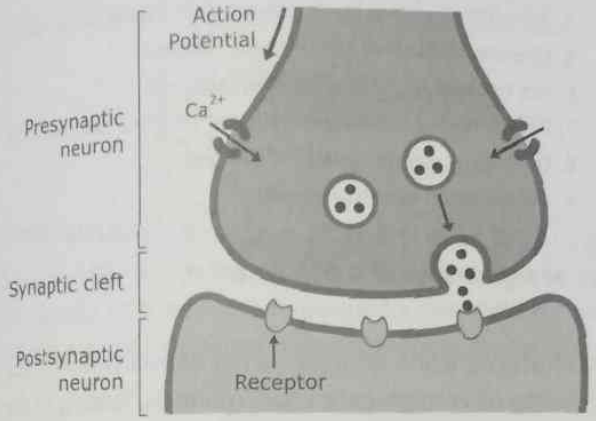


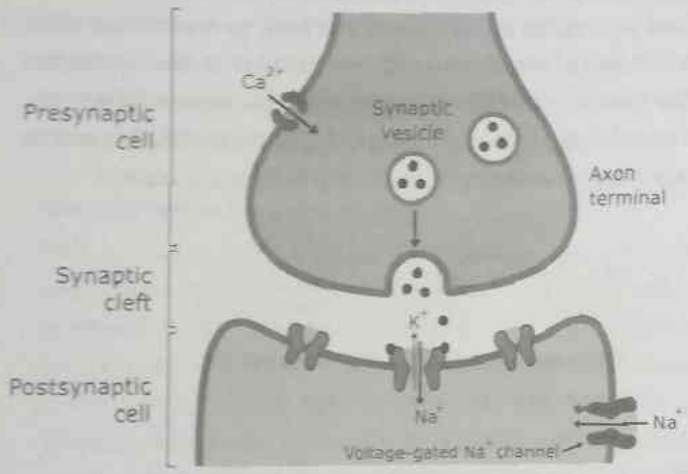

**Figure 3.16** Glucose is actively transported into the cell by $Na^+$-driven glucose symporters at the apical surface, and it diffuses out of the cell by facilitated diffusion mediated by glucose carriers (GLUT) in the basolateral membrane. Tight junctions block the backflow of glucose from the basal side of the epithelium into the gut lumen.




Voltage Gated Channel

1. Action potentials arrive at axon terminal.
2. Voltage-gated $Ca^{2+}$ channels open.
3. $Ca^{2+}$ enters into the cell.
4. $Ca^{2+}$-dependent fusion of synaptic vesicles with the membrane.
5. Synaptic vesicles release neurotransmitter by exocytosis.
6. Neurotransmitter diffuses across the synaptic cleft and binds to receptors present at postsynaptic neurons.
7. Binding of neurotransmitter to receptor generates postsynaptic potential.

**Figure 3.21** Transmission at chemical synapses.




1. Acetylcholine is synthesized in the presynaptic cell.
2. Acetylcholine is packaged into synaptic vesicles.
3. Acetylcholine is released into the synaptic cleft.
4. Acetylcholine binds to its receptor on the postsynaptic cell.
5. Opening of nicotinic acetylcholine receptor.
6. Net influx of $Na^+$ into the postsynaptic cell.
7. Depolarization causes generation of EPSP in postsynaptic cell.
8. Opening of voltage-gated $Na^+$ channel.
9. Generation of action potential.

excitatory post synaptic Potential

**Figure 3.22** Nicotinic acetylcholine receptor and generation of action potential.

membrane potential is measured as a voltage difference across the membrane. In animal cells, the membrane potential varies between −20 and −200 millivolts (mV), depending on the organism and cell type. The sign of the potential (+ or −) always designates whether excess positive or excess negative charges are present, respectively, on the inside of the membrane.

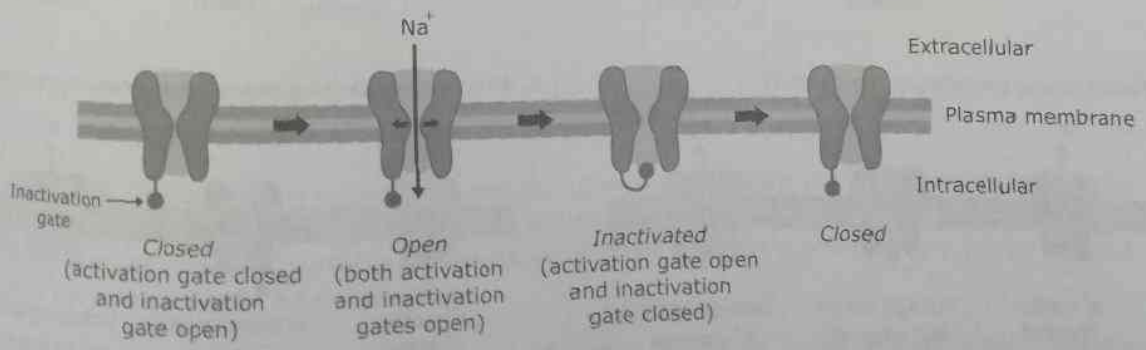
**Neurons** or nerve cells are *excitable* (generate and propagate electrical signals when excited) and *secretory* (secrete neurotransmitters) cells. Its membrane potential changes in the presence and absence of stimulus. Its membrane potential in resting state (when a cell not producing electrical signals) is referred as **resting potential**. The axoplasm inside the neuron contains high concentration of potassium ions and low concentration of sodium ions. In contrast, the fluid outside the axon contains a low concentration of potassium ions and a high concentration of sodium ions. These ionic gradients across the membrane are maintained by the primary active transport of sodium and potassium ions by the $Na^+$-$K^+$ pump. The plasma membrane also contains large number of $K^+$-leaky channels. These channels allow potassium ions to move freely along the electrochemical gradient. Transfer of positive charge to cell's exterior leaves behind unbalanced negative charge within the cell, thereby creating membrane potential. This membrane potential is referred as *resting potential*. Its value is about −70 mV, with the inside of the cell is negative. A nerve cell that exhibits a resting membrane potential is said to be ***polarized.***

### *Generation of action potential*

Changes in membrane potential are brought about by changes in ion movement across the membrane. For example, if the net inward flow of positively charged ions increases compared to the resting state, the membrane **depolarizes**. In this situation, the inside becomes less negative as compared to polarized resting state (for example, a change from −70 mV to −40 mV). This term is also used when the inside becoming positive (for example, a change from −70 mV to +30 mV as during an action potential). By contrast, if the net outward flow of positively charged ions increases compared to the resting state, the membrane **hyperpolarizes** (i.e. inside becomes more negative as compared to the resting state).

A rapid and large changes in membrane potential of nerve cells during which the potential actually reverses so that the inside of the cell transiently becomes more positive than the outside (i.e. depolarized) is termed as **action potential** (*nerve action potential* or *nerve impulse*). Action potentials allow communication over long distances within the body. Let us now consider how the changes in potential occurs during an action potential. Movement of potassium ions along the electrochemical gradient through potassium leaky channels is responsible for the establishment of the *resting potential*. By contrast, action potentials are the direct consequence of the voltage-gated $Na^+$ channels. During an action potential, movement of sodium ions occur through voltage-gated $Na^+$ channels down their electrochemical gradients. This voltage-gated $Na^+$ channel can exist in three conformations:

1. closed but capable of opening (activation gate closed, inactivation gate open)
2. open (both activation and inactivation gates open)
3. closed and not capable of opening or inactivated (activation gate open, inactivation gate closed)

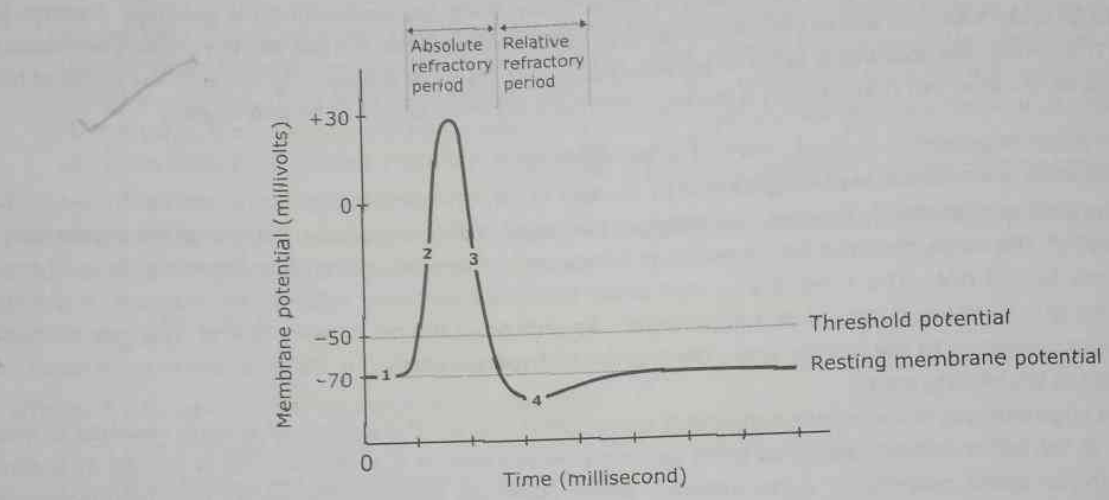



The channel undergoes through these various conformations as a result of voltage changes that take place during an action potential. At resting potential (about −70 mV), voltage-gated $Na^+$ channels are closed. Therefore, sodium ions cannot pass through these voltage-gated channels at resting potential. A stimulus that causes sufficient

depolarization promptly causes voltage-gated $Na^+$ channels to open, allowing sodium ions to enter into the cell down its electrochemical gradient. The influx of positive charge *depolarizes* the membrane. This changes the membrane potential value of about –70 mV to about +30 mV within a fraction of a second.

The voltage-gated $Na^+$ channels have an automatic inactivating mechanism, which causes the channels to reclose rapidly even though the membrane is still depolarized. The voltage-gated $Na^+$ channels remain in this inactivated state, unable to reopen, until a few milliseconds after the membrane potential returns to its initial negative value.

In addition to the inactivation of voltage-gated $Na^+$ channels, another channels operate to help bring the depolarized plasma membrane more rapidly back toward its original polarized state. It is voltage-gated $K^+$ channels. The opening and consequent efflux of potassium ions quickly drives the membrane back toward the potassium ions equilibrium potential. This process is called *repolarization*.



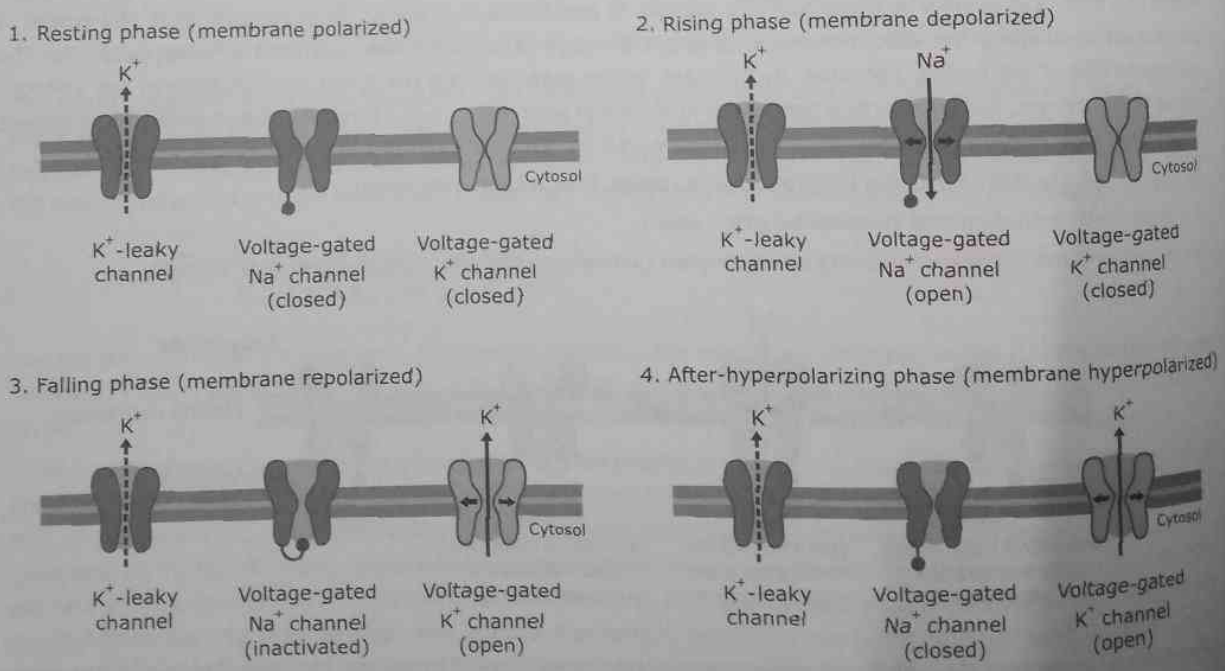




**Figure 3.18** Changes in permeability and ion movement during an action potential.

Voltage-gated $K^+$ channels respond to changes in membrane potential in much the same way as the voltage-gated $Na^+$ channels do, but with slightly slower kinetics; for this reason they are sometimes called *delayed $K^+$ channels*. Unlike voltage-gated $Na^+$ channels, most voltage-gated $K^+$ channels do not exhibit an inactivated state. Instead, they alternate between closed and open states. Hence, an action potential has two main phases: a depolarizing phase (i.e. rising phase) and a repolarizing phase (i.e. falling phase). During the *depolarizing phase*, the negative membrane potential becomes less negative, reaches zero, and then becomes positive. During the *repolarizing phase*, the membrane potential is restored to the resting state of −70 mV. Following the repolarizing phase there may be an *after-hyperpolarizing phase*, during which the membrane potential temporarily becomes more negative (about −90 mV) than the resting state.

The period of time after an action potential begins during which an excitable cell cannot generate another action potential in response to a *normal* threshold stimulus is called the **refractory period**. It can be absolute or relative. During the *absolute refractory period*, even a very strong stimulus cannot initiate a second action potential. This period coincides with the period of voltage-gated $Na^+$ channel activation and inactivation. Inactivated $Na^+$ channels cannot reopen; they first must return to the closed state. The *relative refractory period* is the time period during which a second action potential can be initiated, but only by a larger-than normal stimulus. It coincides with the period when the voltage-gated $K^+$ channels are still open after inactivated voltage-gated $Na^+$ channels have returned to their closed state. The refractory period limit the number of action potentials that can be produced by an excitable membrane in a given period of time.

An action potential occurs in the membrane of the axon of a neuron when depolarization reaches a certain level termed the **threshold potential** (typically between −50 to −55 mV). The generation of an action potential depends on whether a particular stimulus is able to bring the depolarization to threshold. An action potential will not occur in response to a sub-threshold stimulus, a stimulus that causes weak depolarization. However, an action potential will occur in response to a threshold stimulus, a stimulus that is just strong enough to depolarize the membrane to threshold. It means that an action potential either occurs completely or it does not occur at all. This characteristic of an action potential is known as the **all-or-none principle**.

**Box 30.7. Causes of Hypernatremia**

1. Cushing's disease
2. Prolonged cortisone therapy
3. In pregnancy, steroid hormones cause sodium retention in the body.
4. In dehydration, when water is predominantly lost, blood volume is decreased with apparent increased concentration of sodium.
5. Exchange transfusion with stored blood
6. Primary hyperaldosteronism
7. Elderly patients with poor water intake, and inability to express thirst
8. Excessive intake of salt
9. Drugs:
   - Ampicillin
   - Tetracycline
   - Anabolic steroids
   - Oral contraceptives
   - Loop diuretics
   - Osmotic diuretics

**Box 30.8. Causes of Hyponatremia**

1. Vomiting
2. Diarrhea
3. Burns
4. Addison's disease (adrenal insufficiency)
5. Renal tubular acidosis (tubular reabsorption of sodium is defective).
6. Chronic renal failure, nephrotic syndrome
7. Congestive cardiac failure
8. Hyperglycemia and ketoacidosis
9. Excess non-electrolyte (glucose) IV infusion
10. SIADH and defective ADH secretion
11. Pseudo- or dilutional hyponatremia
    - Hyperproteinemia, e.g. myeloma
    - Mannitol
12. Drugs:
    - ACE inhibitors
    - Lithium
    - NSAIDs
    - Vasopressin and oxytocin
    - Chlorpropamide

tubules and ascending limb; (b) Sodium chloride cotransporter in the distal tubules (ascending limb); (c) Sodium channels in the collecting duct; and (d) Sodium potassium exchanger in the distal tubule. These are explained in Chapter 29, under renal regulation of pH.

The rate of sodium excretion is directly affected by the rate of filtration of sodium which is decided by the renal plasma flow and blood pressure (acting through atrial natriuretic peptide). The amount reabsorbed is under the control of aldosterone.

### Edema

In edema, along with water, sodium content of the body is also increased. When diuretic drugs are administered, they increase sodium excretion. Along with sodium, water is also eliminated. **Sodium restriction** in diet is, therefore, advised in congestive cardiac failure and in hypertension.

In the early phases of congestive cardiac failure, hydrostatic pressure on venous side is increased; so water is primarily retained in the body. This causes dilution of sodium concentration, which triggers aldosterone secretion. This is known as **secondary** aldosteronism. Thus sodium is retained, along with further retention of water. This vicious cycle is broken when aldosterone antagonists are administered as drugs.

### Hypernatremia

Increased sodium in blood is known as hypernatremia. Symptoms of hypernatremia include dry mucous membrane, fever, thirst and restlessness. Causes of hypernatremia are enumerated in Box 30.7.

### Hyponatremia

Decreased sodium level in blood is called hyponatremia. Clinical signs and symptoms of hyponatremia include dehydration, drop in blood pressure, drowsiness, lethargy, confusion, abdominal cramps, oliguria, tremors and coma. However, hyponatremia is often asymptomatic. Causes of hyponatremia are shown in Box 30.8, most important causes being vomiting, diarrhea, and adrenal insufficiency.

**Hyponatremia due to water retention** is the commonest biochemical abnormality observed in clinical practice. Hyponatremic patients without edema have water overload and they can be treated by water restriction. Hyponatremia with edema is due to both water and sodium overload and will have to be treated by diuretics and fluid restriction.

**SIADH** (Syndrome of inappropriate secretion of anti-diuretic hormone) is a condition with hyponatremia; normal glomerular filtration rate, and normal serum urea and creatinine concentration. Urine flow rate is less than 1.5 L/day. Symptoms are proportional to the rate of fall of sodium and not to the

**Box 30.10.** Causes of Hypokalemia

1. **Increased renal excretion**
   - Cushing's syndrome
   - Hyperaldosteronism
   - Hyper reninism, renal artery stenosis
   - Hypomagnesemia
   - Renal tubular acidosis
   - Adrenogenital syndrome
   - 17 alpha hydroxylase deficiency
   - 11 beta hydroxylase deficiency
2. **Shift or redistribution of potassium**
   - Alkalosis
   - Insulin therapy
   - Thyrotoxic periodic paralysis (abnormal Na-K-ATPase)
   - Hypokalemic periodic paralysis (abnormal calcium channels)
3. **Gastrointestinal loss**
   - Diarrhea, vomiting, aspiration
   - Deficient intake or low potassium diet
   - Malabsorption
   - Pyloric obstruction
4. **Intravenous saline infusion in excess**
5. **Drugs**
   - Insulin
   - Salbutamide
   - Osmotic diuretics
   - Thiazides, acetazolamide
   - Corticosteroids

**Box 30.11.** Causes of Hyperkalemia

1. **Decreased renal excretion of potassium**
   - Obstruction of urinary tract
   - Renal failure
   - Deficient aldosterone (Addison's)
   - Severe volume depletion (heart failure)
2. **Entry of potassium to extracellular space**
   - Increased hemolysis
   - Tissue necrosis, burns
   - Tumor lysis after chemotherapy
   - Rhabdomyolysis, crush injury
   - Excess potassium supplementation
   - Malignant hypertension
3. **Redistribution of potassium to extracellular**
   - Metabolic acidosis
   - Insulin deficiency (diabetes mellitus)
   - Tissue hypoxia
4. **Transmembrane shift**
5. **Pseudohyperkalemia**
   - Factitious ($K^+$ leaches out when blood is kept for a long time before separation)
   - Improper blood collection (hemolysis)
   - Thrombocytosis (>400 million/ml)
   - Leukocytosis (>11 million/ml)
6. **Hyperkalemic periodic paralysis**
7. **Drugs**
   - Spiranolactone
   - ACE inhibitors
   - Beta blockers
   - Cyclosporine
   - Digoxin

to 5-10 mmol per day or increase excretion to 450 mmol per day depending on the potassium intake. The majority of the filtered $K^+$ (500 mmol) is reabsorbed in the proximal tubule. The control of secretion occurs in the cortical collecting duct. The exchange of potassium for sodium at the renal tubules is a mechanism to conserve sodium and excrete potassium. This is controlled by aldosterone. Aldosterone and corticosteroids increase the excretion of $K^+$. On the other hand, $K^+$ depletion will inhibit aldosterone secretion.

Yet another factor which influences the potassium level is the hydrogen ion concentration. When there is an increase in hydrogen ion concentration of extracellular fluid, there is a redistribution of potassium and hydrogen between cells and plasma. Hydrogen ions are conserved at the expense of potassium ions and *vice versa* depending on hydrogen ion concentration. This may lead to a depletion or retention of potassium. (See Chapter 29).

Urinary potassium excretion varies from 30-100 mmol/day, depending on the intake as well as on the amount of hydrogen ions excreted and acid base status. Renal adaptation maintains potassium balance till the GFR drops to 20ml/mt. In chronic renal failure, hyperkalemia is seen since the failing kidney is unable to handle the potassium load.

## Hypokalemia

This term denotes that plasma potassium level is below 3 mmol/L. A value less than 3.5 mmol/L is to be viewed with caution. Mortality and morbidity are high. Box 30.10 shows the causes of hypokalemia.

**Signs and symptoms:** Hypokalemia is manifested as muscular weakness, fatigue, muscle cramps, hypotension, decreased reflexes, palpitation, cardiac arrhythmias and cardiac arrest. ECG waves are flattened, **T wave is inverted**, ST segment is lowered with AV block. This may be corrected by oral feeding of orange juice. Potassium administration has a beneficial effect in hypertension.

**Redistribution** of potassium can occur following insulin therapy. For diabetic coma, the standard treatment is to give **glucose and insulin**. This causes entry of glucose and potassium into the cell

and hypokalemia may be induced. $K^+$ should be supplemented in such cases.

Redistribution is also seen in **alkalosis**, where the potassium moves into the cell in exchange for $H^+$.

Renal loss of potassium is seen in acute tubular necrosis, renal tubular acidosis and metabolic alkalosis. In metabolic alkalosis, potassium is exchanged with $H^+$, in an attempt to conserve $H^+$.

In turn, hypokalemia can lead to metabolic alkalosis; this is observed in diuretic therapy, and prolonged vomiting, where $K^+$ is lost in exchange for

$H^+$. Non-renal losses are seen in diarrhea.

**Diuretics** used for congestive cardiac failure may cause $K^+$ excretion; hence potassium supplementation is the standard treatment along with diuretics.

## Hyperkalemia

Plasma potassium level above 5.5 mmol/L is known as hyperkalemia. Since the normal level of $K^+$ is kept at a very narrow margin, even minor increase is life-threatening.

In hyperkalemia, there is increased membrane excitability, which leads to ventricular arrhythmia and ventricular fibrillation. Hyperkalemia is characterized by flaccid paralysis, bradycardia and **cardiac arrest**. ECG shows **elevated T wave**, widening of QRS complex and lengthening of PR interval.

Causes of hyperkalemia are shown in Box 30.11. True potassium excess results from decreased urinary output, increased hemolysis and tissue necrosis. Decreased **potassium excretion** can occur in mineralocorticoid deficiency, Addison's disease and potassium sparing diuretics (spironolactone). Potassium channel mutations lead to **long-QT syndrome,** and cardiac arrhythmias.

**Redistribution** occurs in metabolic acidosis, insulin deficiency and tissue hypoxia (Table 30.6).

**Pseudohyperkalemia** is seen in hemolysis, thrombocytosis, leukocytosis or polycythemia; in these conditions, potassium from within the cells will leak out into plasma when the sample is collected.