

# Chloride Channels

## Key References

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## 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, nearly 40 different genes (including those for ligand-gated chloride channels: GABA<sub>A</sub>, GABA<sub>C</sub> and glycine) have been cloned and, when expressed in an appropriate expression system, shown to increase a chloride conductance. 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: phospholemman (a 72 amino acid sarcolemmal protein suggested to express an anion selective channel which mediates taurine efflux during regulatory volume decrease), p-glycoprotein (a member of the 'ATP-Binding Cassette' (ABC) superfamily of transporters), and pICln (a soluble cytosolic protein that has no significant homology with the sequence of any known transporter or ion channel). However, parchorin, p64 and the related chloride intracellular channel (CLIC) proteins are widely expressed candidates for novel, auto-inserting, self-assembling intracellular anion channels. Other chloride channels uncharacterized at a molecular level also exist, such as the background anionic current (I<sub>AB</sub>) in rat and guinea pig ventricular 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 antiestrogens tamoxifen and clomifene, the antidepressants fluoxetine and imipramine, and pyrethroids represent other classes of chloride channel blockers.

To date, compounds identified with putative chloride channel activating properties include: NS004 (a substituted benzimidazolone) a number of xanthine derivatives (e.g. 3,7-dimethyl-1-propyl xanthine), genistein and MPB-07 (reported to activate CFTR), acid-activated omeprazole (activates ClC-2), tamoxifen (activates a large conductance chloride channel) and tefluthrin (activates I<sub>AB</sub>). 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, 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.

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 small conductance 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.

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<b>FAMILY TYPES/SUBTYPES</b>	CIC CIC-1 to 7, CIC-Ka and Kb	CFTR Only one known	CaCC Ca <sup>2+</sup> -activated: four different types to date	GABA/glycine At least 20 subtypes
<b>STRUCTURE</b>	composed of 18 $\alpha$ -helices (in two repeated halves)	12 transmembrane domains Still controversial	4 or 5 transmembrane domains	4 transmembrane domains
<b>TISSUE EXPRESSION</b>	CIC-1 (skeletal muscle) CIC-2 (ubiquitous) CIC-3 (ubiquitous) CIC-4 (ubiquitous) CIC-7 (ubiquitous) CIC-Ka (kidney, inner ear) CIC-Kb (kidney, inner ear)	Epithelium, heart, smooth muscle, CIC-6 (ubiquitous)	Smooth muscle, epithelium	Neuronal tissue
<b>PHYSIOLOGICAL FUNCTION</b>	Cell-volume regulation, transepithelial transport, stabilization of membrane potential (skeletal muscle), intracellular pH regulation, electrogenesis	Transepithelial transport, possibly regulates other ion channels	Neurotransmitter-mediated smooth muscle contraction, transepithelial transport	Neuronal inhibition
<b>CONDUCTANCE</b>	1–9pS <sup>a</sup>	5–8pS	1–10pS	10-90pS
<b>PERMEATION</b>	Cl <sup>-</sup> > Br <sup>-</sup> > I <sup>-</sup>	Br <sup>-</sup> > Cl <sup>-</sup> > I <sup>-</sup>	I <sup>-</sup> > Br <sup>-</sup> > Cl <sup>-</sup>	I <sup>-</sup> > Br <sup>-</sup> > Cl <sup>-</sup>
<b>PHYSIOLOGICAL MODULATORS</b>	Cell swelling (membrane stretch), hyperpolarization, intracellular pH, intracellular Ca <sup>2+</sup> , depolarization	Phosphorylation by PKA (e.g. following $\beta$ -adrenoceptor or hormone stimulation)	Activation through elevation of intracellular Ca <sup>2+</sup> by neurotransmitters, (e.g. norepinephrine, ATP and endothelin)	Ligand-gated by glycine and GABA
<b>DISEASE RELEVANCE</b>	Myotonia, Bartter's III and IV disease, Dent's disease, osteoporosis	Cystic fibrosis	Cancer, asthma (controversial)	Anxiety
<b>PHARMACOLOGICAL BLOCKERS</b>	Examples include: SITS ( <b>A0554</b> ), NPPB ( <b>N4779</b> ), DPC, 9-AC, DIDS ( <b>D3514</b> ), IAA-94 ( <b>I117</b> ), niflumic acid ( <b>N0630</b> ), tamoxifen ( <b>T5648</b> ), chlorotoxin ( <b>C9352</b> ), N-(3'-tri fluoromethylphenyl)-N'-(2-carboxyphenyl)urea, mibefradil ( <b>M5441</b> ), calix[4]arene, clomiphene ( <b>C6272</b> ), Cd <sup>2+</sup> , Gd <sup>3+</sup> , glibenclamide, flufenamic acid ( <b>F9005</b> ), inositol-tetrabishosphate, mefloquin, fluoxetine ( <b>F132</b> )			
<b>GENERAL COMMENTS (PHARMACOLOGY)</b>	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 102 and 116, 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.