VI. THE FUNCTIONS OF PLASMA MEMBRANE

VI.1. CELL JUNCTIONS

Specialized cell junctions occur at many points of cell-cell and cell-matrix contact in all tissues, but they are particularly important and plentiful in epithelia. Most of these junctions are too small to be resolved by light microscopy. They can be visualized, using either conventional or freeze-fracture electron microscopy, both of which show that the interacting plasma membranes (and often the underlying cytoplasm and the intervening intercellular space as well) are highly specialized in these regions. Cell junctions can be classified into three functional groups: 1) **occluding junctions**, which can seal cells together in an epithelial cell sheet in a way that prevents even small molecules from leaking from one side of the sheet to the other; 2) **anchoring junctions,** which mechanically attach cells (and their cytoskeletons) to their neighbors or to the extracelular matrix and 3) **communicating junctions**, which mediate the passage of chemical or electrical signals one interacting cell to its partner.

VI.1.1. A FUNCTIONAL CLASSIFICATION OF CELL JUNCTIONS

1. Occluding junctions (tight junctions)

2. Anchoring junctions

a) actin filament attachement sites
- -cell-cell adherens junctions (adhesion belts)
- -cell-matrix adherens junctions (focal contacts)
- -septate junctions (invertebrates only)

b) intermediate filament attachement sites
- -cell-cell (desmosomes)
- -cell-matrix (hemidesmosomes)

3. Communicating junctions
- a) gap junctions
- b) chemical synapses
- c) plasmodesmata (plants only)

VI.1.2. TIGHT JUNCTIONS/ZONULA OCCLUDENS

It is a diffusion barrier. It is located at the most apical point between adjoining epithelial cells and forms a ring or circumferential band (thus, soul) around the cell.

Examination of the zonula occludes in the electronic microscope reveals a narrow region in which the outer leaflets of the plasma membrane of adjoining cells come in contact to seal off the intercellular space. The seal is created by specific proteins that traverse the outer leaflets of the interacting cells and join in the intercellular space. The arrangement of the protein in forming the seal is best visualized by the freeze fracture technique.

High-resolution transmission electron microscopy similarly reveals that the zonula ocludens is not a continuous seal but, rather, appears as a series of focal fusions between the cells. The points of fusion correspond to the location of the protein particles observed in freeze fracture preparation. Also, observations based on different kinds of epithelia reveal that the complexity and number of strands forming the zonulae occludentes varies. In those epithelia where anastomosing strands or fusion sites are sparse, such as certain kidney tubules, the

intercellular pathway is partially permeable to water and solutes. In contrast, in those epithelia where the strands are numerous and extensively intertwined, intestinal and urinary bladder epithelia, the intercellular region is highly impermeable.

The zonula occludens separates luminal space from intercellular space and connective tissue compartment. It is now evident that the zonula occludens plays an essential role in maintaining selective passage of substances from one side of an epithelium to the other. To the degree that water and solutes are restricted from diffusing between cells by the zonula occludens, transport must occur by active means. This requires specialized membrane transport proteins that move selected substances across the apical plasma membrane into the cytoplasm and then across the basolateral membrane below the level of the junction.

The zonula occludens establishes functional domains in the plasma membrane. As a junction, the zonula occludens restricts not only the passage of water, electrolytes, and other small molecules across the epithelial layer but also the diffusion of molecules within the thickness of the plasma membrane itself. Thus the cell is able to segregate certain enzymes on the apical (free) surface and restrict others to the lateral or basal-lateral surfaces. In the intestine, the enzymes for terminal digestion of peptides and saccharine (dipeptidases and disaccharidases) are localised in the membrane of the microvilli of the apical surface. The Na-K activated ATPase that drives salts and water transport, as well as amino acid and sugar transport, is restricted to the lateral plasma membrane below the zonula occludens.

VI.1.3. ANCHORING JUNCTIONS connect the cytoskeleton of a cell to those of its neighbors or to the extracellular matrix. They are widely distributed in animal tissues. They enable groups of cells, such as those in an epithelium, to function as robust structural units by connecting the cytoskeletal elements of a cell either to those of another cell or to the extracellular matrix. They are most abundant in tissues that are subjected to severe mechanical stress, such as heart muscle and skin epithelium (epidermis).

They occur in three structurally and functionally different forms: 1) adherents junctions; 2) desmosomes and 3) hemidesmosomes. Adherens junctions are connection sites for actin filaments; desmosomes and hemidesmosomes are connection sites for intermediate filaments.

Before we discuss the different classes of anchoring junctions, it is worth considering briefly the general principles of their construction. As illustrated in the following figure, these junctions are composed of two classes of proteins:

- *intracellular attachment proteins*, which form a distinct *plaque* on the cytoplasmic face of the plasma membrane and connect the junctional complex to either actin filaments or intermediate filaments;
- *transmembrane linker proteins*, whose cytoplasmic domains bind to one or more intracellular attachment proteins, while their extracellular domains interact either with the extracellular matrix or with the extracellular domains of transmembrane linker proteins on another cell.

VI.1.3.1. Cell-cell adherens junctions occur in various forms. In many nonepithelial tissues they take the form of small punctate or streaklike attachments that connect actin filaments in the cortical cytoplasm of adjacent cells. In epithelial sheets they often form a continuous **adhesion belt, or zonula adherens** around each of the interacting cells in the sheet, located near the apex of each cell just below the tight junction. The adhesion belts in adjacent epithelial cells are directly apposed, and the interacting plasma membrane are held together by transmembrane

linker proteins that are members of a large family of Ca^{2+}-dependent cell-cell adhesion molecules called ***cadherins***.

Within each cell a contractile bundle of actin filaments lies adjacent to the adhesion belt, running parallel to the plasma membrane, to which it is attached through a set of intracellular attachment proteins that includes α -, β-,γ-catenin, vinculin, alpha-actinin, and plakoglobin. The actin bundles in adjacent cells are thus linked, via the cadherins and attachment proteins, into an extensive transcellular network. The contraction of this network, which depends on myosin motor proteins, is thought to help mediate a fundamental process in animal morphogenesis-the folding of epithelial cell sheets into tubes and other related structures.

VI.1.3.2. Desmosomes and hemidesmosomes

Desmosomes connect intermediate filaments from cell to cell. Hemidesmosomes connect intermediate filaments from cell to the basal lamina.

Desmosomes (macula adherens) are buttonlike points of intercellular contact that rivet cells together. Inside the cell they serve as anchoring sites for ropelike intermediate filaments, which form a structural framework for the cytoplasm of great tensile strength. Thus, through desmosomes, the intermediate filaments of adjacent cells are connected indirectly to form a continuous network throughout the tissue. The particular type of intermediate filaments attached to the desmosomes depends on the cell type: they are *keratin filaments* in most epithelial cells, for example, and *desmin filaments* in heart muscle cells.

The general structure of a desmosome is: -a dense cytoplasmatic plaque composed of a complex of intracellular attachment proteins (including plakoglobin and desmoplakins) responsible for connecting the cytoskeleton to the transmembrane linker proteins, which interact through their extracellular domains to hold the adjacent plasma membrane together. As in adhesion belts, the transmembrane linker proteins belong to the cadherin family of Ca^{2+} -dependent cell-cell adhesion molecules.

The importance of desmosomes in holding cells together is demonstrated by some forms of the potentially fatal skin disease *pemphigus*, in which individuals make antibodies against one of their own desmosomal cadherin proteins; these antibodies bind to and disrupt desmosomes between skin epithelial cells (keratinocytes), causing severe blistering as a result of the leakage of body fluids into the loosened epithelium. The antibodies disrupt desmosomes only in skin, suggesting that these desmosomes are biochemically different from those in other tissues.

Hemidesmosomes or half-desmosomes, resemble desmosomes morphologically but are both functionally and biochemically distinct.

Instead of joining adjacent epithelial cell membranes, they connect the basal surface of epithelial cells to the underlying *basal lamina*-a specialized mat of extracellular matrix at the interface between the epithelium and connective tissue. Moreover, whereas the keratin filaments associated with desmosomes make lateral attachments to the desmosomal plaque, many of those associated with hemidesmosomes have their ends buried in the plaque. As in focal contacts, the transmembrane linker proteins in hemidesmosomes belong to the integrin family of extracellular matrix receptors, rather than to the cadherin family of cell-cell adhesion proteins used in desmosomes.

Thus, although the terminology for the various anchoring junctions is a muddle, the molecular principles are simple. Integrins in the plasma membrane anchor a cell to extracellular matrix molecules; cadherins in the plasma membrane anchor it to cadherins in the membrane of

an adjacent cell. In both cases there is an intracellular coupling to cytoskeletal filaments, which can be either actin or intermediate filaments depending on the types of intracellular attachments proteins employed. Moreover, for all these classes of anchoring junctions, the adhesion depends on extracellular divalent cations, although the significance of this dependence is unknown.

VI.1.4. COMMUNICATING JUNCTIONS - GAP JUNCTIONS

It is one of the most widespread being found in large numbers in most animal tissues. It appears in conventional electron micrographs as a patch where the membranes of two adjacent cells are separated by a uniform narrow gap of about 2-4 nm. This gap is spanned by channel-forming protein molecules that allow inorganic ions and other small water-soluble molecules to pass directly from the cytoplasm of one cell to the cytoplasm of the other, thereby coupling the cells both electrically and metabolically.

The maximal functional pore size for the connecting channels of about 1.5 nm, implying that coupled cells share their small molecules (such as inorganic ions, sugars, amino acids, nucleotides, and vitamins) but not their macromolecules (proteins, nucleic acids, and polysaccharides). When fluorescent molecules of various sizes are injected into one of two cells coupled by gap junctions, molecules smaller than about 1000 daltons can pass into the other cell but larger molecules cannot.

Gap junctions are constructed from transmembrane proteins that form structures called connexons. When the connexons in the plasma membrane of two cells in contact are aligned, they form a continuous aqueous channel, which connects the two cell interiors. The connexons protrude from each cell surface, holding the interacting plasma membranes at a fixed distance from each other-hence the term gap junction, emphasizing the contrast with a tight junction, where the lipid bilayers appear to be in direct contact. A connexon is composed of a ring of six identical protein subunits called *connexins*, each of which contains four putative membrane-spanning alpha helices. The six subunits are thought to associate to form a connexon with a central aqueous pore that is lined by one transmembrane alpha helix from each subunit. The six connexins form a larger and permeable channel.

Like conventional ion channels, individual gap-junction channels do not remain continuously open; instead, they flip between open and closed states. Moreover, the permeability of gap junctions is rapidly (within seconds) and reversibly decreased by experimental manipulations that decrease cytosolic pH or increase the cytosolic concentration of free Ca^{2+}. These observations indicate that gap-junction channels are dynamic structure.

The physiological role of pH regulation of gap-junction permeability is unknown. There is one case, where the reason for the Ca^{2+} control seems clear. When a cell is damaged, its plasma membrane can become leaky. Ions present at high concentration in the extracellular fluid, such as Ca^{2+} and Na^{+}, then move into the cell, and valuable metabolites leak out. If the cell were to remain coupled to its healthy neighbors, these too would suffer a dangerous disturbance of their internal chemistry. But the influx of Ca into the sick cell causes its gap-junction channels to close immediately, effectively isolating the cell and preventing damage from spreading in this way.

VI.2. MEMBRANE TRANSPORT

Because of its hydrophobic interior, the lipid bilayer of cell membranes serves as a barrier to the passage of most polar molecules. This barrier's function is crucially important as it allows the cell to maintain concentrations of solutes in its cytosol that are different from those in the extracellular fluid and in each of the intracellular membrane-bounded compartments. To make use of this barrier, cells have had to involve ways of transferring specific water-soluble molecules across their membranes in order to ingest essential nutrients, excrete metabolic waste products, and regulate intracellular ion concentrations. Transport of inorganic ions and small water-soluble organic molecules across the lipid bilayer is achieved by specialized transmembrane proteins, each of each is responsible for the transfer of a specific ion or molecule or a group of closely related ions or molecules. Cells can also transfer macromolecules and even large particles across their membranes, but the mechanisms involved are different.

VI.2.1 CLASSIFICATION OF MEMBRANE TRANSPORT

1. According to the number and the direction followed by the transported molecules:
- uniport system - a single molecule is transported from one side of the membrane to the other;
- coupled transport - two molecule types are transported:
  - symport - both are transported in the same directions;
  - antiport - are transported in opposite directions.

2. According to the consumption of energy:
- passive transport - without consumption of energy, substances cross the membrane down their concentration gradient;
- active transport - against their concentration gradient, with consumption of energy.

There are two major classes of membrane transport proteins:
- **carrier proteins -** (also called carriers, permeases, or transporters) bind the specific solute to be transported and undergo a series of conformational changes in order to transfer the bound solute across membrane;
- **channel proteins -** need not to bind the solute; they form hydrophilic pores that extend across the lipid bilayer. When these pores are open, they allow specific solutes (usually inorganic ions of appropriate size and charge) to pass through them and thereby cross the membrane. Not surprisingly, transport through channel proteins occurs at a much faster rate than transport mediated by carrier proteins.

VI.2.2. PASSIVE TRANSPORT: it occurs without the consumption of ATP, down their concentration gradient and electrochemical gradient. The passive transport is accomplished by two ways: simple diffusion and facilitated diffusion.

VI.2.2.1. Simple diffusion. Hydrophobic molecules will diffuse across a bilayer. In general, the smaller molecule and the more soluble it is in oil (that is, the more hydrophobic, or nonpolar, it is) the more rapidly it will diffuse across the bilayer.

Hydrophilic molecules will pass across the lipid bilayer with the help of membrane transport molecules (represented by ionophores and ion channels).

A) Ionophores are small hydrophobic molecules that dissolve in lipid bilayer and increase their permeability to specific inorganic ions. Most are synthesized by microorganisms (presumably as biological weapons against competitors or prey). They are used to increase the ion permeability of membranes. There are two classes of ionophores: **mobile ion carriers** and **channel formers.** Both types operate by shielding the charge of the transported ion so that it can

penetrate the hydrophobic interior of the lipid bilayer. Since ionophores are not coupled to energy sources, they permit net movement of ions only down their electrochemical gradients.

Valinomycin is an example of a mobile ion carrier. It is ring-shaped polymer that transports K^+ down its electrochemical gradient by picking up K on one side of the membrane, diffusing across the bilayer, and releasing K on the other side. The ionophore A23187 is another example of a mobile ion carrier, but it transports divalent cations such Ca^{2+} and Mg^{2+}; it acts as an ion-exchange shuttle, carrying two H^+ out of the cell for every divalent cation it carries in. When cells are exposed to A23187, Ca enters the cytosol from the extracellular fluid down a steep electrochemical gradient.

Gramicidin A is an example of a channel-forming ionophore. As a linear peptide of only 15 amino acid residues, all with hydrophobic side chains, it is the simplest and best characterized ion channel. Two gramicidin molecules are thought to come together end to end across the bilayer to form a transmembrane channel, which selectively allows monovalent cations to flow down their electrochemical gradients. Gramicidin A can transport about 2 $x10^7$ captions per open channel in 1 second that can be transported by a single mobile carrier molecule in the same time. Gramicidin is made by certain bacteria, perhaps to kill other microoganisms by collapsing the H, Na, K gradients that are essential for cell survival, and it has been useful as an antibiotic.

B) Ion channels form hydrophilic pores across membranes. For transport efficiency, channels have an advantage over carriers in that more than 1 million ions can pass through one channel each second, which is a rate 1000 times greater than the fastest rate of transport mediated by any known carrier protein. On the other hand, channels cannot be coupled to an energy source to carry out active transport, so the transport they mediate is always passive ("downhill"). Thus the function of ion channels is to allow specific inorganic ions, mainly Na^+, K^+, Ca^{2+}, or Cl^-, to diffuse rapidly down their electrochemical gradients across the lipid bilayer.

Two important properties distinguish ion channels from simple aqueous pores:

- ion selectivity - permitting some inorganic ions to pass but not others;
- ion channels are not continuously open. Instead they have "gates", which open briefly and then close again. In most cases the gates open in response to a specific stimulus. The main types of stimuli that are known to cause ion channels to open are a change in the voltage across the membrane **-voltage-gated channels**, a mechanical stress -**mechanically gated channel**s, or the binding of a ligand -**ligand-gated channels.** The ligand can be either an extracellular mediator - specifically, a neurotransmitter -transmitter-gated channels, or an intracellular mediator, such as an ion –ion-gated channels, or a nucleotide -nucleotide-gated channels. The activity of many ion channels is regulated in addition by protein phosphorylation and dephosphorylation.

More than 100 types of ion channels have been described. They are responsible for the electrical excitability of muscle cells, and they mediate most forms of electrical signalling in the nervous system. A single nerve cell might typically contain 10 kinds of ion channels or more, located in different domains of its plasma membrane. The most common ion channels are those that are permeable mainly to K^+. These channels are found in the plasma membrane of all animal cells.

VI.2.2.2. The facilitated diffusion through carrier proteins.

It is an energy independent transport,"downhill". It is 100000 times faster than the simple diffusion although the transported molecules are larger than those involved in the simple diffusion. Although the molecular details are unknown, carrier proteins are thought to transfer the solute across the lipid bilayer by undergoing reversible conformational changes that

alternately expose the solute binding site first on one side of the membrane and then on the other. Conclusion: the carrier protein can exist in two conformational states-in state "pong" the binding sites for solute A are exposed on the outside of the bilayer; in state "ping" the same sites are exposed on the other side of the bilayer. The transition between the two states is reversible. Therefore, if the concentration of A is higher on the outside of the bilayer, more A will bind to the carrier protein in the pong conformation than in the ping conformation, and there will be a net transport of A down its electrochemical gradient.

Through this mechanism the water, urea, alcohol and anions pass through the red blood cell membrane, and glucose and amino acids passes through the membrane of other cell types.

VI.2.3. ACTIVE TRANSPORT

The active transport can be classified in active transport through ion pumps, active transport driven by ion gradients, and group traslocation.

VI.2.3.1. Ion pumps (Na^+ - K^+ pump)

The concentration of K^+ is typically 10 to 20 times higher inside cells than outside, whereas the reverse is true of Na^+. These concentration differences are maintained by **Na^+ - K^+ pump** that is found in the plasma membrane of virtually all animal cells. The pump operates as an antiporter, actively pumping Na^+ out of the cell against its steep electrochemical gradient and pumping K^+ in.

A major advance in understanding the Na^+- K^+ pump came with the discovery in 1957 of an enzyme that hydrolyzes ATP to ADP and phosphate and requires Na and K for maximal activity. An important clue linking this **Na^+ - K^+ ATPase** with the Na^+ -K^+ pump was the observation that a known inhibitor of the pump, ouabain, also inhibits the ATPase. It was found that (1) the transport of Na^+ and K^+ is tightly coupled to ATP hydrolysis, so that one cannot occur without the other; (2) ion transport and ATP hydrolysis can occur only when Na^+ and ATP are present inside the cells and K^+ is present on the outside; (3) ouabain is inhibitory only when present outside the cells, where it competes for the K^+-binding site; (4) for every molecule of ATP hydrolysed (100 ATP molecules can be hydrolysed by each ATPase molecule each second), three Na^+ are pumped out and two K^+ are pumped in.

Next, we will discuss a schematic model of the pumping cycle of the Na^+ - K^+ ATPase. The binding of Na^+ (1) and the subsequent phosphorylation by ATP of the cytoplasmatic face of the ATPase (2) induce the protein to undergo a conformational change that transfers the Na across the membrane and release it on the outside (3). Then the binding of K^+ on the extracellular surface (4) and the subsequent dephosphorylation (5) return the protein to its original conformation, which transfers the K across the membrane and release it into cytosol (6). These changes in conformation are analogous to the ping - pong transitions, except that here, the phosphorylation and dephosphorylation processes induce these transitions.

Roles of Na^+ -K^+ pump:

- generating and maintaining the cell's membrane potential;
- maintains the osmotic balance and stabilizes cell volume;
- controls the neurotransmitters release from brain;
- is responsible for driving the active transport of sugars and amino acids;
- plays an important role in thermogenesis.

VI.2.3.2. Active transport driven by ion gradients (secondary active transport)

Intestinal and kidney epithelial cells contain a variety of symport systems that are driven by the Na^+ gradient across the plasma membrane; each system is specific for importing a small group of related sugars or amino acids into the cell. In these system the solute and Na^+ bind to different sites on a carrier protein; because the Na^+ tends to move into the cell down its electrochemical gradient, the sugar or amino acid is, in a sense, "dragged" into the cell with it. The grater the electrochemical gradient for Na^+, the grater the rate of solute entry; if the Na^+ concentration in the extracellular fluid is reduced, solute transport decrease.

Another example of secondary active transport is the antiport transport of Na^+ - Ca^{2+} and Na^+- H^+ in proximal convoluted tubule.

VI.2.3.3. Group translocation - it is found in bacteria. The sugars are phosphorilated by the phsospho transferase system. The sugars enter inside the cell, changing their chemical structure, and after that they can not leave it.

VI.2.4. VESICULAR TRANSPORT

Some substances enter and leave cells by processes that involve configurational changes in the plasma membrane at localized sites, namely vesicular transport. This activity involves the formation of vesicles from the membrane or the fusion of vesicles with the membrane. Vesicular transport in its various modes may be defined in more specific terms:

- **endocytosis** - the name given to the process of vesicular transport when it involves substances entering the cell;
- **exocytosis** - the name given to the process of vesicular transport when it involves substances leaving the cell;
- **transcytosis** - substances sleepers the cell without modifying the cell metabolism.

Two forms of endocytosis are recognized: phagocytosis and pinocytosis.

VI.2.4.1. Phagocytosis (Gr. for cell eating) - is the ingestion of particulate matter, including bacteria and other cells.

Phagocytosis steps:

1) chemotaxis - a leaded movement of phagocytic cells to a signal (bacterial components, complement system);

2) the recognition of the bacteria - the phagocytes have receptors that recognize the antigen (non-self). For example, phagocytes cell-surface receptors recognize certain carbohydrate residues in bacteria cell walls, then the specific phagocytes of the bacteria occurs by opsonization. During opsonization, microorganisms are coated with immunoglobulins or complement components.

3) phagocytes adhere to opsonized bacteria;

4) phagocytes engulf the bacteria in membrane - delimited sacs called phagosomes;

5) the degradation of the bacteria (digestion) by the lysosomes.

VI.2.4.2. Pinocytosis (Gr. for cell drinking) is the ingestion of substances initially in molecular dispersion and the ingestion of bulk fluid stimulated by ionic changes.

Two forms of of pinocytosis are recognized with TEM. In one form, smooth **pinocytotic vesicles** are produced; in the other form, **coated vesicles** are produced.

The plasma membrane invaginates to form small pits or caveolae that project into the cell. The opening of the pit constricts into a narrow neck, and further constriction results in the separation of a vesicle from the membrane. These smooth pinocytotic vesicles are especially

numerous in the endothelium of blood vessels, and under the plasma membrane of smooth muscle cells, but are present in nearly every cell type.

The formation of coated vesicles is similar except that the plasma membrane acquires a localized concentration of short bristle-like projections on its inner surface where the vesicle is to form. In the formation of vesicles by this method, the coated membrane first forms a depression, then a small pit; finally, the coated pit pinches off to become a coated vesicle.

Coated vesicles participate in a selective process of absorption referred to as receptor-mediated endocytosis. In receptor -mediated endocytosis, certain molecules within the plasma membrane recognize and bind specific substances that come in contact with the plasma membrane. Smooth pinocytotic vesicles, by contrast, are relatively nonselective. Modification of the environment of the membrane, such as ionic changes, and nonspecific binding of charged particles to the glycocalyx, can stimulate pinocytosis.

There are two general pathways of exocytosis:

-constitutive pathway: identifies a process that is continuous. Proteins that leave the cell by this process are secreted immediately after their synthesis and exit from the Golgi apparatus;

-regulated secretory pathway: proteins that are concentrated and transiently stored in secretory granules pass along the regulated secretory pathway.

VI.3. DIRECT AND DISTANCE INTERCELLULAR COMMUNICATION FUNCTION

VI.3.1. SIGNAL MOLECULES AND RECEPTORS

In order to respond to received messages, the cells contain on their surface, in cytoplasm and nucleus, specific proteins which receive and retain the message. The proteins are called receptor proteins or simply, ***receptors.*** In our organism a number of chemical substances are mobilised and reach on the nervous or unmoral path various organs, "aim" cells, where they bind to the receptors. These substances are named ***"messengers"*** or ***"binders" (ligands).*** The binders act at very low concentrations (10^{-8}) and bind the complementary receptors with a big affinity constant / factor ($k \rightarrow 10^{-8}$ litter / moll). As a result of the receptor-binder binding, a tremendous number of interactions between specialised cells are realised in the organism. Metabolic changes are induced according to the organism's necessities.

There are three categories of signal molecules produced by the organism (endogenous): a) local chemical mediators, b) hormones and c) neurotransmitters.

a) Local chemical mediators are secreted by many cells, but act only upon cells situated in the immediate vicinity, being recognized and captured.

b) Hormones are secreted by the cells of the endocrine glands and by way of the blood stream or lymphatic stream they reach the "aim" cells at great distances, in different organs.

c) Neurotransmitters are produced by neurons and set free at level of chemical synapses, acting only on the adjacent "aim".

The endocrine cells and the neurons are cells highly specialized in chemical signaling and the hormones and neurotransmitters are considered I (first) order messengers of these cells. Thus, the nervous and endocrine way / path realize the functional regulation of all the cells in the organism.

The messages influence the "aim" cells either by decreasing the synthesis rate of the existent proteins or by initiating the synthesis of a new protein. Some cellular answers to the messenger's actions are quick and transitory (ex. insulin), others are slow and long lasting (ex. estradiol). The cellular answer depends in this case on the chemical composition of the

messenger. Thus, the majority of messengers are hydrosoluble molecules and act rapidly; other (steroid and thyroid hormones) are liposoluble, so they have a slow and late intracellular effect.

According to the way they influence the receptors, signal substances can be classified in:

- ***agonistic substances –*** which change totally or partially the structure of their binding protein, activating the receptors (ex. hormones, neurotransmitters);
- ***antagonistic substances –*** which block / obstruct the cellular receptors, without changing their structure (ex. medicines);

VI.3.2 RECEPTOR CLSSIFICATION

According to the origin of the messenger they interact with, there are two main types of receptors for endogenous substances and for exogenous substances.

VI.3.2.1. Receptors for endogenous substances

a) Receptors for neurotransmitters are situated in the postsynaptic membrane of the muscular, nervous or generally, effect cells. They recognize and interact with the mediators of the nervous influx. Among these, we mention: receptors for acetylcoline, noradrenalin, histamine, serotonin.

b) Receptors for hormones are differently localized in the cell, depending on the hormone's nature (hydrophilic or hydrophobic). Liposoluble hormones (steroid hormones, thyroid hormones, retinoic acid) are small molecules which can penetrate the hydrophobic, lipid bilayer and interact with specific receptors from the cytosol or the nucleus. Hydrophilic hormones bind to the receptors in the plasmalema and these send the necessary information to the cell in order to change its metabolism. In this category are included receptors for insulin, hypophysis hormones, parathormone.

c) Receptors involved in immunity reactions are represented by:

- receptors for endogenous antigen – are found on the surface of the cells involved in the immune answer (T lymphocytes) and on the surface of all cells in the organism;
- receptors for antibodies – these are described as the receptors from the surface of the eosinophiles and mastocytes;
- receptors for complement – are situated in the glycocalix of the cells from the mononuclear phagocyte system and induce the immunologically mediated phagocytosis.

d) Receptors for the growing factors – The growing factors are protein molecules which have a basic / fundamental role in cellular multiplication, differentiation and survival, in the course of embryogenesis, the control of tissular homeostasis and repairing of damaged tissues.

VI.3.2.2. Receptors for exogenous substances

There are described four types of receptors:

a) Receptors for viruses;
b) Receptors for "non-self" antigen – are situated on the surface of B lymphocytes.
c) Receptors for lectin;
d) Receptors for drugs – Medicines have an antagonistic action on these receptors.
e) Receptors for bacterial toxins.

VI.3.3. EFFECTS OF BINDER - RECEPTOR BINDING

The events which take place following the messenger – receptor contact are in order:

A. the recognition and attachment of the messenger to the receptor;
B. the answer of the "aim" cell, which consists in:

1. structural changes of the membrane
2. functional changes of the membrane
    a) permeability changes
    b) enzymatic activation
    c) the intracellular penetration of some ions
3. specific changes for the cellular metabolism (specific cellular answer or secondary effect).

VI.3.3.1. Structural changes of the membrane

Right after the contact with the messenger, a redistribution of the receptors takes place; they gather in limited / restricted areas. The mechanism is facilitated by the membrane's fluidity and the movements of the receptor proteins. This is due to the fact, that both the binder and the receptor have at least two binding sites. The binding is usually realized by weak interactions (hydrophobic or H bonds).

In some cells, the binder-receptor complexes cover great surfaces, forming at one of the cell poles a "cupola".

VI.3.3.2. Functional changes of the membrane

a) The permeability changes for Na^+, K^+, Ca^{++} take place at the binding process of the neurotransmitters to the postsynaptic membrane and have a role in transmitting and generating the nervous influx.

b) Activation of enzymes from the plasmalema – Hydrophilic hormones, which represent the majority (hypophysis, epiphysis, parathyroidian, medulosuprarenalian hormones) circulate through the blood with great speed, act rapidly and the effects start a few seconds after the binding to the receptor and end after a few minutes. The binding of the hydrophilic hormone to the specific receptor leads to the formation / appearance of a binder-receptor complex, considered the first (I) order messenger.

At the same time, a structural modification of the receptor molecule takes place, which will determine the appearance of the second (II) order messenger represented by cAMP (cyclic adenosine 3' 5' monophosphate) and cGMP (cyclic guanosine monophosphate). The process of generating II order messengers is based on the activation or inactivaton of the adenylate cyclase, enzyme bound to the plasmalema. This has the role / function to catalyse the cyclic AMP synthesis on the internal side of the cellular membrane or it will determine / cause directly the phosphorilation of cellular proteins (ex. the epidermal growth factor). The activation and inactivation of the adenylate cyclase is realized by going through the following stages:

- the binding of the hormone (H) to the receptor (R) alters its conformation / structure, so that it can associate with the intraplasmatical G protein;
- the hormone-receptor complex associates with the G protein, which later becomes capable of binding the GTP;
- the GTP binding modifies the conformation / structure of G protein so that this can activate the adenylate cyclase (AC). The AC activation leads to the transformation of ATP in cAMP.
- G protein also, hydrolyses then the binding of GTP to GDP, which leads to the inactivation of the adenylate cyclase.

Similarly, the guanylate cyclase enzyme catalyses the formation of cGMP from GTP. cGMP acts in a ten times smaller concentration than cAMP and initiates the increase of Ca^{++} intracellular concentration.

In conclusion, the first order messenger (the binder-receptor complex) determines the activation of the plasmalema enzymes (adenylate cyclase), which catalyse the reactions that produce the second order messengers (cAMP and cGMP).

c) Penetration of ions into the cell – Some surface receptors are functionally coupled to the Ca^{++} channels of plasmalema. The formation of the hormone-receptor complex leads to the opening of these channels and is followed by the increase of the Ca^{++} influx in the cytosol, from the extracellular fluid or from the internal Ca^{++} deposits. The Ca^{++} concentration increase is transitory because the Ca^{++} channels open transitorily and the Ca^{++} that entered in the cytosol is rapidly pumped outside the cell and or its intracellular bound to phosphates, calmoduline or the membranes of intracytoplasmic organelles (endoplasmic reticulum, mitochondria).

VI.3.3. Specific changes of the cellular metabolism

Once synthesized, cAMP is considered a second order messenger which causes a series of specific metabolic phenomena in the cytoplasm. cAMP activates the proteinkinases which determine changes of the cellular metabolism, such as:

- glycogen synthesis by stimulating the glycogensynthetase;
- glycogenogenesis by activating the phosphorylase;
- activation of the protein synthesis in rough endoplasmicum reticulum;
- synthesis activation of nucleic acids in the nucleus.

Ca^{++} is also considered a second order messenger. The Ca^{++} ions penetrate the postsynaptic membranes from the neuromuscular junctions; this phenomenon is followed by the coupling of excitation with contraction and the activation of the actin – myosin system. Second order messengers translate extracellular signals (binding of the binder to the receptor) into intracellular signals and they same time they realize a very great amplification of the initial signal.

Hydrophobic hormones (steroid, thyroid hormones) cross the plasmalema of the “aim” cell by simple diffusion because they are liposoluble. The specific receptors are situated intracytoplasmic for the steroid hormones and intranuclear for the thyroid hormone.