GENERAL AND CELLULAR BASIS OF PHYSIOLOGY

BODY COMPOSITION:

- Young adult male:
  - 18% protein
  - 7% mineral
  - 15% fat
  - Remaining 60% is water
- **60% of body is water**
- Cells of the body exist in an internal sea of EXTRACELLULAR FLUID (ECF), made up of:
  - INTERSTITIAL FLUID
  - CIRCULATING BLOOD PLASMA
- Plasma and cellular elements of blood make up the TOTAL BLOOD VOLUME
- About a third of TOTAL BODY WATER is extracellular and two thirds intracellular
- TBW = 60% of body weight
- ICF = 2/3 of TBW
- ECF = 1/3 of TBW
- Interstitial F = 2/3 of ECF
- Intravascular F = 1/3 of ECF

UNITS OF SOLUTE CONCENTRATION:

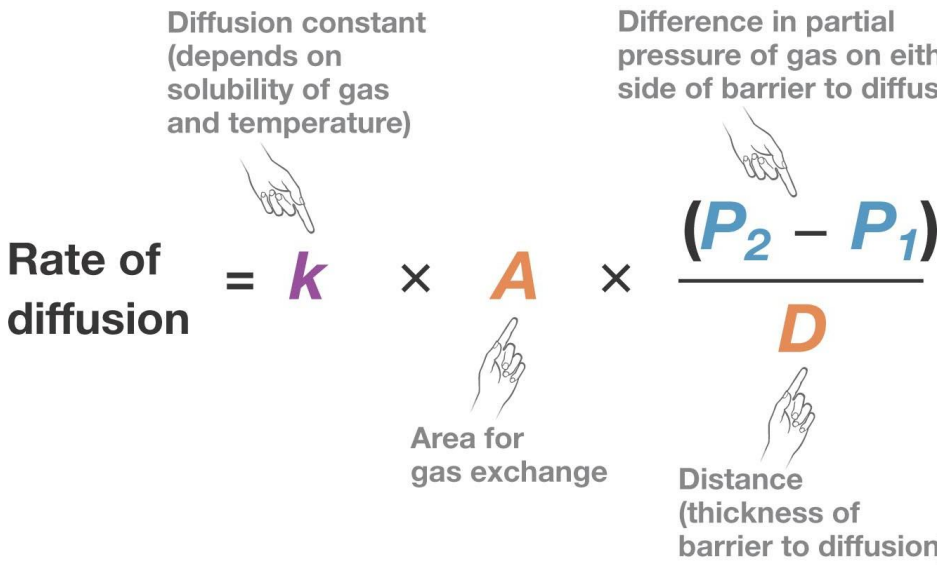
- **MOLES**:
  - **Mole = gram-molecular weight of a substance**
  - **Mole= 6x10^23 molecules (Avagadro constant)**
  - Millmole is 1/1000 of a mole
  - The MOLECULAR WEIGHT of a substance is the ratio of the mass of one molecule of the substance to the mass of one twelfth the mass of an atom of carbon-12
  - Molecular weight often expressed in Daltons
  - THE DALTON (Da), is the mass equal to one twelfth the mass of an atom of carbon-12
  - 1000 Da = 1 kilodalton (kDa)
    - Useful unit for expressing the molecular weight of proteins

- **EQUIVALENTS**:
  - **One mole of an ionised substance divided by its VALENCE**
    - One equivalent of sodium is equal to 23g versus calcium = 40/2 = 20g
    - Milliequivalent is 1/1000 of 1 equivalent

- **PH**:
    - The maintenance of a stable hydrogen ion concentration in the body fluids is essential to life
    - The **pH of a solution is the log to the base 10 of the reciprocal of the hydrogen ion concentration**
        - For each pH unit <7, the concentration of hydrogen is increased tenfold and above 7, it is decreased tenfold
- **BUFFERS:**
    - Intracellular and extracellular pH are generally maintained at very constant levels
    - Body pH is maintained by the buffering capacity of the body fluids
        - A BUFFER is a substance that has the ability to bind or release H+ in solution, thus keeping the pH of the solution relatively constant
        - Carbonic acid is a good example in the human body, moving the equilibrium point depending on additional H+ or OH-

MOVEMENT ACROSS MEMBRANES:

- **DIFFUSION**:
    - Diffusion is the process by which a gas or a substance in solution expands, because of the motion of its particles to fill all of the available volume
    - The particles of a substance dissolved in a solvent are in continuous random movement
        - A given particle is equally likely to move into or out of an area in which it is present in high concentration
        - However, since there are more particles in the area of high concentration, the total number of particles moving to area of lower concentration is greater
            - NET FLUX
    - **Time to reach equilibrium is the SQUARE OF THE DIFFUSION DISTANCE**
    - The magnitude of the diffusing tendency is directly proportional to the cross-sectional area across which diffusion is taking lace and the concentration gradient or chemical gradient
        - **FICK'S LAW OF DIFFUSION**



- **OSMOSIS**:
  - When a substance is dissolved in water, the concentration of water molecules in the solution is less than that in pure water
  - If the solution is placed on one side of a membrane permeable to water but not to the solute, and an equal volume of water is placed on the other, water molecules diffuse down their concentration gradient
    - **OSMOSIS:**
      - **DIFFUSION OF <u>SOLVENT</u> MOLECULES FROM A REGION OF LOW CONCENTRATION OF <u>SOLUTE</u> INTO A REGION IN WHICH THERE IS A HIGHER CONCENTRATION OF SOLUTE ACROSS A MEMBRANE WHICH IS IMPERMEABLE TO THE SOLUTE**

  - The **pressure necessary to prevent solvent migration is known as the <u>OSMOTIC PRESSURE</u>**
    - Osmotic pressure **depends on the NUMBER of molecules**, rather than the type of particles
    - In an IDEAL SOLUTION, the **osmotic pressure is related to temperature and volume** (**ideal gas equation**)
      - **P = nRT/V**
      - Where n is the number of particles, R is the gas constant, T is the absolute temperature and V is the volume
      - **If T is held constant, then the osmotic pressure is proportionate to the number of particles in solution per unit volume**
    - In the body, it is the effective concentration (activity) in the body fluids rather than the number of equivalents that determines its osmotic effect

        - THE **NUMBER OF OSMOTICALLY ACTIVE PARTICLES IS EXPRESSED IN OSMOLES**
        - OSMOLARITY = number of osmoles per litre of solution
        - OSMOLALITY = number of osmoles per kilogram of solvent

        - Osmolarity is affected by the volume of various solutes in the solution and the temperature, while the osmolality is not

- **TONICITY:**
    - The **term used to describe the osmolality of a solution relative to PLASMA**
    - Same osmolality as plasma = ISOTONIC
    - Greater osmolality = HYPERTONIC
    - Lesser osmolality = HYPOTONIC
    - All solutions that are initially isotonic would stay that way were it not for the fact that some solutes diffuse into cells and others are metabolised
        - Normal saline thus remains isotonic as there is no net movement of the osmotically active particles into the cells and the particles are not metabolised
        - HOWEVER, 5% glucose solution is isotonic initially, but glucose is metabolised, so the nett effect is infusing a hypotonic solution
    - All but 20 milliosmoles in each litre of normal plasma is contributed by sodium and its associated anions (chloride and bicarbonate)
        - Proteins contribute very little due to their size
    - Glucose and urea also contribute

- **REGULATION OF CELL VOLUME:**
    - Animal cells swell when exposed to extracellular hypotonicity and shrink when exposed to hypertonicity
        - **Swelling** activates channels permitting **efflux of potassium and chloride**, with water following and returning cell to normal volume

- **NON-IONIC DIFFUSION:**
    - Some weak acids and bases are soluble in cell membranes in the undissociated form, whereas they cross membranes poorly in the ionic form
    - If substances **diffuse then dissociate, there is appreciable net movement of the undissociated substance**

- **DONNAN EFFECT:**
    - **When an ion on one side of a membrane cannot diffuse through the membrane, the distribution of other ions to which the membrane is permeable are effected in a predictable way**
        - E.g. the negative charge of a nondiffusible anion (protein) hinders diffusion of the diffusible cations and favours diffusion of the diffusible anions
    - GIBBS AND DONNAN showed that in the presence of a non-diffusible ion, the diffusible ions distribute themselves so that at equilibrium their concentration ratios are equal
    - Hence, **because of intracellular proteins, there are MORE OSMOTICALLY ACTIVE PARTICLES IN THE INTERSTITIAL FLUID**
        - Cells maintain their normal volume because of the Na/K ATPase
    - Also, there is an asymmetrical distribution of permeant ions across the membrane, the magnitude of which is calculated using the **NERNST EQUATION:**
    - Third, since there are **more proteins in plasma than in the interstitium, there is a Donnan effect on ion movement across the capillary wall**

    Non-diffusible proteins:
    Intracellular > extracellular
    Plasma > interstitium

- **GENESIS OF THE MEMBRANE POTENTIAL:**
    - An equilibrium is reached in which the tendency of potassium to move out of the cell is balanced by its tendency to move into the cell
        - At equilibrium, there is a slight excess of cations on the outside and anions on the inside
        - This condition is maintained by the Na/K ATPase
            - This pumps potassium back into the cell and keeps the intracellular concentration of sodium LOW

<u>TRANSPORT ACROSS CELL MEMBRANES:</u>

EXOCYTOSIS:

- Vesicles containing material for export are ticketed to the cell membrane via the **v-SNARE/t-SNARE** arrangement
- The area of fusion breaks down, leaving the contents of the vesicle outside the cell and the cell membrane intact
    - **CONSTITUTIVE PATHWAY -> little or no processing prior exocytosis** -> more rapid
    - NON-CONSTITUTIVE PATHWAY ->prohormones are processed to mature hormones prior to exocytosis

ENDOCYTOSIS:

- Reverse of exocytosis
- Material makes contact with the cell membrane, which then INVAGINATES
- Invagination is then pinched off, leaving the engulfed material in the membrane-enclosed vacuole and the cell membrane intact
- **CLATHRIN**-MEDIATED endocytosis occurs at membrane indentations where the protein Clathrin accumulates
    - Responsible for internalisation of many receptors and the ligands bound to them

Exocytosis: SNARE
Endocytosis: Clathrin

COATS AND VESICLE TRANSPORT:

- All vesicles involved in protein transport have protein coats
- Certain amino acid sequences or attached groups on the transported proteins ticket the proteins for particular locations

MEMBRANE PERMEABILITY AND MEMBRANE TRANSPORT PROTEINS:

- **Small, nonpolar molecules (O2 and N2) and small polar molecules such as CO2 diffuse across lipid membranes**
- However, membranes have very limited permeability to other substances
- INSTEAD, they cross the membranes by endocytosis, exocytosis and by passage through highly specific TRANSPORT PROTEINS, that form channels for ions or other substances (glucose, urea and amino acids)
- There are even transport channels for water (AQUAPORINS)
- Many transport proteins are continuously open, but the rate at which they transport ions can be varied, that is they are GATED:
    - By alterations in membrane potential (VOLTAGE GATED)
    - When they bind a ligand (LIGAND-GATED)
    - Some are opened by mechanical stretch
- Other transport proteins are carriers that bind ions and other molecules and then change their configuration, moving the bound molecule from one side to the other
- Molecules move by their:
    - CHEMICAL GRADIENT (from high to low concentration)
    - ELECTRICAL GRADIENT (cations move to negatively charged areas and vice versa)
    - When carrier proteins move substances in the direction of their chemical or electrical gradients, no energy input is required and the process is called FACILITATED DIFFUSION
        - E.G. GLUCOSE TRANSPORT

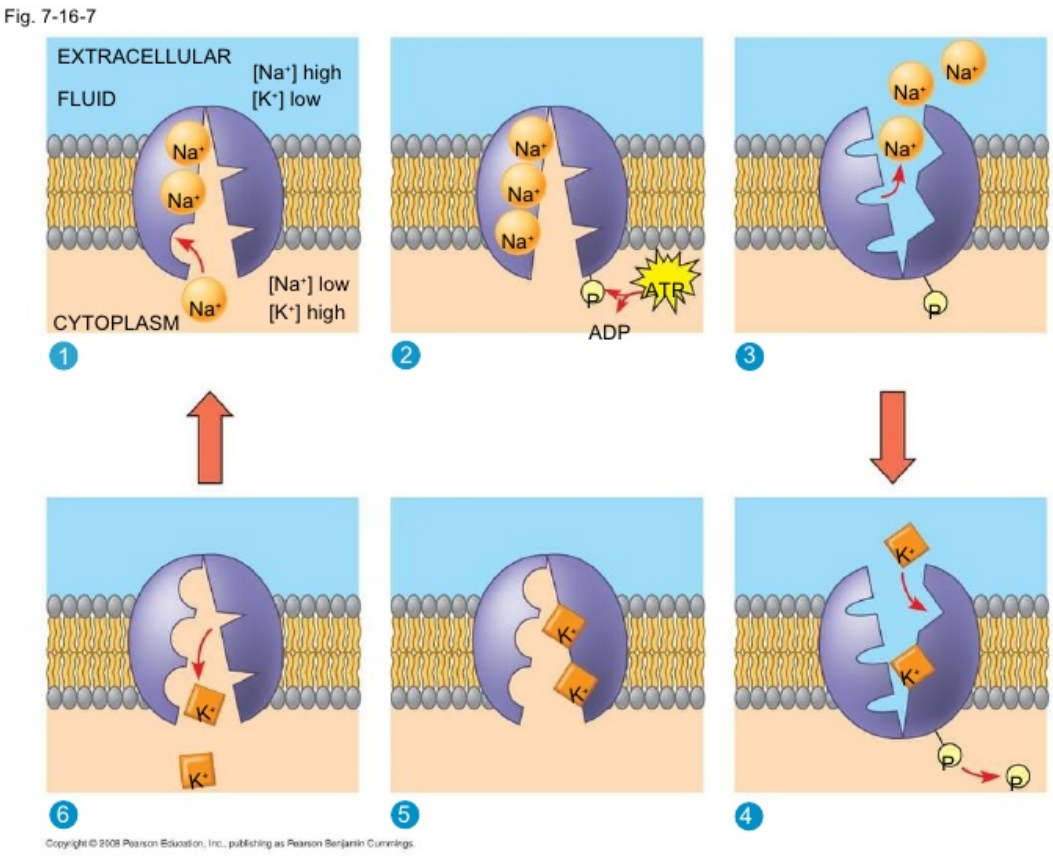
    - If going against chemical or electrical gradient, then this will require energy and is called ACTIVE TRANSPORT
        - In animal cells, the energy is provided almost exclusively by hydrolysis of ATP
        - E.g. Na/K ATPas
- Some transport proteins are called UNIPORTS as they transport only one substance
- Others are called SYMPORTS, because transport requires the binding of more than one substance and they cross the membrane together
    - E.g. facilitated diffusion of sodium and glucose from the intestinal lumen into mucosal cells
- Others are called ANTIPORTS, because they exchange one substance for another

ION CHANNELS:

- There are ion channels for potassium, sodium, calcium and chloride
- Each exists in many forms with diverse properties

SODIUM/POTASSIUM ATPase:

- Uses the energy from hydrolysis of ATP to ADP to extrude three sodium from the cell and take tow potassium into the cell for each molecule of ATP hydrolysed
    - An ELECTROGENIC PUMP
- When **sodium binds to the alpha subunit, ATP also binds and is converted to ADP, with a phosphate being transferred to the phosphorylation site**
    - This causes a **change in conformation**, **extruding sodium into the ECF**
    - **Potassium then binds extracellularly**, **dephosphorylating the alpha** subunit, which **returns to its previous conformation**, releasing potassium into the cytoplasm



SECONDARY ACTIVE TRANSPORT:

- The active transport of sodium is coupled to the transport of other substances
- E.g. the symport in the GI mucosa that transports glucose into the cell only if sodium binds to the protein and is transported into the cell at the same time
    - The electrochemical gradient for sodium is maintained by the active transport of sodium out of the mucosal cell into the ECF
- In the heart the Na-K ATPase indirectly affects calcium transport:
    - An antiport in the membranes of cardiac muscle cells normally exchanges intracellular calcium for extracellular sodium

TRANSPORT ACROSS EPITHELIA:

- In the GIT, the pulmonary airways, the renal tubules, substances enter one side of a cell and exit another, producing movement of the substance from one side of the epithelium to the other

<u>INTERCELLULAR COMMUNICATION:</u>

- Cells communicate with one another via chemical messengers
- Within a given tissue, some messengers move from cell to cell via GAP JUNCTIONS

- Cells are also affected by substances secreted in to the ECF
- Three general types of **intercellular communication** are mediated by messengers in the ECF:
    - **NEURAL COMMUNICATION**:
        - Neurotransmitters are released at synaptic junctions from nerve cells and act across a narrow synaptic cleft on the post-synaptic cell
    - **ENDOCRINE COMMUNICATION**:
        - Hormones and growth factors reach cells via the circulating blood
    - **PARACRINE COMMUNICATION**:
        - Products of cells diffuse in the ECF to affect neighbouring cells
- Special example is **AUTOCRINE COMMUNICATION**:
    - Cells secrete chemical messengers that bind to receptors on the same cell

MECHANISMS BY WHICH CHEMICAL MESSENGERS ACT:

- Ligands such as acetylcholine bind directly to ion channels in the membrane, changing their conductance
- Thyroid and steroid hormones enter cells and act on one or another member of a family of structurally related cytoplasmic or nuclear receptors
- Activated receptor binds to DNA and increases transcription of selected mRNAs
- Many ligands trigger release of intracellular mediators such as CAMP, IP3 and DAG
- Extracellular ligands are called FIRST MESSENGERS and intracellular mediators are known as SECOND MESSENGERS
    - Second messengers bring about many short-term changes in cell function by altering enzyme function and transcription of various genes
    - The second messengers generally activate **PROTEIN KINASES**, which are enzymes that catalyse the **phosphorylation** of amino acid residues:
        - **Tyrosine**
        - **Serine**
        - **Threonine**
- In some instances, the intracellular portions of the receptors themselves are protein kinases, and in others, they phosphorylate themselves (receptor tyrosine kinases)
- **PHOSPHATASES are obviously important in that they inactivate phosphorylated enzymes**

STIMULATION OF TRANSCRIPTION:

- When steroid hormones (and thyroid) bind to their receptors inside cells, the conformation of the receptor protein is changed and a DNA binding domain is exposed
    - The receptor-hormone complex moves to DNA, where it **binds to enhancer elements** and upregulates transcription

- **Steroids have more rapid, non-genomic action which are mediated by putative membrane receptors**

INTRACELLULAR CALCIUM:

- **The free Ca2+ concentration in the cytoplasm** at rest is maintained at about **100nmol/L**
    - The **Ca2+ concentration in the interstitial fluid is about 12,000 times the cytoplasmic concentration,** so there is a marked inwardly directed concentration gradient as well as inwardly directed electrical gradient
    - **Massive electrochemical gradient for calcium influx into cells**
    - Much of the intracellular calcium is bound by the ER and other organelles, providing a store from which calcium can be mobilised

- Increased cytoplasmic Ca2+ binds to and activates calcium-binding proteins and these in turn activate a number of protein kinases
- The voltage-gated Ca2+ channels are often divided into T (transient) or L (long-lasting) on the basis of **whether they do or do not inactivate during maintained depolarisation**
- Many second messengers act by increasing intracellular Ca2+, produced by releasing Ca2+ from intracellular stores, principally the ER
- **IP3 is the major second messenger that causes Ca2+ release from the ER**

Calcium control of VSM contraction

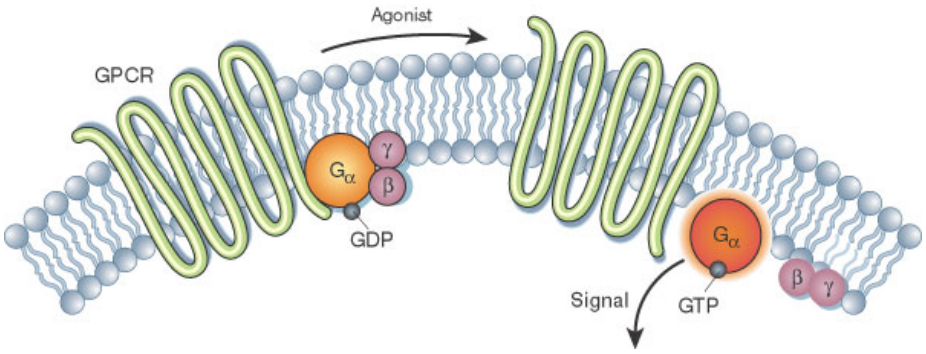
- ↑Ca → Ca-CaM; 4 calcium ions bind to each CaM
- Ca-CaM activates of MLCK → phospohrylation MLC

→ disinhibition of myosin ATPase, and initiation of cross-bridge cycling

G PROTEINS:

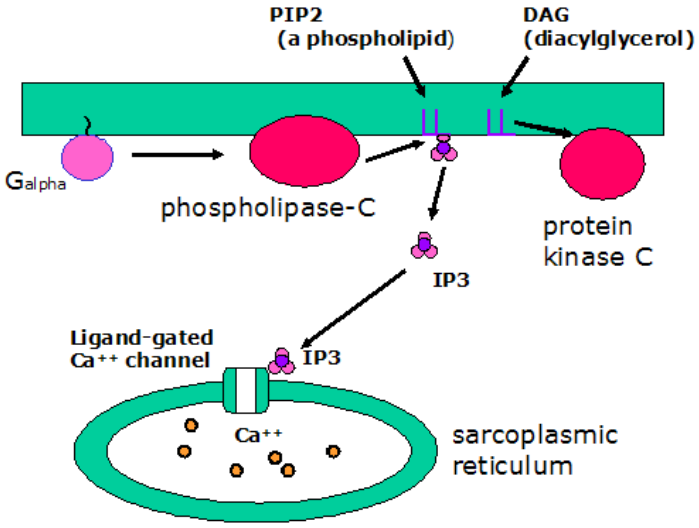
- A common way to translate a signal to a biologic effect inside cells is by way of nucleotide regulatory proteins (G –proteins) that bind **GTP**
    - GTP is the guanosine analogue of ATP
    - When the signal reaches a G protein, the **protein exchanges GDP for GTP**
    - The **GTP-protein complex brings about the effect**

- The **HETEROTRIMERIC G PROTEINS** couple cell surface receptors to catalytic units that catalyse the intracellular formation of second messengers or couple the receptors directly to ion channels
    - These are made up of three subunits, **alpha, beta and gamma**
    - **Alpha subunit is bound to GDP**
    - When a ligand binds to a G-protein coupled receptors, this **GDP is exchanged for GTP on the alpha subunit**
    - **Alpha subunit separates from beta and gamma**, both of which have many biologic effects
    - **Alpha subunit has intrinsic GTPase** then converts GTP to GDP and leads to reassociation



INOSITOL TRIPHOSPHATE AND DIACYLGLYCEROL AS SECOND MESSENGERS:

- IP3 is often the link between ligand binding and increased Ca2+ concentration in the cytoplasm
- Ligand binding activates PHOSPHOLIPASE C which catalyses the hydrolysis of PIP2 to IP3 and DAG
- **IP3 diffuses to the ER where it triggers the release of Ca2+**
- **DAG stays in the membrane, where it activates PROTEIN KINASE C**

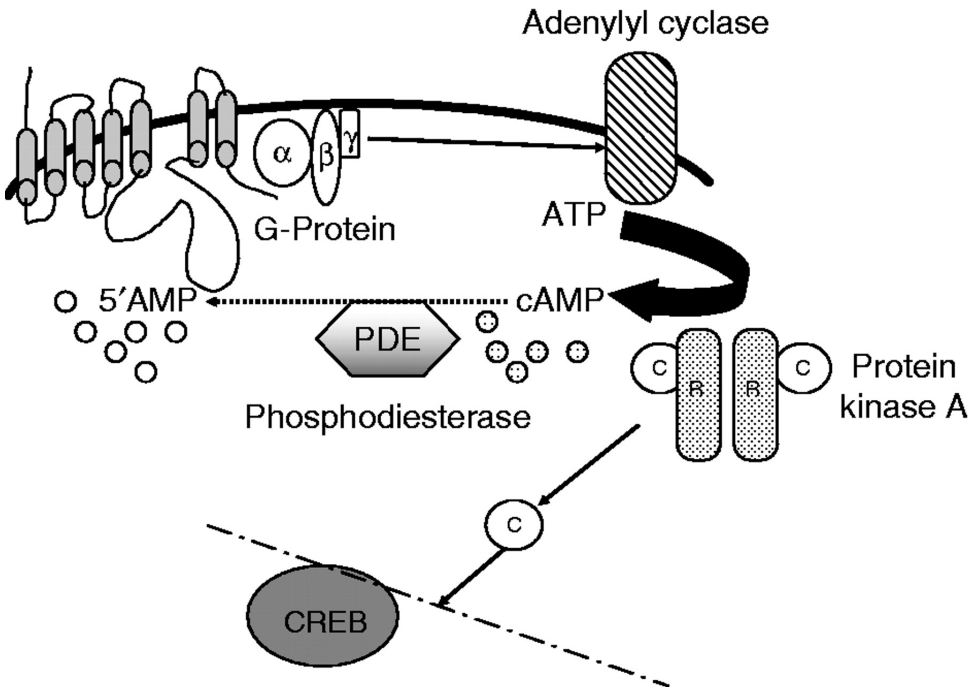


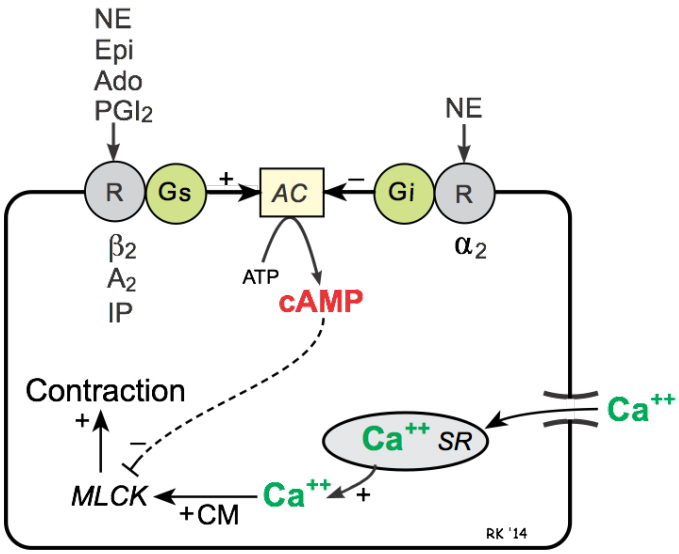
CYCLIC AMP:

- **Formed from ATP** by the action of the enzyme **ADENYLYL CYCLASE**
- **cAMP is inactivated** by the enzyme **PHOSPHODIESTERASE**
- **CAMP activates protein kinase A**, which catalyses the phosphorylation of proteins, changing heir conformation and altering their activity
    - The active catalytic subunit of PKA moves to the nucleus and **phosphorylates the CAMP-responsive element binding protein (CREB)**

- **Phosphodiesterase (which inactivates cAMP)** is **inhibited by methylxanthines** such as **caffeine and theophylline**, augment the hormonal and transmitter effects mediated via CAMP
- **Methylxanthines (caffeine / theophylline) → inhibition of phosphodiesterase → potentiation of effects of cAMP**

- Eight isoforms of adenylyl cyclase are known:
    - Combined with many different G proteins, this permits the CAMP pathway to be customised to specific tissue needs
    - When the appropriate ligand binds to a stimulatory receptor, a **Gs alpha subunits activates one of the adenylyl cyclases**
        - Conversely, when the appropriate ligand binds to the inhibitory receptor, a **Gi alpha subunit inhibitis adenylyl cyclase**





R, receptor; Gs, stimulatory G-protein; AC, adenylyl cyclase; CM, calmodulin; MLCK, myosin light chain kinase; SR, sarcoplasmic reticulum; NE, norepinephrine; Epi, epinephrine; Ado, adenosine; PGI , prostacyclin; β_2, beta-2-adrenoceptor; α_2, alpha-2-adrenoceptor; A_2, adenosine receptor; IP, prostacyclin receptor.

GUANYLYL CYCLASE:

- A family of enzymes that catalyse the formation of **cyclic GMP**
  - Important in **vision in both rods and cones**
  - There are cyclic GMP regulated ion channels
  - Cyclic GMP also activates a number of cyclic GMP dependent kinases, which produce a number of physiological effects

GROWTH FACTORS:

- Groups of polypeptides conveniently divided into THREE GROUPS:
  - Those that foster the multiplication or development of various types of cells:
    - E.g. nerve growth factor, insulin-like growth factor, epidermal growth factor, activins and inhibins
    - Have a single transmembrane domain and intracellular tyrosine kinase domain, which autophosphorylates on enzyme binding
  - Cytokines, involved in regulation of the immune system
  - Colony-stimulating factors, that regulate the proliferation and maturation of red and white blood cells
    - Cytokines and CSF activate the so-called Janus tyrosine kinases (JAKs) in the cytoplasm
    - These in turn phosphorylate signal transducer and activator of transcription (STAT) proteins, which form dimers and move to the nucleus, where hey act as transcription factors

- **Growth hormone activates the JAK-STAT pathway**