

Chloride Channels

Key References

- Becq, F. et al. "Development of substituted benzo-[c]-quinolinium compounds as novel activators of the Cystic Fibrosis chloride channel." *J. Biol. Chem.* **274**, 27415-27425 (1999).
- Cabantchik, Z.I., Greger, R. "Chemical probes for anion transporters of mammalian cell membranes." *Am. J. Physiol.* **262**, C803-C827 (1992).
- Chappe, V. et al. "Structural basis for specificity and potency of xanthine derivatives as activators of the CFTR chloride channels." *Br. J. Pharmacol.* **123**, 683-693 (1998).
- Clement, Y. "Structural and pharmacological aspects of the GABA_A receptor: Involvement in behavioural pathogenesis." *J. Physiol. (Paris)* **90**, 1-13 (1996).
- Kozlowski, R.Z. "Chloride channels: Potential therapeutic targets." In *Chloride Channels* Kozlowski, R.Z., ed., pp.177-186, ISIS Medical Media Ltd, Oxford (1999).
- Large, W.A., Wang, Q. "Characteristics and physiological role of the Ca²⁺-activated Cl⁻ conductance in smooth muscle." *Am. J. Physiol.* **271**, C435-C454 (1996).
- Mulvaney, A.W. et al. "Cardiac chloride channels: Physiology, pharmacology and approaches for identifying novel modulators of activity." *Drug Discov. Today* **5**, 492-505 (2000).
- Nilius, B. et al. "Volume-activated Cl⁻ channels." *Gen. Pharmacol.* **27**, 1131-1140 (1996).
- Nishizawa, T. et al. "Molecular cloning and characterization of a novel chloride intracellular channel-related protein, parchorin, expressed in water-secreting cells." *J. Biol. Chem.* **275**, 11164-11173 (2000).
- Rajendra, S. et al. "The glycine receptor." *Pharmacol. Ther.* **73**, 121-146 (1997).
- Sieghart, W. "GABA_A receptors: Ligand-gated Cl⁻ ion channels modulated by multiple drug-binding sites." *Trends Pharmacol. Sci.* **13**, 446-450 (1992).
- Strange, K. et al. "Cellular and molecular physiology of volume-sensitive anion channels." *Am. J. Physiol.* **270**, C711-C730 (1996).

Overview

From a functional viewpoint, several different types of chloride channels showing different electrophysiological and regulatory characteristics have been described. These can be loosely grouped into five categories: cAMP-, calcium-, volume- and voltage-activated chloride channels as well as ligand-gated chloride channels. In addition to being differentially regulated, chloride channels can be discriminated by their molecular structure.

To date, some 30 different genes (including those for ligand-gated chloride channels, e.g. GABA and glycine) have been cloned which result in an increase in chloride conductance following expression in an appropriate system. In addition, there are a number of other candidates that are thought to be either chloride channels or regulators of chloride channels, since they also give rise to a chloride current when expressed. Such proteins include; P-glycoprotein (a member of the ABC superfamily which is topologically similar to the cystic fibrosis transmembrane regulator (CFTR), a chloride channel activated by cAMP), a protein termed ICl_n which has no significant homology with the sequence of any known transporter or ion channel, CLIC-1, a member of a family of proteins related to the bovine intracellular chloride channel p64, and Parchorin (a novel protein with significant homology to so-called CLIC channels) which elicits chloride efflux from parietal cells. Other chloride channels uncharacterized at a molecular level also exist, such as a novel background anionic current in rat ventricular cell myocytes that plays a role in regulating action potential duration.

A number of chloride channel blockers have been identified, although none of these are used therapeutically. These blockers represent a selection of heterogeneous molecules, including the stilbene disulphonate derivatives such as the amino reactive agent 4-acetamido-4'-isothiocyanato-stilbene-2,2'-disulfonic acid (SITS) and the diphenylamine-2-carboxylate (DPC) derivatives such as 5-nitro-2-(3-phenylpropylamino) benzoic acid (NPPB). Indanylyl oxyacetic acid (IAA-94) is an example of a third group of chloride channel blockers. In addition, the triphenyl-nonsteroidal anti-estrogens tamoxifen and clomifene, the antidepressants fluoxetine and imipramine, and pyrethroids represent other classes of chloride channel blockers.

To date, the only compounds identified with putative chloride channel activating properties are NS004, a substituted benzimidazolone, and a number of xanthine derivatives (e.g. 3,7-dimethyl-1-propyl xanthine) which have been reported to activate CFTR. All the chloride channel modulators noted above display fairly low affinity (mid μM-mM) and possess poor selectivity for the different classes of chloride channel. For example, 4,4'-diisothiocyanatostilbene-2,2'-disulfonic acid (DIDS) is also a potent inhibitor of anion exchangers and of the potassium/chloride co-transporter. Similarly, NPPB is an effective inhibitor of this co-transporter and the lactate transporter. However, recent findings suggest that it should be possible to develop agents specific for a given type of chloride channel since it appears that some blockers may discriminate between calcium-activated chloride channels and CFTR. For example, the CFTR channel is blocked by glibenclamide (originally thought to be specific for certain

types of potassium channel), but is relatively insensitive to DIDS, NPPB or tamoxifen (an anti-estrogen used in the therapy of breast cancer).

Among the most recent modulators of chloride secretion in epithelia, calixarene derivatives have been shown to reversibly block outwardly rectifying chloride channels at subnanomolar concentrations without effects on CFTR. Cyclic AMP has been reported to inhibit volume-regulated chloride channels from mammalian heart. Calcium-activated and volume-activated chloride channels on the other hand are both blocked by DIDS and NPPB, although tamoxifen and clomifene act selectively on volume-activated channels.

With regard to potency, chlorotoxin (a 36-amino acid peptide isolated from the venom of the scorpion *Leiurus quinquestriatus*) has been reported to block chloride channels with a much higher affinity (nM) than the blockers described above. Recent work has also identified a number of novel phenyl derivatives containing acid groups such as N-(3'-trifluoromethylphenyl)-N'-(2-carboxyphenyl)urea with reported blocking capacities in the submicromolar range. Interestingly, mibefradil, a T-type calcium channel blocker, has also been reported to block chloride channels in the submicromolar range.

Chloride Channels ^a

FAMILY	CIC	CFTR	Unknown	GABA/glycine
TYPES/SUBTYPES	CIC-1 to 7, CIC-Ka and Kb	Only one known	Ca ²⁺ -activated: four different types to date	At least 20 subtypes
STRUCTURE	12 transmembrane domains	12 transmembrane domains	2-4 transmembrane domains Still controversial	4 transmembrane domains
EXPRESSION	Virtually ubiquitous	Epithelium, Heart	Smooth muscle, Epithelium	Neuronal tissue
FUNCTION	Cell-volume regulation, Transepithelial transport, Stabilization of membrane potential (skeletal muscle)	Transepithelial transport, Possibly regulates other ion channels	Neurotransmitter-mediated smooth muscle contraction, Transepithelial transport	Neuronal inhibition
CONDUCTANCE	1–9pS ^b	5–8pS	1–10pS	10-90pS
PERMEATION	Cl ⁻ > Br ⁻ > I ⁻	Br ⁻ > Cl ⁻ > I ⁻	I ⁻ > Br ⁻ > Cl ⁻	I ⁻ > Br ⁻ > Cl ⁻
PHYSIOLOGICAL MODULATORS	Cell swelling (membrane stretch), Hyperpolarization, Intracellular pH, Intracellular Ca ²⁺ , Depolarization	Phosphorylation by PKA (e.g. following β-adrenoceptor or hormone stimulation)	Activation through elevation of intracellular Ca ²⁺ by neuro- transmitters, (e.g. norepinephrine, ATP and endothelin)	Ligand-gated by glycine and GABA
PHARMACOLOGICAL BLOCKERS	Examples include: SITS (A 0554), NPPB (N 4779), DPC, 9-AC, DIDS (D 3514), IAA-94 (I-117), niflumic acid (N 0630), tamoxifen (T 5648), chlorotoxin (C 9352), N-(3'-trifluoromethylphenyl)-N'-(2-carboxyphenyl)urea, mibefradil (M 5441), calixarene and flufenamic acid (F 9005)			
GENERAL COMMENTS (PHARMACOLOGY)	No radiolabeled blockers available → Selectivity of blockers generally poor → NS004 and a number of xanthine derivatives identified as activators for CFTR →			

ABBREVIATIONS

9-AC: 9-Aminocamptothecin

DIDS: 4,4'-Diisothiocyanatostilbene-2,2'-disulfonic acid

DPC: Diphenylamine-2-carboxylate

IAA-94: Indanylyl oxyacetic acid

NPPB: 5-Nitro-2-(3-phenylpropylamino) benzoic acid

NS004: 5-Trifluoromethyl-(5-chloro-2-hydroxyphenyl)-1,3-dihydro-2H-benzimidazo le-2-one

SITS: 4-Acetamido-4'-isothiocyanato-stilbene-2,2'-disulfonic acid

FOOTNOTES

a Ligand-gated chloride channels (i.e. those regulated by GABA and glycine) are discussed on pages 28 and 42, respectively.

b Large 20-50pS outwardly-rectifying chloride channels underlie volume-activated chloride currents in various preparations. These may belong to an unknown gene family. Recent evidence has shown that CIC-3 may be responsible for native swelling-activated chloride currents in many mammalian cells, but not in human. Swelling-activated chloride currents are known to be regulated by phosphorylation and dephosphorylation.