ENVIRONMENTAL TOXINS AND ION CHANNELS

KRZYSZTOF DOŁOWY
Department of Biophysics, Agricultural University SGGW, 02-526 Warszawa, Rakowiecka 26/30, Poland, dolowy@delta.sggw.waw.pl

Organisms inhabiting the same ecological niche compete with each other. In the case of the lower organisms this is an electro-chemical war. The weapons are chemical substances affecting the ability to maintain an electrical gradient across the cell membrane.

Cells use an electrochemical gradient to transport necessary substances from the environment into the cell and to produce ATP. Specialized cells of higher organisms use electrochemical gradients to get information from the environment, to communicate between parts of their bodies via their nervous systems and to coordinate their muscles' work. The chemical substances used in the electrochemical war can be divided into a few categories: ionophores, pore formers and specialized toxins, which block or modify the ion channels in the cell membrane.

Ionophores and pore formers

Ionophores [1] are the lipid soluble substances produced by microorganisms. They are able to transport ions across the lipid bilayer. Ionophores are not forming the water filled pores since in their presence the membrane remains osmoticaly tight. The ionophores usually form a cyclic ring made of aminoacids (often rear ones) or hydroxy-acids bound together via peptide or ether bonds (or both). In spite of the large number of oxygen atoms in the ionophore molecule, all these compounds are extremely insoluble in water and soluble in lipids. This is due to the screening of oxygen atoms from the external environment, arising from the ring structure of the compound. Ionophores can transport ions across the cell membrane only when the lipid bilayer remains in its fluid state [1].

The ionophore molecule dissolves in the lipid bilayer, binds the ion on one side, moves to the other side of the bilayer and then releases the ion. The best known ionophores are valinomycin, nonactin and nigericin. Each ionophore form a specific complex with a particular ion e.g. valinomycin with potassium and dianemycin with sodium. Some ionophores are neutral (valinomycin and nonactin) i.e. their complexes with cations are positively-charged and can only be transported towards the negative side of the membrane. Neutral ionophores destroy the membrane potential. The other ionophores are negatively-charged e.g. nigericin. The complex of a negatively-charged ionophore with a cation is neutral and thus can diffuse back and forth across the membrane. Nigericin is known to discharge the ion gradient across a cell membrane transporting a

potassium ion for exchange with a proton [1]. There are dozens of different ionophores produced by bacteria and many more synthetic ones. There is no clear answer to the question of how the organisms which produce ionophores are immune to their effect. It is likely that this immunity has something to do with the fluidity of the membrane.

Another group of substances affecting the cell membrane are polyene antibiotics, such as filipin, nystatin and amphotericin B produced by bacteria [2]. Polyene antibiotics are used against topical infections of fungal origin. These compounds have multiple conjugated double bonds on one side of the molecule and an array of hydroxyl groups on the other. They form large pores in the cell membrane in the presence of sterols [3]. Since bacteria do not contain sterols [4] they are immune to polyene antibiotics. Polyene antibiotics make the membrane permeable to ions and larger molecules (up to mono-sugars) and are known to produce pores (of radius 0.55 nm) in the membrane leading to the osmotic lysis of the cell [1].

Short linear peptides are another group of pore-forming antibiotics produced by microorganisms [2]. These peptides contain a chain of 20 to 35 aminoacid residues (often of a rear type). For example, gramicidin A is a linear polypeptide made of 15 neutral aminoacid residues, produced by *Bacillus brevis.* Gramicidin A is toxic to Gram positive bacteria [2]. It is likely that two gramicidin A molecules form the cation selective pore.

Alamecithin is produced by the fungus *Trichoderma viride*, which is the best-known member of the peptaboil class antibiotics. Peptaboils are linear polypeptides neutral or negatively-charged, made of 20 aminoacid residues, and capable of producing voltage-dependent cation conductance in lipid bilayers [2]. Magainins are linear polypeptides positively-charged, made of 23 aminoacid residues, and isolated from the skin of the African clawed frog *Xenopus laevis*. Magainins show bactericidal activity and are capable of forming anion selective conductance in lipid bilayers [2].

Nicin is the best known representative of the lantibiotics class of antibiotics used as food preservatives. Nicin is a linear, 34 aminoacid residue polypeptide, rich in positively-charged histidine and lysine aminoacids. Nicin forms anion selective pores [2].

Some constituents of venom e.g. melittin of the venom of the bee *Apis mellifera*, have similar pore forming properties. Melittin is also a linear, positively-charged, 26 aminoacid residue polypeptide forming an anion selective channel [2]. In the venom of the wasp *Paravespula lewisii* there is mastoparan a 14 aminoacid residue positively-charged polypeptide which apparently forms a cation selective pore. δ-Hemolysin produced by *Staphylococcus aureus* is a linear, 26 aminoacid residue polypeptide, with four positively and four negatively charged residues forming channels leading to cell lysis [2].

All these linear polypeptides form multi-state channels in the lipid bilayer. Each state of the pore has different conductance. The conductance most likely depends on the number of molecules forming the pore. This phenomenon was first described for alamecthin [5]. Depending on the charge of the polypeptide, the pores formed are selective to the counter ions. The few exceptions from the rule might arise due to lipid adsorption. The antibiotic effect is probably due to dissipation of the membrane potential. How the producers of the pore forming molecules are immune to their action still remains unclear.

Toxins – substances which block or modulate the ion channels

In nature, there are many chemical substances which act on ion channels. Many of these substances are toxins used for defense or as an offensive weapon. Snakes, spiders, scorpions and other predators often use venom to kill their prey. This venom is usually a mixture of different substances. The most effective of these at killing are those substances which influence either the sodium channel of the nerve (responsible for action potential formation), or the acetylcholine receptor channel (AChR - responsible for transferring nerve impulses from nerve cell to muscle), or the voltage-gated potassium channel of the nerve cell (which terminates the nerve impulse). Often, the toxins produced as defensive weapons are effective against the same ion channels as venom.

The Japanese delicacy fugu - the puffer fish of the family *Tetraodontidae* produces tetrodotoxin in its poisonous organs. Tetrodotoxin is a small heterocyclic compound which effectively blocks the sodium channel effecting conduction of the nerve impulses, and paralyzes the respiratory muscles [6]. μ-conotoxin of the predatory molluscs – the cone shells *Conus*, which inject their cocktail of venom via a harpoon-like disposable tooth - has a similar effect to tetrodotoxin. Conotoxins are peptides of 10 to 30 aminoacid residues, containing 2 or 3 disulfide links [6].

Batrachotoxin is the alkaloid secreted by the skin of the Columbian arrow poison frogs of the genus *Phyllobates*. A similar compound was found in the skin and feathers of birds of the genus *Pitohui* from New Guinea [6]. Batrachotoxin activates the sodium channel permanently leading to repetitive nerve firing. Plant alkaloids have a similar effect to that of barachotoxins for example, varatridine from the lily genus *Veratrum*, aconitine from the monk's hood of the buttercup family, and grayanotoxins found in rhododendrons of the heather family. Another group of substances which activate the sodium channel permanently are scorpion α- and β-toxin of the *Androcotonis*, *Buthus*, *Leirus* and *Centruroides* genera [6].

The toxin present in scorpion venom blocks the potassium channel – this is the effect of charybdotoxin of *Leiurus quinquestriatus*, iberitoxin of *Buthus tamulus* and noxiustoxin of *Centuroides noxius*. Apamin in bee venom and the dendrotoxins of the green mamba snake *Dendroaspis augusticeps* have similar effects [6].

The most effective antagonist of the nicotinic acetylcholine receptor (nAChR) is α-bungarotoxin of the banded krait *Bungurus multicinctus* and related snakes. A similar effect is produced by the α-conotoxin of *Conus*. Another compound which blocks the nAChR channel, is d-turbocurrarine (and others of the curare complex) from the plant *Chondodendron tomentosum* [6].

It would be difficult to claim that the substances discussed above (both pore formers and toxins) are present in the environment. However, there are substances produced by microorganisms living in fresh water which might cause serious or even fatal health problems to the general population. In certain coastal areas, mussels and clams become toxic sporadically or constantly in some month of the year and in human produce a neurotoxic syndrome known as "paralytic shellfish poisoning" (PSP) [7]. Toxins produced by marine dinoflagellates (*Gonyaulax* species *Gymnodinium tamarensis*, *Gymnodinium catentum* and a few others responsible for 'red tides') are responsible for PSP. PSP has been documented in many parts of the world - in Florida, California, Mexico, Tasmania, Portugal, Spain, Norway, England, France, Italy, Philippines and Thailand. Toxic dinoflagellates produce saxitoxin, neosaxitoxin and gonyautoxin (small heterocyclic compounds). Plankton-eating molluscs concentrate these toxic substances [7].

PSP causes a widespread inhibition of impulse-generation in peripheral nerves and skeletal muscles by blocking the sodium channel, which may result in respiratory paralysis leading to death. The fatal dose of saxitoxin is 1-2 mg and it is one of the most potent toxins. The symptoms appear within 30 minutes of consuming the toxic bivalve molluscs [7].

A milder syndrome known as neurotoxic shellfish poisoning (NSP) occurs in Florida after the ingestion of bivalve mollusks. The occurrence of the syndrome is associated with a 'red tide' due to a bloom of *Gymnodinium breve*, which produces brevetoxins. Brevetoxins are lipid soluble toxins (saxitoxin is water-soluble). Brevetoxins permanently open the sodium channel. The presence of brevetoxin in water leads to extensive fish death and respiratory irritation in humans [7].

Saxitoxin and neosaxitoxin are also produced by blue-green algae responsible for the green bloom of lakes – *Nodularia spumigena*, *Anabaena floss-aquae* and *Microcystis aeruginosa*. These algae also produce two other classes of neurotoxins: anatoxin-a and anatoxin-s [8]. Anatoxin-a opens the AChR channel leading to muscle contraction. Anatoxin-s blocks the enzyme acetylcholine esterase, which is responsible for destroying acetylcholine and terminating the natural opening of the AChR channels [8].

It is not clear why these extremely potent toxins are produced. A decrease in the number of plankton grazers is observed in the presence of toxins. But the toxins are especially effective against higher animals. Maybe the toxins are not a defensive but an offensive weapon. It has been observed [9] that fish excrement

induces the transformation of algae into aggressive toxin-producing forms which attach to fish, kill them and eat their flesh.

REFERENCES

1. Gomperts, B. **The Plasma Membrane. Models for the Structure and Function.** Academic Press. London 1977.
2. Marshall, G.R. and Beusen, D.D. The structural basis of peptide channel formation. In **Biomembrane Electrochemistry** M. Blank & I. Vodyanoy eds. **Adv. Chem. Ser.** 235 (1994) 259-314.
3. de Kruiff, B. and Demel, R.A. Polyene antibiotic-sterol interactions in membranes of *Acholeplasma laidlawii* cells and lecithin liposomes: molecular structure of the polyene antibiotic-cholesterol complexes. **Biochim. Biophys. Acta** 339 (1974) 57.
4. Gloldfine, H. Lipids of Prokaryotes – Structure and Distribution. **Curr. Topics Membr. Transp.** 17 (1982) 1-44.
5. Gordon, L.G.M. and Haydon, D.A. The unit conductance channel of alamecthin. **Biochim. Biophys. Acta** 255 (1972) 1-14-1018.
6. Aidley, D.J. and Stanfield, P.R. **Ion channels. Molecules in action**. Cambridge University Press 1996.
7. Viviani, R. Eutrophication, marine biotoxins, human health. **Sci. Tot. Environ. Supplement** (1992) 631-662.
8. Dow, C.S. and Swoboda, U.K. Cyanotoxins. In **The Ecology of Cyanobacteria.** B.A. Whitton & M. Potts eds. Kluwer Academic Publishers. Dordrecht 2000, pp. 613-632.
9. Burkholder, J.A.M. Implications of harmful microalgae and heterotrophic dinoflagellates in management of sustainable marine fisheries. **Ecol. Appl.** 8 (1998) 537-562.