Ion channel modulators

By
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Introduction

- Ionic Channels are integral proteins permitting ions to pass through membrane.
- Ionic channels are either:

  1) Non-gating channel (Always open permit continuous movement of ions.
     A) Leak channels between extracellular and intracellular compartments.
        - Decrease outward conductance appears as inward current e.g. Potassium leak channel

Fig. 1) Membrane proteins

B) Gap junction channels:
    - Present between the adjacent cells (e.g. in the heart to enable it to work as a functional one unit).
    - Protein of the channels called connexin e.g. Cx26, Cx32, Cx37, Cx40, Cx43, Cx45,

Fig. 2) Gap junction and and CAMs
NB: Cell adhesion molecules (CAMs) are membrane molecules that mediate signal transduction between cells. They need direct surface contact.

2) Gating channels: (Has the ability to close and open to control ions passage).
   I) Voltage gating channels are responsible for propagating action potentials across the axonal membrane.
   II) Ligand gating channels has a binding site for a specific extracellular neurotransmitter.
   III) Signal gating channels which responds to intracellular signals induced by binding of neurotransmitter to a separate receptor protein.
   IV) Acid-sensing ion channel (ASIC)
   V) Mechanical gating;
      a) non-selective cation channel in hair cells of inner ear activated by sound vibration.
      b) mainly Calcium, involved in neurotransmitter release and blocked by gentamycin.
      - Most ion channels have a similar basic structure:

Fig.3) Channels gating

NB: • All voltage gated ion channels have a large pore forming subunit, which sits within the membrane.
   • The pore forming subunit (also called the a-subunit) contains a central aqueous pore through which the relevant ion passes in response to voltage change induced activation, also known as gating.
   * Some channels are dual gating: NMDA require glutamate and depolarization to open.
   * Only voltage-gated ion channel are highly ion selective. The majority of Ligand gated ion channels provide permeation paths wide enough for fully hydrated ion to pass according for their relatively poor selectivity.
NB; Hydrated ion radii: K < Na << Ca
Mechanism of ion selectivity;
1) Electric field repulsion (cation-selective channels have negatively charged residues lining the pores).
2) Sieving of hydrated ions (can not select for larger over smaller ions)
3) Specific, ion binding sites, can select for small differences in cation radii; dehydration by carboxyl groups (Na channels) or carbonyl group (K channels).


Strategies to modulate voltage-gated channel function (Phillip et al, 2004)
Several types of block might be seen:
• Voltage-dependent block – blocking of the channel occurs via a charged drug molecule binding to a site within the electrical field of the membrane. Because the drug must move through the electric field to get to the binding site, the rate constants for drug binding and unbinding are voltage-dependent and the $K_d$ of the drug changes according to the membrane potential of the cell.

![Schematic diagram illustrating the different ways in which modulation of voltage-gated ions channels can occur.](image)
• **State-dependent block** – blocking of the channel occurs when the channel is in a defined state, which can be either resting, activated or inactivated.
   I) Open-state block (also known as open channel block) – occurs when the affinity for the drug molecule is highest with the channel in the open conformation.
   II) Inactivated-state block – occurs when the drug preferentially binds to the channel in the inactivated conformation. Here channel open time is not altered, but the reduced probability of channel reopening reduces macroscopic current flow

• **Use-dependence** (also known as phasic block) – occurs if the drug has a higher affinity for the activated pre-open, the activated and the inactivated state. Here repetition of the pulse might result in an increase in the observed block.

• **State-independent block** (also known as tonic block) – that simply occlude the ion channel pore and prevent ions from moving through the channel. The drug binds irrespective of the state of the channel and the block remains constant with repetitive test pulses.

**I) Voltage gated channels and its modulators**

**A) Channel Activated by depolarization**

![Diagram of voltage gated channels](image)

**Sodium channel** consists of FOUR transmembrane domain, each has SIX transmembrane α helices, the forth helice is believed to be the voltage sensor.

**Potassium channel** has ONE molecule of only SIX transmembrane α helices

**Calcium channels:** similar to sodium channels

**Fig.4)** Sodium, potassium and calcium voltage gated channels

**1) Voltage gated Sodium channels:**
- Nav1.4, 1.5 found in muscle
- Nav1.7, 1.8, 1.9 found in peripheral nervous system
- Nav1.1, 1.2, 1.4, 1.6 found in central nervous system
- Nav1.2, 1.6 are in Nodes of Ranvier, axon initial segment

**Sodium Currents:** 1-Transient, \( I_{\text{NaF}} \). Responsible for Action Potential, Blocked by TTX

A Block of Na+ channel suppresses excitability, reduces conduction velocity and prolongs the refractory period by delaying the re-availability of Na+ channels. This is the mechanism of class I antiarrhythmic drugs, further classification details depend on additional properties of the Na+ channel blockers. Most clinically useful antiarrhythmics of this class are by no means selective for Na+ channels only but can impair additional ion channels thus altering action potential duration (APD) to a variable extent (quinidine, disopyramide, procainamide). The kinetics of Na+ channel blockade governs the extent of block. The physicochemical properties of a channel blocker will determine binding affinity or access to the channel in its closed, open or inactivated state. Since Na+ channel blocker often bind to the open or inactivated channel, binding and hence block depends on heart rate. Channel block is stronger at high rates because Na+ channels open more frequently, become inactivated for the duration of the systole and hence can bind a lot of drug resulting in strong channel block. Channels will remain blocked until the drug dissociates when the channels return to their resting state during diastole. The dissociation of blocker determines how many channels will be available for the following excitation. Drugs with rapid dissociation kinetics (e.g. lidocaine) will be effective against early extrasystoles. Conversely, drugs with slow dissociation kinetics (e.g. flecainide) will maintain considerable block at the end of diastole and this residual block is enhanced with increasing heart rates. (Hála et al, 2004)

Fig.5) Voltage gated Sodium channels. Positively charged portion of the channel is usually attracted to the negative interior of the cell. Depolarization weakens that attraction. The pore changes shape, and opens (Voltage-gated). the channel have 2 gates and 3 states.
In experimental cardiology compounds that promote the open state of Na\(^+\) channels have been known for a long time and delayed inactivation of Na\(^+\) channels has been observed as one common underlying mechanism. Pharmacologically modified Na\(^+\) channels allow Na\(^+\) to enter the cytosol even during the plateau phase leading to considerable prolongation in action potential duration. The extra Na\(^+\) entry may impose a considerable Na\(^+\) load leading to an increase in intracellular Na\(^+\) concentration accompanied by a positive inotropic effect. In fact, several Na\(^+\) channel modulators (DPI 201-107, BDF 9148, BDF 9196) have been synthesised as potential drugs for putative therapeutic use in chronic heart failure. However, in animal experiments these agents produced very long lasting action potentials with early after depolarisations and these effects were particularly pronounced at low stimulation frequencies. Ischemia or hypoxia induce a non-inactivating state of the Na\(^+\) channel, which will allow persisting Na\(^+\) influx, and induce excessive cellular Na\(^+\) load. The benzothiazolamine derivative R56865 preferentially blocks this channel state, but leaves the physiological state of Na\(^+\) channels unaffected. (Hála et al, 2004)

Fig.6) Procaine Blocks Na\(^+\) Channels from inside the cell

Fig.7) Voltage gated sodium channel and toxin Binding Sites
2) Voltage gated Calcium channels:

The majority of channel subtypes present in the sinus and atrioventricular (AV) nodes and in cardiomyocytes are L- and T-type Ca\(^{2+}\) channels. L-type Ca\(^{2+}\)-channels are blocked by phenylalkylamine (e.g. verapamil), benzothiazepine (e.g. diltiazem) and dihydropyridine derivatives (e.g. nifedipine). Verapamil and diltiazem have antiarrhythmic properties (class IV of the Vaughan Williams classification, because they prolong the refractory period of the Ca\(^{2+}\)-dependent action potentials in the sinus node and the atrio-ventricular node. Like Na\(^+\) channel blockers, Ca\(^{2+}\) channel blockers also bind preferentially to open and inactivated channels. Recovery from block is slow due to slow drug dissociation during diastole, and accumulation of block occurs at high frequencies. Verapamil and diltiazem delay AV-nodal conduction. Dihydropyridine derivatives lack this effect because dissociation from the channels is too rapid for development of sufficiently large block. (Ravens et al, 2004)

The selective T-type Ca\(^{2+}\) channel blocker mibefradil has been introduced as an antihypertensive and antianginal drug. Interestingly, this compound lowers the beating frequency. Mibefradil was withdrawn from the market because in vitro selectivity for T-type Ca\(^{2+}\) channels could not be exploited for a clinically differentiated therapy. In clinically relevant concentrations, the compound blocks almost all voltage-dependent Ca\(^{2+}\) channel subtypes. In addition, mibefradil is toxic to the liver. Other experimental T-type Ca\(^{2+}\) channel blockers prolong action potential duration because they also block K\(^+\) channels. (Ravens et al, 2004)

T-type Ca\(^{2+}\) channels are distributed throughout smooth muscles, although not as ubiquitous as their L-type counterpart. In general they co-exist with L-type channels and form the smaller component of net inward Ca\(^{2+}\) current. However, their voltage-range of activation and inactivation, and the presence of a significant window current near to the resting membrane potential of most smooth muscle cells, mean that they could play a role in generating a significant Ca\(^{2+}\) influx and, if coupled to other current components, could underlie spontaneous and rhythmic activity. The ability to manipulate T-channel activity in different tissues means that they could be the targets for agents designed to modulate tissue function under normal and pathological conditions. Perhaps an outstanding issue is to characterise the channels subtypes and present in various tissues, to enable better tissue specificity to be generated when developing targeted channel modulators (Fry et al, 2006).

L-type Ca\(^{2+}\) channels are modulated by physiological neurotransmitters. The catecholamine norepinephrine and epinephrine stimulate β-adrenoceptors to activate cAMP-dependent protein kinase A which phosphorylates L-type Ca\(^{2+}\) channels and thus enhances their open probability. This enhances Ca\(^{2+}\) entry and causes a positive inotropic effect. Concomitant shortening in action potential duration is explained by enhanced inactivation of the depolarising Ca\(^{2+}\) current due to increased intracellular Ca\(^{2+}\) concentration. Several animal toxins like maitotoxin and special dihydropyridine derivatives (Bay K 8644) enhance activation of Ca\(^{2+}\) channels. These drugs prolong action
potential duration and produce a positive inotropic effect. At high concentrations, Ca$^{2+}$-channel openers may trigger arrhythmias due to Ca$^{2+}$ overload of myocardial cells. (Ravens et al., 2004)

Fig 8) Types of Calcium channels
3) Voltage gating Potassium channels:

• \( K^+ \) channels open more slowly than \( Na^+ \) channels
• Voltage-gated \( K^+ \) channels are closed at rest and during rising phase
• The n-gate opens during falling phase (1 msec after depolarization)
  e.g. \( K_A, K_V (1-5), K_V (r), K_V (s), \)
  \( K_{SR}, B K_Ca, I K_Ca, S K_Ca, K_M, \)

Potassium channels constitute a family of proteins with diversified functions. Depending on the specific type of channel and the electrical environment in which it resides, potassium channels can repolarize cells after a depolarization event, modulate the shape of action potentials, determine the frequency of cell firing, and participate in potassium transport in epithelia. Consequently, the activity of potassium channels is associated with control of neuronal excitability and neurotransmitter release, heart rate, cardiac and smooth muscle contraction, endocrine secretion, epithelial electrolyte transport, T cell proliferation, apoptosis, and tumor progression. In electrically excitable cells, opening of potassium channels will decrease cell excitability and agents that increase the activity of these proteins should have utility in the treatment of disorders such as epilepsy, pain, hypertension, angina, and stroke. In contrast, inhibitors of potassium channels would promote cell excitability; the consequent increase in neurotransmitter or hormone release could be exploited in the treatment of Alzheimer’s disease or type 2 diabetes. *(Garcia and Kaczorowski, 2005)*

\( K^+ \) channels are classified by the gene that encodes for the \( \alpha \)-subunit or according to characteristic properties of the currents they give rise to. The four \( \alpha \)-subunits of \( K^+ \) channels may be homomers or heteromers, i.e. encoded by different genes. Since this causes some difficulties in nomenclature, the names for the characteristic currents are often used synonymously for the channels. *(Nerbonne, 2000)*

Because of the abundance of cardiac \( K^+ \) channel subtypes, the number of drugs that block \( K^+ \) channels is especially large. \( K^+ \) channel blockers possess antiarrhythmic properties since they prolong action potential duration and refractory period (class III, ). However, block of \( K^+ \) channels may also produce proarrhythmic effects. Prolonged refractory period will indeed suppress early extrasystoles, nevertheless, excessive prolongation of the action potential destabilises the plateau phase (early afterdepolarisation) and increases the risk of Tdp arrhythmias. Several factors predispose for drug-induced Tdp, these include female gender, bradycardia, low \( K^+ \) (and \( Mg^{2+} \)) plasma levels and old age. Patients with congenital or drug-induced acquired long QT interval appear to be at particularly high risk. *(Ravens et al 2004).*
1. $I_{to}$

The transient outward current $I_{to}$ passes through channels that in the human heart are encoded mainly by Kv4.3 and Kv1.4. The additional $\beta$-subunit KChiP (“K Channel interacting Protein”) is necessary to produce the typical time course. Prolonged action potential duration as observed in chronic congestive heart failure is associated with low expression of Kv4.3 and KChiP, as well as with reduced amplitude of $I_{to}$. It is not clear whether there is a direct relationship between reduced $I_{to}$ amplitude and prolonged action potential duration, because $I_{to}$ should be already inactivated during the late repolarisation. Experimental results with $I_{to}$ blockers such as tedisamil are not conclusive because these drugs lack sufficient selectivity for $I_{to}$. Nevertheless in the failing dog heart with low amplitude $I_{to}$ the long action potential duration could be normalised by adenoviral overexpression of $I_{to}$ channels (Barth, 2003).

2. $I_{Kur}$

Selective blockers of the rapidly activating outwardly rectifying $K^+$ current $I_{Kur}$ (Kv1.5 channel) are used against supraventricular tachyarrhythmias because this current is present only in the atria and is not detectable in the ventricles. Blockers of $I_{Kur}$ can suppress atrial arrhythmias without impairing the electrophysiology of the ventricles. Low concentrations
4-aminopyridine will selectively block $I_{Kur}$ in human atrial myocytes. In human atrial trabeculae block of $I_{Kur}$ elevates the plateau phase of the action potential. Action potential duration at 90% of repolarisation is shortened in trabeculae from patients in sinus rhythm but prolonged in atrial fibrillation (Hála et al., 2003). Many drug companies currently develop compounds with preferential block of $I_{Kur}$, and preliminary reports about successful cardioversion of atrial fibrillation in animal experiments have been published (Blaauw et al., 2002 and Gögelein et al., 2003)

3. $I_{Kr}$

HERG channels (“human ether-a-go-go related gene”) conduct the rapidly activating component of the outwardly rectifying current $I_{Kr}$ that was first identified as an individual current by its sensitivity against the selective blocker E-4031. Further selective blockers of HERG channels are the methanesulfonanilide derivatives dofetilide and ibutilide which all prolong action potential duration and have been developed as class III antiarrhythmic drugs. The antiarrhythmic efficacy of $I_{Kr}$ blockers is however limited due to their characteristic reverse frequency dependence. The APD-prolonging effect of $I_{Kr}$ blockers is lost at high beating rates, whereas it is very strong at low frequencies. Reverse frequency dependence may serve as an explanation for the lack of efficacy of this class of drugs against malignant ventricular arrhythmias. D-Sotalol for instance increased mortality in patients with myocardial infarction and left ventricular dysfunction and dofetilide was not able to reduce mortality (Ravens et al., 2004).

HERG channels are sensitive to block by a surprisingly large variety of agents with very different chemical structure. Non-cardiac drugs that block HERG channels include antibiotics (macrolides: erythromycin; gyrase inhibitors: sparfloxacin; cotrimoxazol; antimalarial drugs: halofantrine, chloroquine); antiemetic and prokinetic drugs (cisapride, domperidone); antihistaminics (astemizole, terfenadine); neuroleptics (haloperidol, chlorpromazine, phenothiazine, risperidone); antidepressive drugs (desipramine, amitriptyline, imipramine, fluoxetine) (Clancy et al., 2003).

4. $I_{Ks}$

$KvLQT1$ and minK protein form the channel complex for the slowly activating component of the delayed outward rectifier $I_{Ks}$. This current is blocked selectively by the chromanol derivatives chromanol 293B and HMR 1556 and by the benzodiazepine derivatives L-735,821 and L-768,673], whereas it is activated by L-364,373. Catecholamines also enhance the amplitude of $I_{Ks}$. Prolongation of action potential duration by block of $I_{Ks}$ is independent of heart rate. On the other hand, $I_{Ks}$ blockers increase the risk of delayed afterdepolarisations when activity of the sympathetic nervous system is high (Gögelein et al., 2000 and Várro et al., 2000).
Table 1) Factors that relate to K channels being therapeutic targets

<table>
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<th>Properties</th>
<th>Comments</th>
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<td>K⁺ channels</td>
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<tr>
<td>Function</td>
<td>Suppress or prevent cell excitability</td>
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<td>Diversity</td>
<td>At least 80 subtypes due to heterogeneous assembly of pore forming proteins and accessory proteins</td>
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<td>Location</td>
<td>Heterogeneous distribution suggests selective tissue management, especially where restricted expression is observed</td>
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<td>Channelopathies</td>
<td>Dysfunctional channels due to mutations in genes encoding channel proteins or regulated channels</td>
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<td>Cloning and expression</td>
<td>Avoids complexity within the data as found when obtained from native cells, but expression systems may not replicate native cells/tissues</td>
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<th>Modulators</th>
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<td>K⁺ channel family selectivity</td>
<td>Common identity between subtypes within a family due to heterogeneous assembly limits single subtype selectivity</td>
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<tr>
<td>K⁺ channel subtype selectivity</td>
<td>Enables subtle modulation of cellular homeostasis to influence physiology</td>
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<tr>
<td>Single versus multiple channel subtype modulation</td>
<td>Benefits dependent on tissue expression and potential compensation mechanisms especially following single subtype modulation</td>
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Table 2) K channel modulators (Lawson and McKay, 2006)

<table>
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<tr>
<th>Channel</th>
<th>Location</th>
<th>Target conditions</th>
<th>Channelopathies</th>
<th>Modulators</th>
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<td>Endothelial cells</td>
<td>Proliferative disorders (cancer, restenosis)</td>
<td>Chlorzoxazone</td>
<td>ICA-17043</td>
</tr>
<tr>
<td></td>
<td>Epithelial cells</td>
<td>Hypertension</td>
<td>Riluzole</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Secretory diarrhea</td>
<td>NS-309</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Cystic fibrosis</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Peripheral vascular disease</td>
<td></td>
<td></td>
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<tr>
<td></td>
<td></td>
<td>Urinary incontinence</td>
<td></td>
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<tr>
<td>SKCa</td>
<td>Neuronal</td>
<td>Myotonic muscular dystrophy</td>
<td>Schizophrenia</td>
<td>UCL-1684</td>
</tr>
<tr>
<td></td>
<td>Smooth muscle</td>
<td>Gastrointestinal disorders</td>
<td>Ataxia</td>
<td>UCL-1530</td>
</tr>
<tr>
<td></td>
<td>Skeletal muscle</td>
<td>Memory disorders</td>
<td>Anorexia nervosa</td>
<td>Dequalinium</td>
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<tr>
<td></td>
<td></td>
<td>Epilepsy</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Narcolepsy</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td>Schizophrenia</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Urinary incontinence</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Kv1.3</td>
<td>Lymphocytes</td>
<td>Immunosuppression</td>
<td>NS-309</td>
<td>Correolide</td>
</tr>
<tr>
<td></td>
<td>Neurons</td>
<td>Multiple sclerosis</td>
<td>Riluzole</td>
<td>H-37</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Diabetes</td>
<td>1-EBIO</td>
<td>WIN-17317-3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Obesity</td>
<td></td>
<td>CP-339618</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>UK-78282</td>
</tr>
</tbody>
</table>

Table: K channel modulators, continues, Lawsan and McKay, 2006.
4) **Voltage gated Chloride channels: ClC-0 - ClC-8**

Chloride channels are widely found anion pores that are regulated by a variety of signal and that play various roles as sown in table.

<table>
<thead>
<tr>
<th>Ligand-gated Channel Family</th>
<th>Group</th>
<th>Tissue</th>
<th>Proposed Function</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetylcholine</td>
<td>M</td>
<td>Nerve/muscle</td>
<td>Neuronal inhibition/muscle tension</td>
</tr>
<tr>
<td>GABA</td>
<td>I,M,N,V</td>
<td>Nerve (all)/muscle (LN)</td>
<td>Neuronal inhibition/muscle tension</td>
</tr>
<tr>
<td>Glutamate</td>
<td>I,N</td>
<td>Nerve/muscle</td>
<td>Regulate glutamate excitability</td>
</tr>
<tr>
<td>Glycine</td>
<td>V</td>
<td>Nerve</td>
<td>Neuronal inhibition</td>
</tr>
<tr>
<td>Histamine</td>
<td>I</td>
<td>Nerve</td>
<td>Visual system neurotransmission</td>
</tr>
<tr>
<td>Serotonin</td>
<td>N</td>
<td>Nerve</td>
<td>Neuronal inhibition</td>
</tr>
<tr>
<td>Voltage-gated ClC&lt;sup&gt;2&lt;/sup&gt;</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ClC-0</td>
<td>V</td>
<td>Fish electric organ</td>
<td>Stabilize membrane potential</td>
</tr>
<tr>
<td>ClC-1</td>
<td>V,N</td>
<td>Muscle</td>
<td>Stabilize membrane potential</td>
</tr>
<tr>
<td>ClC-2</td>
<td>V,N</td>
<td>Ubiquitous</td>
<td>Chloride secretion/regulation</td>
</tr>
<tr>
<td>ClC-3</td>
<td>V,N</td>
<td>Ubiquitous</td>
<td>Cell volume regulation</td>
</tr>
<tr>
<td>ClC-4</td>
<td>V,N</td>
<td>Nerve/muscle</td>
<td>Acidification of endocytotic vesicles</td>
</tr>
<tr>
<td>ClC-5</td>
<td>V,N</td>
<td>Kidney</td>
<td>Acidification of endocytotic vesicles</td>
</tr>
<tr>
<td>ClC-K</td>
<td>V</td>
<td>Kidney</td>
<td>Chloride Reabsorption</td>
</tr>
</tbody>
</table>

<sup>2</sup>Group refers to taxonomic group where I = insect, M = mollusc, N = nematode, and V = vertebrate.

<sup>2</sup>Although a similar numbering system is proposed for vertebrate (Fahike. 2001) and nematode (*C. elegans*) ClC-tvve channels, they are not strict homologs of one another (Schriever et al., 1999)

Quoted from **Bloomquist (2003)**

**B) Voltage gated Activated by Hyperpolarization (HCN)**

Fig.11) e.g Cardiac "Funny" Channel (IF)
- Inward IF current causes slow depolarisation (Pacemaker)
- Rate of IF activation influences rate of depolarization
  - Faster activation = faster depolarisation = more frequent action potentials
  - Rate of activation modulated by:
    - Norepinephrine (increases rate)
    - Acetylcholine (decreases rate)
    - Depolarisation → Action potential in cardiac → deactivates IF
    - Repolarisation re-activates IF current and the Cycle begins again.
- Blocked by ivabradine which is used to slow the heart rate. (*Savelieva and Camm, 2006*)

II) Ligand gating channels

- Has a binding site for a specific extracellular neurotransmitter. It is subdivided into:
  
  A - G protein linked receptor coupled to ion channel (metabotropic): -
  
  • Acetylcholine (muscarinic)
  • Adrenaline & noradrenaline
  • Glutamate (quisqualate)
  • Cholecystokinin & gastrin
  • Dopamine
  • GABA (GABAB)
  • 5-Hydroxytryptamine (1,2)
  • Neurotensin
  
  • Adenosine & adenine nucleotides
  • Angiotensin
  • Bradykinin
  • Cannabinoid
  • Endothelin
  • Histamine
  • Leukotriene
  • Opioid peptides
  
  • Bombesin
  • Calcitonin
  • Galin
  • Chemokine
  • Melatonin
  • VIP
  • Neuropeptide Y
  • Prostanoid

![Diagram of ligand gating channels]

**Fig 12** Direct (ionotropic) and indirect (metabotropic) ligand gating channel.
e.g **1-Muscarinic (m) receptor gated K.chs**: which mediate outward (hyperpolarizing) K⁺ currents. They are classified into:

<table>
<thead>
<tr>
<th>M1 gated K channel (Kir 3-1)</th>
<th>M2 gated K channel (Kir 3-4)</th>
</tr>
</thead>
<tbody>
<tr>
<td>- Defective in epilepsy</td>
<td>- It is defective in parkinsonism, Alzheimer, long Q-T syndrome.</td>
</tr>
<tr>
<td>- Closing by m1 agonist oxytemorin, pilocarpine) causing depolarization of neuron (may cause seizures)</td>
<td>- blocked by galamine</td>
</tr>
<tr>
<td>- Opened by retigabine which is also GABAA agonist (used in epilepsy)</td>
<td>- Opened by m2, Adenosine (A₂) and GABA_B agonists causing hyperpolarization of neurons and cardiac cells(IKACh).</td>
</tr>
</tbody>
</table>

![m2 channel](image)

Fig.13) Acetylcholine-activated K current

2) **SUIPONYLUREA receptor (SUR) gated K. ch (KArP):**

- Mediate outward (hyperpolarizing) K current
- Blocked by ATP, tolbutamide, glyburide - - etc (oral hypoglycemic)
- They are Classified into:
  a- **SUR-1 gated K.ch.** (in Pancreas, neurons and Smooth muscle)
    - Selectively opened by diazoxide.
    - Defective in hyperinsulinemic hypoglycemia of infancy.
  b- **SUR-2 gated K.ch:**
    - SUR-2A in heart and skeletal muscle; (HMR 1883 & 1098 are blockers in research to treat heart failure, ventricular arrhythmia and cardiac arrest.
    - SUR-28 present in:

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Opener</th>
<th>Opener indication</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood vessels</td>
<td>Nicorandil</td>
<td>Hypertension</td>
</tr>
<tr>
<td>Bladder</td>
<td>WaY-1333537</td>
<td>Bladder overactivity</td>
</tr>
<tr>
<td>Bronchi</td>
<td>Aperkalium</td>
<td>Bronchial asthma</td>
</tr>
<tr>
<td>Hair</td>
<td>Minoxadil</td>
<td>Baldness</td>
</tr>
</tbody>
</table>

c- **Mitochondrial KATP ch:** Opened by nicorandil
B) Ionotropic (receptor directly linked to the channel):

1) ATP (P2X) modulate excitatory non-selective cation channel
2) Epithelial Na channel,

3) Proton sensing channel (ASIC)
4) Nicotinoid receptor: a) Cationic (nAChR, 5HT3)
   b) Anionic (GABA$_A$, GABA$_C$, glycine)

A-Axis blockade
B-competitive blockers (compete with Ach).
C-Depolarizing blockers (excessive stimulation)

Fig 15) Nicotinic acetyl choline receptor linked channel and its modulators
Table: Serotonin receptors subtypes and major signaling pathway

<table>
<thead>
<tr>
<th>Subtypes</th>
<th>5-HT&lt;sub&gt;1&lt;/sub&gt;</th>
<th>5-HT&lt;sub&gt;2&lt;/sub&gt;</th>
<th>5-HT&lt;sub&gt;3&lt;/sub&gt;</th>
<th>5-HT&lt;sub&gt;4&lt;/sub&gt;</th>
<th>5-HT&lt;sub&gt;5&lt;/sub&gt;</th>
<th>5-HT&lt;sub&gt;6&lt;/sub&gt;</th>
<th>5-HT&lt;sub&gt;7&lt;/sub&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>5-HT&lt;sub&gt;1A&lt;/sub&gt;, 5-HT&lt;sub&gt;1B&lt;/sub&gt;, 5-HT&lt;sub&gt;1D&lt;/sub&gt;, 5-HT&lt;sub&gt;1E&lt;/sub&gt;, 5-HT&lt;sub&gt;1F&lt;/sub&gt;</td>
<td></td>
<td>5-HT&lt;sub&gt;2A&lt;/sub&gt;, 5-HT&lt;sub&gt;2B&lt;/sub&gt;, 5-HT&lt;sub&gt;2C&lt;/sub&gt;</td>
<td>5-HT&lt;sub&gt;3A&lt;/sub&gt;, 5-HT&lt;sub&gt;3B&lt;/sub&gt;</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Major signalling pathway</td>
<td>cAMP↓</td>
<td>1P&lt;sub&gt;3&lt;/sub&gt;↑</td>
<td>ion channel</td>
<td>cAMP↑</td>
<td>cAMP?</td>
<td>cAMP↑</td>
<td>cAMP↑</td>
</tr>
</tbody>
</table>

**5HT3 antagonist**

- Ondansetron, Dolasetron, granisetron & tropisetron (Used in chemotherapy induced emesis)
- Alosetrone, Clansetron, mosapride, rinzapride (used in IBS with diarrhea)

Fig.14) GABA is - Widely distributed throughout the CNS (brain, retina). It is implicated in numerous disorders: e.g. Huntington’s chorea, Parkinson’s disease, Epilepsy, Schizophrenia, Tardive Dyskinesia, Dementia, Sleep disorders, Affective disorders, anxiety.

GABAergic Receptors (A and C are Ionotropic and B is Metabotropic)

b- Modulation of GABA<sub>A</sub> function:
- Increase- by Benzodiazepine’s, barbiturates, anesthetics, alcohol, neurosteroids
- Decrease by - various alkaloids (picrotoxin, bicuculline, convulsants (flurothyld)

**Glycine antagonist** : (licostinol)
5) Ionotropic glutamate receptor (AMPA, Kainate, NMDA)

Fig. 15) Two types of glutamate receptors in the brain: one that opens when it binds glutamate (AMPA/kainate) which is blocked by YM872 and one that opens only when it binds glutamate and the cell is depolarized (the NMDA receptor) which is modulated by many agents (shown in the figure).

**Glutamate antagonists**: (potential use is neuroprotection) Soskic and Schrattenholz (2006)

- AMPA receptor antagonist (YM872).
- NMDA receptor antagonist
  * Competitive (Selfotel, Aptiganel)
  * Channel blocker CP-101, 606 and dextorphan – remacemide and Magnesium (Block voltage gated calcium channels and NMDA receptors)

6) Acid-sensing ion channel (ASIC)

Acid-sensing channel and demonstrated that it is a member of a large family of channels that have been extensively studied. The family is commonly called the degenerins/epithelial sodium channels (ENaC) (18) and is characterized by amiloride sensitivity and sodium selectivity. These channels have no homology to voltage-gated sodium channels but, instead, have about 500 amino acids, two putative transmembrane domains, intracellular N and C terminals, and a substantial extracellular domain. At least three subunits must bind together
to form a functional channel. The most studied members of this family are the ENaCs. ENaCs are constitutively active, sodium selective channels that are expressed on epithelia and support selective sodium transport across them. Degenerin channels are expressed in \textit{C. elegans} worms. A mutation that causes them to be overactive causes degeneration of mechanosensing neurons, this being one reason why they are considered to be mechanosensing channels in worms.

![Fig.16) Schematic diagram demonstrating the role of ASIC1a channels in ischemic neuronal injury. Lower area (green) represents neurons in non-ischemic conditions where the concentration of the extracellular protons is low and the ASIC1a channels remain closed. Upper area (red) represents neurons in ischemic conditions where the concentration of the extracellular proton increases, resulting in activation of ASIC1a channels and the flux of large amounts of Ca$^{2+}$ into neurons, which leads to neuronal injury. PeTX1, a specific ASIC1a blocker isolated from venom of tarantula \textit{Psalmodus cambridgei}, blocks the ASIC1a channels, resulting in neuroprotection. The membrane lipid bilayer is represented by E. B. White's well-known poem.](image)

Extensive cloning work has found four distinct genes with appropriate homology to be considered in the ASIC family. There are also two known splice variants of ASIC1 and two of ASIC2. ASIC4 forms no functional channel and it takes fairly extreme acid (pH 5 and below) to open ASIC2. But ASIC1 and ASIC3 are opened by pH changes that are clearly relevant physiologically. ASIC3 is expressed predominately in the sensory peripheral nervous system and is not seen in the central nervous system. ASIC1 is the predominant ASIC in the central nervous system and is also present in sensory neurons. ASIC2 is present both in the central and peripheral nervous systems and forms heteromultimers with ASIC1 and ASIC3, causing detectable changes in either kinetics or pharmacology (\textit{Naves and McCleskey}, 2005).
Table) ASIC subtypes

<table>
<thead>
<tr>
<th></th>
<th>ASIC1</th>
<th>ASIC2</th>
<th>ASIC3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Character</td>
<td>Rapid activation / inactivation</td>
<td>Rapid activation / moderate inactivation</td>
<td>Biphasic response rapidly inactivating</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>transient and sustained components</td>
</tr>
<tr>
<td>Activators</td>
<td>Extracellular ( H^+ )</td>
<td>Extracellular ( H^+ )</td>
<td>Extracellular ( H^+ )</td>
</tr>
<tr>
<td>Blockers</td>
<td>Psalmotoxin, amiloride, benzamil, ibuprofen</td>
<td>amiloride</td>
<td>Amiloride (transient component only)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>diclofenac &amp; aspirin (sustained components only)</td>
</tr>
</tbody>
</table>

**Transient receptor potential (TRP) channel**

Transient receptor potential) family consists of six main subfamilies termed the TRPC (canonical), TRPV (vanilloid), TRPM (melastatin), TRPP (polycystin), TRPML (mucolipin), and TRPA (ankyrin) groups. These subfamilies encompass 28 ion channels that function as diverse cellular sensors. All of the channels are permeable to monovalent cations, and most are also permeable to \( Ca^{2+} \) (Nilius et al., 2005). *Transient receptor potential (TRP) channels are involved in a wide range of processes ranging from osmoregulation, thermal, chemical and sensory signalling, and potentially in the pathophysiology associated with several diseases* (Hicks, 2006)

There are strong indications that TRP channels are involved in many diseases. At this point, four channelopathies have been identified in which a defect in a TRP channel–encoding gene is the direct cause of disease. TRPs are also involved in some systemic diseases because of their role as receptors for irritants, inflammation products, and xenobiotic toxins. Other indications of the involvement of TRPs in several diseases come from correlations between the levels of channel expression and disease symptoms or from the mapping of TRP-encoding genes to susceptible chromosome regions. Finally, the phenotypes of TRP knockout mice and other transgenic models allow a degree of extrapolation to human diseases (Nilius et al., 2005)

Patents for TRPV1 antagonists alone span diseases ranging across chronic pain, neuropathies, headache, bladder disorders, irritable bowel syndrome (IBS), gastro-oesophageal reflux disease (GORD), and cough amongst others. Most research is currently focused around those TRP channels involved in sensory processes, with the neurogastroenterology and motility field playing a major role, for example, through recent discoveries of differential roles for TRPV receptor subtypes in chemosensitivity and mechanosensitivity of visceral afferents (Hicks, 2006)

Now there are at least 28 known TRP channels, with a variety of modalities for activation allowing them to function as cellular sensors of heat, cold, protons, mechanical force,
tonicity, inflammation and all transduce these signals via conducting currents carried by monovalent cations, and in some cases calcium. Some show selectivity for cold stimuli (TRPM8 and TRPA1), and these are activated by menthol and peppermint oil, leading to speculation around these channels as the target for the relief of some gastrointestinal (GI) symptoms provided by these substances. Patents for TRPV1 antagonists alone span diseases ranging across chronic pain, neuropathies, headache, bladder disorders, irritable bowel syndrome (IBS), gastro-oesophageal reflux disease (GORD), hypersensitivity and cough amongst others. (HICKS, 2006)

TRP channels have a wide expression pattern throughout the body. Whenever this is the case, the risk for adverse events increases. Even in the case of TRPV1, expression has been reported in several cells besides visceral afferents and so the potential for side effects in the thermoregulatory, vascular, cognitive and motor function, and somatic sensory systems, must all be considered. (HICKS, 2006)

Gastrointestinal motility and sensory disorders, such as IBS, dyspepsia, GORD, chronic idiopathic constipation, are known to involve complex aetiologies, and to comprise symptom complexes that overlap to some degree. Unlike the treatment of chronic pain disorders, the reduction of sensory symptoms, albeit highly desirable, may not be sufficient for significant overall symptom relief. The IBS area is good example of the difficulty of a single target approaches to therapy for complex disorders, and TRP channel proponents are well aware of the need to consider whether modulators of individual targets are going to be effective for overall symptom relief. For example, the serotonin system was targeted for the treatment of IBS based upon the known critical role of serotonin signalling in the three major functions of motility, secretion and sensory signalling between 'big brain' and the 'little brain'. Similarly, corticotrophin releasing factor (CRF), and tachykinins, have been ascribed multiple roles in the gut and the brain–gut axis, which have made these systems attractive targets for research. We do not know enough yet about the role of the TRP channel family in these systems to be confident that they have the potential to yield sufficiently broad therapeutic efficacy. (HICKS, 2006)
Table 2. Overview of the involvement of TRP channels in disease. (Nilius et al, 2005)

<table>
<thead>
<tr>
<th>Possible association with disease</th>
<th>TRP channel</th>
<th>Disease</th>
</tr>
</thead>
<tbody>
<tr>
<td>Channelopathy (mutations, known gene defects)</td>
<td>TRPC6</td>
<td>Focal segmental glomerulosclerosis, gain-of-function mutations, proteinuria with progressive kidney failure</td>
</tr>
<tr>
<td></td>
<td>TRPM6</td>
<td>Defective Mg$^{2+}$ reabsorption, HSH (autosomal-recessive, hypomagnesemia with secondary hypocalcemia)</td>
</tr>
<tr>
<td></td>
<td>TRPP2</td>
<td>ADPKD (autosomal dominant polycystic kidney disease), cardiac septal defects</td>
</tr>
<tr>
<td></td>
<td>TRPML1</td>
<td>Mucolipidosis IV</td>
</tr>
<tr>
<td>Candidate genes (based on chromosomal location)</td>
<td>TRPV4</td>
<td>ADNSHL (autosomal nonsyndromic hearing loss)</td>
</tr>
<tr>
<td></td>
<td>TRPM2</td>
<td>BP-I, II (bipolar disorder), nonsyndromic hereditary deafness, holoprosencephaly?, Knobloch syndrome</td>
</tr>
<tr>
<td></td>
<td>TRPM3</td>
<td>Amyotrophic lateral sclerosis with frontotemporal dementia, early-onset pulmonary cataract, HLH (hemophagocytic lymphohistiocytosis), nephronophthisis</td>
</tr>
<tr>
<td></td>
<td>TRPM5</td>
<td>BWS (Beckwith-Wiedemann syndrome)</td>
</tr>
<tr>
<td></td>
<td>TRPML2</td>
<td>Candidate gene for neurosensory disorders</td>
</tr>
<tr>
<td></td>
<td>TRPML3</td>
<td>Candidate gene for neurosensory disorders</td>
</tr>
<tr>
<td>Systemic diseases associated with changes in channel activity</td>
<td>TRPC1</td>
<td>Asthma, COPD, defective immune response involving both B cells and T cells</td>
</tr>
<tr>
<td></td>
<td>TRPC3</td>
<td>Asthma, COPD, defective immune response involving both B cells and T cells</td>
</tr>
<tr>
<td></td>
<td>TRPC5</td>
<td>Duchenne muscular dystrophy, idiopathic pulmonary arterial hypertension (IPAH), heart hypertrophy, essential hypertension</td>
</tr>
<tr>
<td></td>
<td>TRPV1</td>
<td>Thermal hyperalgesia, allodynia, functional bowel disease (FBD), Crohn’s disease, vulvodynia, osteoarthritis, pancreatitis, GERD (gastro-esophageal reflux disease), bladder disease, cystitis, asthma</td>
</tr>
<tr>
<td></td>
<td>TRPV4</td>
<td>Asthma, bronchial hyperresponsiveness</td>
</tr>
<tr>
<td></td>
<td>TRPM2</td>
<td>Target for excitotoxicity in brain</td>
</tr>
<tr>
<td></td>
<td>TRPM7</td>
<td>Neuronal cell death</td>
</tr>
<tr>
<td>Correlation of disease with abnormal channel expression</td>
<td>TRPV6</td>
<td>Increased in prostate cancer</td>
</tr>
<tr>
<td></td>
<td>TRPM1</td>
<td>Down-regulated in malignant melanomas</td>
</tr>
<tr>
<td></td>
<td>TRPM8</td>
<td>Up-regulated in tumors of prostate, breast, colon, lung, skin</td>
</tr>
<tr>
<td>Knockout mouse model</td>
<td>TRPC4</td>
<td>Impaired endothelium-dependent vasorelaxation and endothelial barrier function</td>
</tr>
<tr>
<td></td>
<td>TRPC6</td>
<td>Defective vasomotor control, sensitized myogenic response</td>
</tr>
<tr>
<td></td>
<td>TRPV1</td>
<td>Suppression of inflammatory thermal hyperalgesia, impaired bladder function</td>
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<tr>
<td></td>
<td>TRPV2</td>
<td>Cardiomyopathy, cardiac hypertrophy</td>
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<td>TRPV3</td>
<td>Defective thermosensation</td>
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<td></td>
<td>TRPV4</td>
<td>Impaired osmoregulation</td>
</tr>
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<td>TRPV5</td>
<td>Defective Ca$^{2+}$ reabsorption</td>
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<td></td>
<td>TRPM7</td>
<td>Defect in skeletogenesis</td>
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<td>TRPP3</td>
<td>krd mouse with kidney-retina defects</td>
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<td>TRPML3</td>
<td>Varintin-waddler mouse</td>
</tr>
<tr>
<td>Classes</td>
<td>Compounds</td>
<td>Natural origin</td>
</tr>
<tr>
<td>-------------------------------</td>
<td>--------------------</td>
<td>------------------------------</td>
</tr>
<tr>
<td>Capsaicinoids and related compounds</td>
<td>Capsaicin</td>
<td>Capsicum sp.</td>
</tr>
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<tr>
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<td>Drimianal and polygallic</td>
<td>Drimia venosa, Polygonum hydropiper</td>
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<td>Isevernella</td>
<td>Lactorus selloreus</td>
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III) Signal gating channels:

which responds to intracellular signals induced by binding of neurotransmitter to a separate receptor protein:

- They are subdivided according to the site of the channel into:

  A) Channel at cell membrane:

  • HCN Channel (Cyclic nucleotide activating channel (e.g. cAMP cation, cGMP cation, cAMP chloride)

  • Ca²⁺-gated K⁺:
    - Blocked by: Calcium buffers: BAPTA, EGTA, Calcium channel blockers: cadmium, Cobolt,
    - Barium flows through calcium channels, but can’t bind to KCa channels
    - Specific blockers: Apamin (SK), Iberiotoxin, charybdotoxin

  • leukotriene C₄-gated Ca²⁺

  • arachidonic acid-activated K⁺

  • Ca²⁺-gated Cl⁻

  B) Ionic channels inside the cell (Intracellular):

  • ryanodine receptor Ca²⁺

  • IP₃-gated Ca²⁺

Fig. 17) HCN Channel Structure

Fig. 18) Intracellular Ionic channels
• **Inward rectifiers: \( \text{I}_K^{1}, \text{I}_K^{\text{ACh}}, \text{I}_K^{\text{ATP}} \)**  
  Activation of inward rectifying channels stabilises cardiac rhythm by hyperpolarising the cell membrane and is proarrhythmic by shortening action potential duration. This is the underlying mechanism of atrial fibrillation triggered by vagal stimulation that activates the inward rectifier \( \text{I}_K^{\text{ACh}} \) by release of the neurotransmitter acetylcholine.

  Sarcolemmal ATP-sensitive \( \text{K}^+ \) channels (\( \text{K}_{\text{ATP}} \) channels) were first discovered in the myocardium. Their octameric structure consists of four pore-forming Kir6.2 subunits and four regulatory SUR2A subunits (sulfonyl urea receptors). Under physiological conditions these channels are closed. They open when the intracellular ATP concentration falls to very low values as for instance found during hypoxia. Sarcolemmal \( \text{K}_{\text{ATP}} \) channels are considered to protect the myocardium against ischaemic injury. When activated they drastically shorten the cardiac action potential, reduce contractility and hence lower myocardial energy demand. However, this mechanism need not necessarily be only protective but could also lead to life threatening arrhythmias. In addition, mitochondrial \( \text{K}_{\text{ATP}} \) channels may also contribute to protection against ischaemic myocardial damage (Light et al., 2001 and Kovoor et al., 2001).

  ATP-dependent \( \text{K}^+ \) channels regulate release of insulin from the \( \beta \)-cells of isles of Langerhans in the pancreas. Sulfonylurea derivatives block these channels and have a good clinical standing in therapeutic use to treat diabetes mellitus. Agents with higher affinity for cardiac over pancreatic \( \text{K}_{\text{ATP}} \) channels should be useful antiarrhythmic drugs that protect against ventricular fibrillation. Opening of ATP-dependent \( \text{K}^+ \) channels in smooth muscle reduces vascular tone. Several \( \text{K}^+ \) channel openers as diazoxide, cromakalim and pinacidil have been developed as drugs against coronary heart disease or high blood pressure. Present research is directed towards compounds with preferential cardioprotective over vasodilatory action (Sato et al, 2000).

**Zinc and copper ions**

Zinc and copper ions are of key physiological importance in mammalian tissue. Zinc is a vital nutrient and with the exception of iron, it is the most abundant trace element in the body (Takeda, 2001). Copper is also a vital trace element, the third most abundant in humans, and is present at low levels in a variety of cells and tissues with the highest concentrations in the liver (Gaetke & Chow, 2003). Both ions have a range of physiologically important roles in humans. They are a key structural component of many proteins and act as co-factors for the activity of many enzymes that are critical for brain function. These include enzymes involved in antioxidant defense (superoxide dismutase; SOD) cellular respiration (cytochrome c oxidase) and catecholamine synthesis (dopamine-\( \beta \)-hydroxylase) and a plethora of other enzymes involved in multiple biological processes required for growth, development, and maintenance of the nervous system (Gaetke & Chow., 2003, Barnes et al., 2005, Frederickson et al., 2005).
There are increasingly compelling arguments that both ions can also function as signaling molecules, with evidence of release from synaptic terminals and measured actions on a wide range of membrane proteins. As such, in the nervous system, both ions might be predicted to have modulatory roles in regulating neuronal excitability. In this review, we will consider the roles of zinc and copper ions in the nervous system, both in terms of their physiological actions and in terms of their potential use as pharmacological mediators of proteins that regulate neuronal excitability. (Frederickson et al., 2005).

**Fig. 19** Zinc release from zinc containing neuron terminals. (A) During normal stimulation, zinc (red circles) is released from presynaptic terminals. It can act on postsynaptic channel proteins such as GABA$_A$ receptors, NMDA receptors, or many other different ion channels to alter their activity. In addition, it may permeate through certain channel proteins (such as voltage-gated Ca$^{2+}$ channels, trp channels, or AMPA/kainate receptors as indicated in the schematic) to enter the postsynaptic cell. (B) Following excessive excitation, zinc is depleted from presynaptic vesicles so less is available for release; however, the intracellular concentration of zinc in the postsynaptic cell is increased, which may be protective or detrimental to the neuron depending on the particular neuron considered (Takeda, 2000).

## Zinc and copper in the brain

In the plasma, zinc and copper are present at concentrations of around 15 μM. For zinc, almost all of this is bound to proteins such as albumin, hence free, ionic, zinc is in the nanomolar range (Takeda, 2001). The brain has the highest zinc content compared to other organs (Mocchegiani et al., 2005) about 10-fold higher than that found in plasma (around 100–150 μM). This has been localized to several specific brain regions; however, the vast majority remains protein bound. The binding of zinc to L-histidine in the plasma and cerebrospinal fluid (CSF) promotes transport of zinc to target sites, from where its uptake across the blood–brain barrier is tightly regulated. Following uptake, passage of zinc through the CSF and brain extracellular fluid compartments is unrestricted. Entry routes into glia and neurons are still to be clarified, although several zinc transporters have
been identified (Mocchegiani et al., 2005). Even within the brain, 90% of total brain zinc is bound to zinc metalloproteins, with much of the remaining 10% found in presynaptic vesicles, either loosely bound or free (and therefore, histochemically reactive) (Takeda, 2001). Indeed, it is known that free ionic zinc is a potent killer of neurons and glia, with prolonged exposure to growth media containing in excess of 100 nM leading to cell death (Frederickson et al., 2005).

Like zinc, copper accumulates in the brain. The average copper concentration in the CSF has been estimated to be around 70 μM. While, like zinc, most of this is protein bound, loosely bound copper is estimated at around 0.1–0.8 μM, whereas the normal extracellular copper concentration in the brain is of the order of 0.2–1.7 μM (Stuerenberg, 2000, Schumann et al., 2002 and White et al., 2004). However, these values are frequently exceeded in the synaptic cleft and during neurodegenerative disease where concentrations of copper may reach 200 μM and 400 μM, respectively (White & Cappai, 2003).

Zinc is not uniformly distributed about the brain. Higher concentrations are present in the grey than white matter, while the highest concentrations are located in specific forebrain regions including the hippocampus, amygdala and neocortex (Takeda, 2000). Histochemically stainable zinc (free plus loosely bound zinc) is found in particularly high concentrations in certain synaptic vesicles. Neurons that contain such vesicles have been termed zinc-enriched neurons (ZEN). It is clear that these neurons are not associated with a single “primary” synaptic neurotransmitter. For example, GABAergic ZEN terminals have been found in the cerebellum (Wang et al., 2002), whereas in the cerebral cortex, amygdalar nuclei, olfactory bulb, and hippocampal formation, ZEN terminals are glutamatergic (Frederickson & Bush, 2001). This has given rise to the term “gluzinergic” neurons, used by some. The hippocampus is particularly rich in glutinergic neurons in regions such as the dentate gyrus, granule cell mossy fibers, and in CA3 and CA1 neurons. Zinc-containing fibers from hippocampal region innervate the cerebral cortex, amygdala, striatum, or limbic regions (Mocchegiani et al., 2005).

Again, just like zinc, copper is found to have a differential distribution in the central nervous system (CNS) with certain synaptic vesicles showing particularly high levels of copper. Copper is also primarily associated with glutaminergic or adrenergic neurons, particularly in regions such as the hippocampus, olfactory bulb, and locus coeruleus. For now, at least, we have been spared the term “glucupergic” neurons. Giant boutons of hippocampal mossy fibers contain ~300–350 μM of zinc (Takeda, 2001). Release of zinc occurs from presynaptic, small clear round vesicles within neurons (see Frederickson et al., 2000). Pools of zinc and copper can be released following membrane depolarization or neural activity in a calcium (Ca^{2+})-dependent manner (Horning & Trombley, 2001).

A recent review by Frederickson et al. (2005) has illustrated how 3 primary methods have been used to show synaptic release of zinc. These are imaging of zinc in boutons to show depletion following nerve stimulation; detection of zinc in the perfusate following
nerve stimulation; and direct imaging of released zinc using fluorescent probes. Of the 3 methods, it is clear that the third method is the most direct, the fastest, and the most informative. For adult preparations at least, fluorescent zinc probes reveal clear increases in synaptic zinc following nerve stimulation and estimates of concentration in the range 10–30 μM (Thompson et al., 2002 and Li et al., 2003, Kay, 2003).

Both zinc and copper are essential for correct development and functioning of the brain. Furthermore, both zinc and copper levels in the immature brain increase with age until adulthood when a constant concentration is maintained (Tarohda et al., 2004, Takeda, 2001).

When zinc concentrations rise inside either neurons or glial cells, the ions can be expelled from the cell or else concentrated in synaptic vesicles by ZnT transporters, taken up by mitochondria or incorporated into zinc binding proteins. A family of zinc transporters are involved in transporting zinc across cell membranes (Colvin et al., 2003). In terms of the nervous system, perhaps the most interesting of these are ZnT3 and ZnT4, which are required for the transport of zinc into synaptic vesicles. The distribution of ZnT3, in particular, has been widely studied and its presence is taken to be evidence in favor of synaptic release of zinc in such neurons (Harris, 2002).

While relatively little is currently known about copper transport in the CNS, copper-transporting ATPases (ATP7A and ATP7B) play a central role in distribution of copper (Puig & Thiele, 2002). Genetic mutations in ATP7A and 7B lead to severe neurodegenerative disorders, Menkes and Wilson diseases, respectively (Strausak et al., 2001). These 2 proteins are distributed in a cell-specific manner in accordance with their distinct functional properties. Furthermore, a recent study by Barnes et al. (2005) demonstrated that cerebellar expression of the 2 copper ATPases is regulated individually during development. ATP7B is constantly localized to Purkinje neurons, allowing steady transport of copper to ceruloplasmin. However, the expression profile of ATP7A is variable such that during early development expression occurs in Purkinje neurons, while during late development and into adult life expression switches to Bergmann glia. Current data indicate that ATP7A is responsible for the overall supply of copper to the brain due to its presence in the choroid plexus (Barnes et al., 2005). Intracellular copper homeostasis is mediated by copper-induced trafficking to the plasma membrane (ATP7A) or to vesicles (ATP7B) followed by expulsion of excess copper from cells (Llanos & Mercer, 2002).

3. Protein targets

How do zinc and copper alter the function of proteins?

Zinc binds directly to proteins to alter their function. It particularly targets histidine, cysteine, aspartate, and glutamic acid residues. Unlike the situation for zinc, there are
several ways in which copper can modify the activity of a protein. Copper is therefore potentially much more complicated than zinc and perhaps much more interesting, at least from a mechanistic perspective. Like zinc, it may bind directly to an amino acid (again most likely cysteine, histidine or glutamic acid residues) to alter protein function. However, because it is a redox metal a second action may be to bind to cysteine residues and oxidize them. This may catalyse the formation of disulphide bonds between physically adjacent cysteine residues thereby changing protein function. A third more indirect way that copper can modulate protein function is through the generation of free radicals, which can profoundly alter protein and cell function (Yu & Catterall, 2004);

2. Ligand-gated ion channels

The most well-studied membrane proteins regulated by zinc (and to a lesser extent copper) are the receptors for fast excitatory (glutamate) and fast inhibitory (γ-aminobutyric acid; GABA) transmission in the CNS. Many of the details of these regulations have been worked out over the last few years (Smart et al., 2004) to the extent that, for some receptor subunits, the exact amino acids zinc binds to, have been identified (Hosie et al., 2003). There is strong evidence from studies in hippocampal mossy fibers that synaptically released zinc can modulate both excitatory and inhibitory synaptic transmission under physiological conditions (Vogt et al., 2000 and Ruiz et al., 2004).

a. Glutamate receptors

Excitatory amino acid receptors are the mediators of synaptic transmission at many synapses that can undergo use-dependent modifications of synaptic efficiency. Ionotropic excitatory amino acid receptors can be divided into 2 large families, the N-methyl-D-aspartate (NMDA) and the (S)-alpha-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA)/kainate receptor family.

With regard to NMDA receptors, it is well established that both zinc and copper are potent inhibitors). While the copper binding site has not yet been elucidated, the inhibition of NMDA receptor currents by zinc has been shown to be mediated by 2 separate mechanisms, a highly sensitive (low nanomolar range) voltage-independent site on the NR2A subunit and a less sensitive (μM) voltage-dependent site on the NR2B subunit (Rachline et al., 2005).

AMPA/kainate receptors are also blocked by copper in the low μM range, with binding again proposed to occur at 2 separate binding sites). Unlike copper, zinc elicits biphasic current responses from AMPA/kainate receptors that are characterized by potentiation at low concentrations (50 μM) and inhibition at high concentrations (1 mM). However, a recent study by has highlighted the existence of a zinc-insensitive population of AMPA/kainate receptors, in addition to the well-documented zinc-sensitive
AMPA/kainate receptor population within the rat olfactory bulb. Application of zinc to the insensitive population of AMPA/kainate receptors produced uniphasic, inhibitory responses or occasionally had no effect at all. The differential effects of zinc on AMPA/kainate receptors have primarily been attributed to the varying subunit compositions expressed by individual receptors. Blakemore and Trombley (2004)

As well as agonist and antagonist actions on excitatory amino acid receptors, it has been demonstrated that zinc is permeable through these receptors Marin et al., 2000 and Jia et al., 2002). The Ca\(^{2+}\)-permeable subtype of AMPA/kainate receptors is a primary route of zinc entry/uptake into neurons with maximum translocation expected during intense neuronal activity. Related to this, NMDA receptor activation has been proposed to regulate copper homeostasis in hippocampal neurons through the release of copper via translocation of the copper transporter ATP7A to hippocampal neuronal processes (Schlief et al., 2005.).

b. γ-Aminobutyric acid\(_A\) receptors

After glutamate receptors, GABA\(_A\) receptors have been studied most extensively for zinc and (to a lesser extent) copper sensitivity (Hosie et al., 2003). While normal synaptic GABA\(_A\) receptors are thought to be relatively zinc-insensitive, tonic GABA\(_A\) receptors have a much higher sensitivity to block. This occurs because different GABA\(_A\) receptor subunit combinations have different zinc sensitivities. For example, the α1β3 splice variant of the GABA\(_A\) receptor is most sensitive to block by zinc, with other α and β variants having lower sensitivity. GABA\(_A\) receptors that contain γ subunits have greatly reduced sensitivity due to the interposition of the γ subunit and structural changes at the α–β interface site (Hosie et al., 2003). GABA\(_A\) receptors containing γ subunits are by far the most prevalent subtypes found at GABAergic synapses. The effects of endogenous zinc on GABA\(_A\) receptors has also been shown and modulation of GABA\(_A\) receptors by zinc is probably a vital factor in normal brain function but this probably occurs through extrasynaptic rather than synaptic GABA\(_A\) receptors and/or following changes in GABA\(_A\) receptor composition in disease states such as epilepsy (Dudek, 2001). Again, much less information is available for copper effects. At least in some cases, copper is thought to block GABA\(_A\) receptors through the same mechanism as zinc (Sharanova et al., 2000).

Inhibitory glycine receptors are also modulated by zinc; however, zinc has a biphasic effect on these receptors producing a potentiation of response at low concentrations but an inhibition at higher concentrations . (Laube et al., 2000)

c. P2X receptors

The P2X receptors are a family of ionotropic receptors that are widely distributed in the brain, peripheral nerves, and many other cell types, existing as both homomers and
heteromers. There are currently 7 members of the P2X family classed as P2X₁–P2X₇. The co-localization of zinc with P2X receptors in the nervous system suggests a physiological role in zinc modulation of ATP-evoked currents (North, 2002).

Trace metals have been known to enhance the cationic currents elicited by most extracellular excitatory ATP receptors in native tissues, including those in rat superior cervical ganglion nodose and coeliac ganglion neurons and PC12 cells). However, not all P2X receptors respond to zinc in the same manner. While the activity of the P2X₂ and P2X₃ receptors is potentiated by zinc as expected from studies in native tissues, 1–10 μM copper or zinc has been found to inhibit the activity of homomeric P2X₇ receptors or P2X₁ receptors (Coddou et al., 2002).

P2X₂ receptor currents are also potentiated with equal affinity by copper, whereas zinc and copper differentially modulate the P2X₄ receptor. While zinc potentiates P2X₄ receptor ATP-gated currents, copper inhibits them in a time- and concentration-dependent manner, (Acuna-Castillo et al., 2000 and Coddou et al., 2003).

Although relatively little is known about the P2X₆ receptor (North, 2002), increased expression of the P2X₆ purinergic receptor has been demonstrated in the hippocampus of zinc diet-restricted rats (Chu et al., 2003).

3. Voltage-gated-like ion channels

a. Sodium channels

Voltage-gated sodium (Na⁺) channels are present in the membrane of most excitable cells. There are 9 primary α subunits (Naᵥ1.1–Naᵥ1.9) which show differential expression throughout the body (Alexander et al., 2004). The majority of Na⁺ channels are highly sensitive to block by tetrodotoxin (TTX) but some are much less sensitive. For example, Naᵥ1.5, the so-called “cardiac” Na⁺ channel, and Naᵥ1.8 and Naᵥ1.9 (expressed particularly highly in peripheral nociceptive neurons) are much less sensitive to TTX than other Na⁺ channels. (Goldin, 2001).

Modulation of Na⁺ channels by zinc has been widely studied. showed that millimolar concentrations of external zinc modified the kinetics of squid giant axon Na⁺ currents. Despite the low potency of this effect, it has been suggested to arise from zinc binding to a specific site within the channel rather than from a screening of negative surface charges (Hank & Sheets, 1992, Schild et al., 1991).

b. Potassium channels

Potassium (K⁺) channels play a key role in a number of different aspects of the electrical responses of the nervous system. K⁺ channel activity determines neuronal action potential frequency and waveform and regulates the excitability of individual neurons.
(Hille, 2001). This physiological importance coupled with their diversity (with well over 70 different α subunits expressed in the mammalian nervous system) (see makes them fundamental regulators of neuronal excitability. It is important then to understand the nature and consequences of actions of compounds such as zinc and copper on these channels. (Alexander et al., 2004)

c. Voltage-gated potassium channels

As for Na⁺ channels, modulatory effects of divalent metal ions such as zinc and copper on the gating of K⁺ channels are well characterized In a number of studies, divalent cation effects have been shown to cause equal shifts in the voltage-dependent kinetics of K⁺ channels, explained by surface charge effects (Hille, 2001 and Elinder & Arhem, 2003). Again, as for Na⁺ channels, these effects were seen to occur at millimolar concentrations and were originally envisaged as having little physiological relevance.

More recent studies, however, on subtypes of voltage-gated K⁺ channels have revealed responses that occur at much lower ion concentrations. Furthermore, there are examples of particular K⁺ channel subtypes that are expressed in neurons, showing notable sensitivity to these ions (Horning & Trombley, 2001).

Horning and Trombley (2001) considered the excitatory effect of both zinc and copper on rat olfactory bulb neurons. They found that zinc (100 μM) produced modest but biphasic effects on voltage-gated K⁺ currents, potentiating peak current amplitudes of an Iₐ current by 17% (presumably by shifting the voltage dependence of inactivation as above) but inhibiting steady-state current amplitudes of delayed rectifier-type currents by 15%. Copper (30 μM), however, inhibited both peak and steady-state amplitudes of delayed rectifier-type currents by an average of 20% and 17%, respectively. Thus, copper and zinc can differentially influence neuronal excitability and synaptic transmission in the rat olfactory bulb.

For many K⁺ channels, the site(s) of action of zinc and copper remains to be determined. However, Kehl et al. (2002) have shown that inhibition of human Kv1.5 channels expressed in HEK293 cells by both hydrogen ions and zinc was substantially reduced by a mutation of histidine 463 (H) or arginine 487 (R), amino acids located either in the channel turret (H) or near the mouth of the pore (R) of the channel. (Cusimano et al. (2004)

d. Background or “leak” potassium conductances

Background K⁺ currents regulate the resting membrane potential of neurons and are fundamental regulators of neuronal excitability (Mathie et al., 2003). As such, compounds that regulate the activity of these channels will have a huge influence on
neuronal excitability. Two pore domain potassium channel (K2P) is thought to underlie such leak conductances in many neurons (Goldstein et al., 2001 and Lesage, 2003).

Because very few pharmacological agents are available to distinguish TASK-1 from TASK-3 channels, zinc was seen as a useful diagnostic tool to distinguish the two. A number of subsequent studies identified TASK-like conductances in native cells and attributed the current to TASK-1 channels at least in part on the basis of zinc sensitivity (Hartness et al., 2001, Barbuti et al., 2002, Gurney et al., 2003, Cooper et al., 2004 and Johnson et al., 2004).

In fact more recent experiments from our laboratory and elsewhere suggest that TASK-3 channels are much more sensitive to block by zinc than TASK-1 with an IC$_{50}$ of around 10–20 μM depending on the recording conditions used Clarke et al., 2004, Gruss et al., 2004 and Kim et al., 2005).

A mutation of a glutamate at position 70 and a histidine at position 98, of TASK-3, left the channel with a reduced sensitivity to zinc block. Conversely, mutation of a lysine at position 70 in TASK-1 to a glutamate (as is present in TASK-3) induced zinc sensitivity on the TASK-1 channel (Clarke et al., 2004). TASK-3 is highly expressed in the brain and has been implicated in neuronal apoptosis (Lauritzen et al., 2003). Thus, modulation by a compound such as zinc could be a useful pharmacological tool in the treatment of neurodegenerative and proliferative diseases.

In contrast, Kim et al. (2005) found that a different K2P channel, TREK-2 was enhanced rather than inhibited by zinc with an EC$_{50}$ of around 90 μM. Related to this, Gruss et al. (2004) had previously found that the K2P channel, TREK-1, was activated by copper with an EC$_{50}$ of 3 μM. As for TASK-3, zinc inhibited TREK-1, with an IC$_{50}$ of 3 μM. K2P channels are widely distributed throughout brain and are involved in setting the resting membrane potential and modulating neuronal excitability. The differential effects of zinc and copper on different K2P channels are useful diagnostically. Furthermore, the influence of these ions on neuronal excitability could be either increased or decreased dependent upon the K2P channel expressed in the neuron of interest.

e. Other potassium currents

There is much less information available about the effects of zinc and copper on other K$^+$ channels, such as inward rectifier K$^+$ channels or Ca$^{2+}$-activated K$^+$ channels. Showed that the inward rectifier K$^+$ channel HIR (Kir2.3) was weakly sensitive to block by external zinc, having an IC$_{50}$ between 100 and 200 μM at physiological pH. Another strongly rectifying channel IRK1 (Kir2.1), however, was completely insensitive to zinc at similar concentrations. Morera et al. (2003) found that copper concentrations of 20 μM and above induced a concentration- and time-dependent decrease in the channel open probability. Zinc, at concentrations up to 100 μM had no effect on these channels. Copper
was shown to act via oxidation of extracellular cysteine residues, involved in gating of the channel.

3. Calcium channels

Voltage-gated calcium (Ca$^{2+}$) channels can be divided into 3 families based on their structural and functional characteristics and within each family there are several different subunits. Functionally, these differences are reflected in distinct inactivation kinetics; activation and inactivation gating; and single channel conductances. This diversity allows each type to play a different but critical role in aspects of neuronal function. (Ertel et al., 2000 and Alexander et al., 2004)

1. T-type Ca$^{2+}$ channels

In terms of block by zinc and copper ions, T-type or low voltage-activated Ca$^{2+}$ channels (LVAs) are arguably the most important group. They constitute a family of 3 channel isoforms, Ca$_{V}$3.1, Ca$_{V}$3.2, and Ca$_{V}$3.3. Most brain regions express more than 1 isoform and some neurons, such as olfactory granule cells and hippocampal pyramidal neurons, express all 3 genes (Talley et al., 1999). The activation and inactivation curves of T-type Ca$^{2+}$ channels overlap and cross at $\sim$ 60 mV, allowing T-type Ca$^{2+}$ channels to sustain a continuous Ca$^{2+}$ influx in neurons and glia due to a small number of channels being continuously open. T-type Ca$^{2+}$ channels are generally thought of as a generator of pacemaker activity and/or regulators of hormone and neurotransmitter secretion. They are also known to contribute to pathophysiological conditions such as cardiac hypertrophy and absence epilepsy.

A number of papers have been published on the LVA T-type Ca$^{2+}$ channel, demonstrating inhibition by micromolar zinc. showed that the T-type Ca$^{2+}$ channel current in rat aorta smooth muscle cells in primary culture was reversibly inhibited by zinc with an IC$_{50}$ of 30 μM, while showed in CA1 pyramidal cells from acutely isolated rat hippocampal neurons that zinc inhibited the T-type Ca$^{2+}$ channel with an IC$_{50}$ of around 20 μM. Similarly, showed that zinc was highly specific for T-type Ca$^{2+}$ channels in rat dorsal root ganglion cells, with 20 μM zinc producing > 80% block. N- and L-type channels were less potently inhibited by zinc, with an IC$_{50}$ of 69 μM. More recently, Jeong et al. (2003) looked at the effect of copper and zinc on recombinant T-type Ca$^{2+}$ channels (Ca$_{V}$3.1, Ca$_{V}$3.2, Ca$_{V}$3.3). Copper and zinc blocked all 3 T-type channels but showed a high affinity for Ca$_{V}$3.2 channels (IC$_{50}$ = 0.9 μM and 2.3 μM for copper and zinc, respectively). Much higher concentrations were required to block Ca$_{V}$3.1 and Ca$_{V}$3.3 channels (IC$_{50} \geq 200$ μM).

2. L-, N-, P-, and Q-type Ca$^{2+}$ channels
The L-, N-, P-, and Q-type (high voltage activated, HVA) voltage-gated Ca\(^{2+}\) channels are, for the most part, as sensitive as T-type Ca\(^{2+}\) channels to inhibition by copper but are generally less sensitive to zinc inhibition at least compared to recombinant Cav3.2 channels). N- and L-type Ca\(^{2+}\) channels in mouse neuroblastoma and rat glioma hybrid cell line (NG108-15) were particularly sensitive to block by copper with IC\(_{50}\)s of 7 \(\mu\)M and 14 \(\mu\)M, respectively. (Jeong et al., 2003)

In pyramidal neurons from rat piriform cortex, 20 \(\mu\)M zinc inhibited each of the 4 components of HVA current (corresponding to L-, N-, P-, and Q-type currents) by around 35–57% whereas copper had an IC\(_{50}\) of less than 1 \(\mu\)M for each component of the HVA current. In the study with zinc, a higher degree of block was observed when the concentration of the permeant ion (either Ca\(^{2+}\) or barium) was lowered. Thus, the action of zinc is consistent with competitive binding of zinc and the permeant ion to an extracellular binding site, the occupancy of which by zinc results in Ca\(^{2+}\) channel block. It is worth noting that many studies on zinc block of Ca\(^{2+}\) channels may underestimate the effectiveness of the ion because they are done in artificially high concentrations of permeating ion (barium or Ca\(^{2+}\)) to allow reliable measurement of current through the channels. (Castelli et al., 2003 and Magistretti et al., 2003).

3. Permeation of zinc through voltage-gated Ca channels

As well as being blocked by zinc, Ca\(^{2+}\) channels can mediate the entry of zinc into neurons, at least under certain recording conditions. For example, looked at the effect of zinc on the neurones isolated from the subesophageal ganglia of Helix aspersa. In magnesium and Ca\(^{2+}\)-free media, 25 mM zinc generated all-or-none action potentials. Ca\(^{2+}\) antagonists such as verapamil and cobalt reduced these action potentials. Thus, the voltage-gated Ca\(^{2+}\) channel identified in Helix nerve cell bodies is permeable to zinc. Similar zinc permeation through Ca\(^{2+}\) channels has been observed for mammalian neurons (Kerchner et al., 2000 and Sheline et al., 2002).

The fact that zinc both blocks Ca\(^{2+}\) channel current and carries charge through the channel suggests that its interaction with the channel pore may be similar to that of Ca\(^{2+}\), which also blocks current through the channel carried by divalent or monovalent cations. Furthermore, this carrying of zinc by voltage-gated Ca\(^{2+}\) channels supports the notion that blockade of these channels may have therapeutic utility in pathological conditions, such as cardiac arrest or sustained seizures, where excessive zinc influx may contribute to neuronal death.

4. Store-operated calcium channels and transient receptor potential channels

In addition to voltage-gated Ca\(^{2+}\) channels, another major route for Ca\(^{2+}\) entry into cells is through store-operated calcium channels (SOCCs). Ca\(^{2+}\) entry and the subsequent refilling
of intracellular stores in many cells, particularly cells that lack or express small numbers of voltage-gated Ca\(^{2+}\) channels, has now been established to occur through such SOCCs. This may be of particular relevance in the CNS when considering the role of glial cells.

This so-called “capacitive calcium influx” plays an important role in shaping the Ca\(^{2+}\) response of various tissues and cell types. Inhibition by heavy metals is a hallmark of SOCC activity. The first demonstration of a Ca\(^{2+}\) current that corresponded with SOCC activity was recorded in rat peritoneal mast cells and was termed I\(_{\text{CrAC}}\). One characteristic of I\(_{\text{CrAC}}\) was inhibition by 1 mM zinc and this has been borne out by subsequent studies (Gore et al., 2004). Prothero et al., 2000 showed that a rise in intracellular Ca\(^{2+}\) via SOCCs following activation of metabotropic receptors in rat cortical glial cells was powerfully inhibited by 100 μM zinc. More recently, Kresse et al (2005) showed a similar zinc inhibition of capacitive Ca\(^{2+}\) entry in mouse hippocampal astrocytes, following activation of the metabotropic receptors, and suggested that the site of action of zinc may involve a change in the redox potential, possibly through an action on cysteine residues in the SOCCs (Gore et al., 2004).

Transient receptor potential (trp) channels have been identified as major pathways for cation movement in non-excitatory cells (Clapham et al., 2001, Montell et al., 2002 and Vennekens et al., 2002)... Recently, however, there has been much debate over the exact correlation between functional SOCCs and recombinant trp channels (Clapham, 2003 and Nilius, 2003). It is possible that block by zinc could provide a useful diagnostic tool to help in this debate (Gore et al., 2004).

Like voltage-gated Ca\(^{2+}\) channels, at least some trp channels are permeable to zinc. For example, 2 channels of the TRPM subfamily, TRPM6 and TRPM7, have been shown to be permeable to various divalent cations, including zinc (Hermosura et al., 2002, Monteilh-Zoller et al., 2003, Schmitz et al., 2003 and Voets et al., 2004). Indeed, Monteilh-Zoller et al. (2003) showed that TRPM7 was 2-fold more permeant to zinc than to Ca\(^{2+}\). This suggests that TRPM7, in a similar manner to voltage-gated Ca\(^{2+}\) channels or zinc-permeable AMPA/kainate receptors, could potentially play a major role in mediating the severe neurotoxic effects associated with high levels of zinc in the brain during ischemia.

4. Physiological consequences of zinc and copper actions

1. Neuronal excitability

As shown in the previous section, physiologically relevant concentrations of zinc and copper can modulate several ligand-gated ion channels including glutamate receptors, GABA\(_A\) receptors, and P2X receptors, as well as affecting many members of the voltage-gated-like ion channel family including K\(^+\), Na\(^+\), and Ca\(^{2+}\) channels. With such a wide range of actions, it is difficult to predict (and indeed measure) what the net effects on
neuronal excitability of these ions might be. Nevertheless, a number of striking features emerge from studies of these receptors and channels, principally the differential sensitivity of certain members of each family to zinc and, although much less thoroughly studied, occasionally to copper too. For ligand-gated ion channels, for example, NMDA receptors that lack the NR2B subunit are much more sensitive to zinc modulation than all other glutamate receptors (Tovar et al., 2000), whereas, for GABA_A receptors, it is those that lack the γ subunit; that is, those expressed primarily extra-synaptically, that are most sensitive to zinc. Similarly for Na^+ channels, zinc is a much more effective blocker of 1 subtype of TTX-insensitive Na^+ channel (NaV1.5) than other Na^+ channels, whereas for Ca^{2+} channels a certain T-type channel subunit (CaV3.2) is particularly sensitive. For K^+ channels, zinc can have profound effects on the gating of certain Kv channel subtypes (particularly those that show fast inactivation such as Kv1.4), whereas it displays rather selective blocking actions on closely related members of the K2P channel family (compare the effects on TASK-3 and TASK-1).

There are 2 main consequences of such differential effects of zinc. Firstly, zinc can provide a useful diagnostic tool to identify channels in mammalian neurons that underlie observed currents, when other pharmacological tools are lacking. This has been exemplified recently by the use of zinc to identify TASK-3 channels as underlying background K^+ currents in cerebellar granule cells (Clarke et al., 2004 and Aller et al., 2005). In a more physiological sense, these differences are useful because they allow the possibility of differential responses of neurons to either exogenously applied zinc or synaptically released zinc depending on the particular ion channels that neuron expresses at a given time and where they are localized. Kv1.4, for example, has been found highly localized in axons and terminals, with a possible role in modulating the excitability of nerve terminals. In the mossy fiber terminals of the hippocampus, high concentrations of zinc are also found and thus zinc may play a physiological role in hippocampal transmission through regulation of Kv1.4. In terms of changes with time, a good example is the altered sensitivity of GABA_A receptors to released zinc that can occur in temporal lobe epilepsy (Dudek, 2001).

2. Neurotoxicity

Neurotoxicity can be observed following unwanted rises in extracellular copper or zinc concentration. Extracellular levels of copper are significantly elevated during aging and some neurodegenerative disorders. The ability of copper to undertake redox cycling to activate molecular oxygen is used by a variety of enzymes. Although normally bound to proteins, excess copper may be released and become free to catalyze the formation of highly reactive hydroxyl radicals, implicated in disorders associated with abnormal copper metabolism and neurodegenerative changes (Barnham et al., 2004 and Valko et al., 2005). Ironically, one of the most important roles of copper, physiologically, is in
controlling free radical reactions particularly as part of the enzyme, copper/zinc superoxide dismutase (SOD1). So, for example, a dietary deficiency of copper increases cellular susceptibility to oxidative damage. Thus, either too little or too much copper in the brain can lead to an increased vulnerability to oxidative damage.

The brain is particularly susceptible to oxidative stress due to its high energetic requirement (utilizing 20% basal oxygen consumption), in addition to the high levels of transition metals and reduced antioxidant defenses compared to other organs (Maynard et al., 2005). Within the brain, neurons are the most metabolically active cells having the greatest oxygen requirement. Thus, SOD expression is most prominent amongst neuronal populations consisting of large neurons that are continually vulnerable to oxidative damage (Peluffo et al., 2005). In the adult CNS, SOD1 is expressed in numerous regions, the most notable being hippocampal pyramidal neurons, granule neurons of the dentate gyrus, cortical neurons (especially pyramidal cells), neurons of the substantia nigra, as well as distinctly high expression in motor neurons of the spinal cord (Peluffo et al., 2005).

Although not a redox-active metal-like copper, zinc too is required to allow optimal antioxidant responses and for DNA repair. Zinc is a functional and structural component of several enzymes and transcription factors involved in the antioxidant response and DNA integrity. Inadequate levels of zinc in the brain lead to abnormal functioning of SOD, which in addition to its dismutase activity, acquires peroxidase activity resulting in generation of peroxynitrite and neuronal death (Mocchegiani et al., 2005). The physiological intracellular concentration of free zinc in eukaryotic cells lies in the low picomolar range. Increases in free intracellular zinc (nanomolar concentrations), following ischemia, for example, can lead to neurotoxicity (Frederickson et al., 2005).

However, intracellularly accumulated zinc may be neurotoxic or neuroprotective depending on its concentration and the particular neuron of interest (Cote et al., 2005). For example, within the strata oriens and lucidum of the CA3 region of the hippocampus, high intracellular concentrations of zinc resulted in cell death, an effect that could be ameliorated by reducing zinc levels. Conversely, moderate intracellular concentrations of zinc in neurons of the CA3 pyramidal layer resulted in cell survival. Surprisingly, zinc chelation led to an increase in the mortality rate of these CA3 pyramidal cells (Cote et al., 2005). Similarly, if the free intracellular zinc is decreased in some neurons, for example, through the use of zinc chelators such as TPEN, this can trigger apoptosis (Frederickson et al., 2005). Thus, the intracellular concentration of these ions also needs to be tightly regulated by neurons.

5. Clinical consequences

In the previous section, a number of potential physiological and pathophysiological consequences of zinc and copper actions were considered. It is of interest, finally, to
consider these ideas in terms of known disease states, specifically Alzheimer's disease (AD) and certain forms of epilepsy.

1. Alzheimer's disease

Alzheimer's disease (AD) is a progressive neurodegenerative disorder characterized by amyloid plaques and neurofibrillary tangles in conjunction with neuronal cell loss or dysfunction. The primary constituent of amyloid deposits associated with all cases of AD is amyloid-β protein (Aβ), derived from the proteolytic cleavage of amyloid precursor protein. In healthy individuals, Aβ is predominantly membrane associated; however, in the AD brain there is a marked augmentation in the proportion of aggregated (diffuse and plaque amyloid) and soluble Aβ peptides (Bush, 2003).

A number of recent studies have demonstrated that the toxicity and aggregation of Aβ during AD is promoted by aberrant interactions with metals, in particular copper and zinc (Maynard et al., 2005). Aβ contains selective high and low affinity copper and zinc binding sites that facilitate its precipitation via interactions with these metal ions. The AD-affected brain contains increased concentrations of copper and zinc within the core and peripheral regions of amyloid plaques (Zecca et al., 2004 and Maynard et al., 2005).

Interaction between Aβ and oxidized metal ions renders the Aβ peptide toxic to neurons in cell culture (Maynard et al., 2005), an effect that is abolished under copper-free conditions (Bush, 2003). If copper has a primary role in the toxicity of Aβ peptide actions, zinc seems to play a key role in aggregation of amyloid deposits. At neutral pH, interactions between zinc and Aβ result in the formation of insoluble aggregates, whereas binding of copper is competitive resulting in soluble Aβ complexes. In contrast, within the slightly acidic environment of an elderly or inflamed brain, copper causes insolubilization and aggregation of Aβ (Maynard et al., 2005).

Neuronal death in AD occurs selectively in the hippocampus and neocortex as well as in particular subcortical regions. Because zinc-releasing neurons are also seen in these regions high concentrations of free, ionic zinc may be released during synaptic transmission. Under conditions of reduced metabolism in the aged or AD-affected brain, pools of extracellular zinc could accumulate, promoting aggregation of Aβ. In support of this idea, studies using mice that are deficient in the synaptic zinc transporter, ZnT3 showed an ~50% reduction in amyloid load compared to wild-type animals (see Maynard et al., 2005).

2. Epilepsy

Several of the ion channel proteins (such as Na⁺ channels, T-type Ca²⁺ channels, and GABA_A receptor channels), which zinc and copper act on, have been implicated in certain forms of epilepsy, thus it is difficult to make simple predictions about the pro- or anti-
convulsive actions of these ions. This is compounded by experimental observations where zinc has been documented to act as either an anticonvulsant Seizure activity in human epileptics and model animals influences the distribution of essential trace elements, including copper and zinc, in the brain and peripheral tissues Hirate et al., 2002). A number of studies suggest that altered zinc homeostasis in the brain contributes to the occurrence of epileptic seizures (Takeda et al., 2003). Prior to seizure activity, zinc concentrations are higher in the brain of epileptic (EL) mice than control mice. The enhanced uptake of zinc into the brain may reflect a compensatory mechanism for maintaining correct brain function in EL mice (Hirate et al., 2002 and Takeda et al., 2003). Conversely, postseizure activity, the zinc concentration in the brain of EL mice is significantly lower than that in control mice (Hirate et al., 2002). The likelihood of seizure activity in EL mice is diminished by zinc supplementation, whereas it is augmented by zinc deprivation (Hirate et al., 2002 and Takeda et al., 2003). Kainate-induced seizures in EL mice trigger a substantial, but tissue-specific loss of zinc from the brain. A reduction of zinc occurs in the hippocampus, amygdala, and cerebral cortex where there is an abundance of gluzinergic neuron terminals but not in the cerebellum (Takeda et al., 2003). Perhaps the single most interesting observation to date concerning the importance of zinc in epilepsy arises from observed structural and receptor distribution changes that occur in the hippocampus of patients with temporal lobe epilepsy or in animal models of this condition (Dudek, 2001. Two quite distinct changes occur, but the relationship between the two has clear functional consequences. Firstly, the composition of the GABA_A receptors in dentate granule cells changes from relatively zinc-insensitive receptors (incorporating γ subunits) to receptors comprised, primarily, of zinc-sensitive subunits (see Section 3.2.2). Secondly, mossy fiber terminals (which release zinc) are reorganized to innervate the dentate granule cells. It has been proposed that zinc released from mossy fibers during intense stimulation will block these novel GABA_A receptors, decrease inhibition, and contribute to the generation of seizures in the dentate gyrus (Hamed & Abdellah, 2004. This represents an excellent example of the functional consequences that arise from the combination of an alteration of zinc release from synaptic terminals and the selective sensitivity of particular protein subunits to modulation by zinc.

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