

# Chloride transport and the actions of nedocromil sodium and cromolyn sodium in asthma

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*Nedocromil sodium has been shown to be capable of inhibiting chloride ion flux in mast cells, epithelial cells, and neurons. This feature may explain how it can prevent responses such as mast-cell degranulation, the effects of osmolarity changes in the airways, and neuronal activation. This mechanism may also provide a unifying hypothesis to explain the effects of nedocromil sodium on a range of cell types involved in asthma, such as sensory and efferent neurons and cells involved in inflammation. (J Allergy Clin Immunol 1996;98:S102-6.)*

**Key words:** Chloride channel, cromolyn sodium, epithelial cell, mast cell, nedocromil sodium, neuronal activity

It is well established that symptoms can be induced in persons with asthma by a wide range of indirectly acting stimuli such as antigens, chemicals, exercise, and sensory nerve stimulants. Many of these responses can be prevented by short-term administration of either cromolyn sodium or nedocromil sodium.<sup>1,2</sup> However, neither of these agents inhibits the bronchospasm caused by histamine or methacholine. The wide spectrum of activity of these agents suggests that they may be affecting a pathway that is common to several cell types (mast cells, eosinophils, epithelial cells, and sensory neurons), in keeping with the findings of many in vitro studies.<sup>3-5</sup> Elucidation of the modes of action of nedocromil sodium and of cromolyn sodium may provide insight into the pathogenesis of asthma. This article reviews briefly the increasing evidence that suggests an effect of these agents on chloride transport in several cell types.

Activation and degranulation of cells such as mast cells and eosinophils have long been associated with accumulation of inflammation in the asthmatic airways. Mediator secretion appears to depend on the synergistic action of an increase in intracellular calcium and a guanosine triphosphate-stimulated event.

## Abbreviations used

cGMP: Cyclic guanosine monophosphate  
IC<sub>50</sub>: Concentration producing 50% inhibition  
IP<sub>3</sub>: Inositol trisphosphate

Secretagogue-induced increases in intracellular calcium occur in two stages. An initial increase mediated by inositol trisphosphate (IP<sub>3</sub>) through the release of calcium from intracellular stores and a secondary influx of extracellular calcium result in a sustained increase in calcium.<sup>6</sup> The latter event, which lasts for several minutes, is probably mediated through a highly calcium-specific channel, described by Penner et al.,<sup>7</sup> which can be activated by intracellular application of IP<sub>3</sub><sup>8</sup> and which requires membrane hyperpolarization to support calcium influx. It also has been shown that a small-conductance chloride channel in rat peritoneal mast cells,<sup>9</sup> activated by secretagogues, intracellular application of cyclic adenosine monophosphate, and high intracellular calcium ion concentration, can provide the negative membrane potential or hyperpolarization necessary to maintain calcium influx through this mechanism and thus the sustained increase leading to degranulation. (After the initial IP<sub>3</sub>-induced increase in intracellular calcium, the cell interior is relatively depolarized and would no longer favor calcium influx unless a negative or hyperpolarizing force were introduced inside the cell.) Hoth and Penner<sup>10</sup> have subsequently identified the calcium current that is activated by this mechanism and have described it as

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the "calcium release-activated calcium current," because it is likely to be responsible for the eventual refilling of depleted intracellular stores of calcium in addition to the facilitation of cell degranulation. A large-conductance, nonspecific cation channel that can be activated by antigen or by compound 48/80 and regulated by guanosine triphosphate also allows divalent cations to enter the cell but probably does not produce sufficient calcium influx to influence degranulation.<sup>9</sup>

## **MAST CELLS**

Neither cromolyn sodium nor nedocromil sodium is believed to act directly on calcium channels, but each has been shown previously to reduce calcium influx into cells.<sup>11</sup> The first insight into their possible mode of action in this respect came from the observation that sodium cromoglycate blocks intermediate-conductance chloride channels in patches of cultured mucosal-like mast cells (RBL-2H3) ( $IC_{50}$  15  $\mu$ mol/L)<sup>12</sup> when applied to the cytoplasmic side of the inside-out patches. Studies on RBL cells have demonstrated a similar activity with nedocromil sodium ( $IC_{50}$  8  $\mu$ mol/L) (Romanin C, et al., unpublished data). In the experiments carried out with RBL cells it was shown that a parallel inhibition of serotonin release and chloride channel activity occurred when cromolyn sodium or the chloride channel-blocking agent NPPB was applied to the patches. Although the biophysical characteristics of this channel do not resemble those described in the original studies by Mathews et al.<sup>9</sup> and although its precise role is unknown, this finding led the investigators to believe that chloride channels play a functional role in mediator release from mast cells. Furthermore, pharmacologic studies<sup>13</sup> have demonstrated that antigen-stimulated histamine secretion from rat peritoneal mast cells is inhibited when extracellular chloride ions are replaced by the nonpermeant anions isethionate or gluconate, although some histamine secretion still occurs.

## **EPITHELIAL CELLS**

Further evidence that the antiasthma drugs have an effect on chloride transport is provided by studies on epithelial cells. Cromolyn sodium has been shown to block chloride channels present in the intestinal cell line HT29 ( $IC_{50}$  19  $\mu$ mol/L),<sup>14</sup> again applied to the cytoplasmic side. This channel is likely to be analogous to the one identified in RBL cells.<sup>12</sup> More recently, our own studies have demonstrated that cromolyn sodium, nedocromil sodium, and frusemide are able to block the activ-

ity of a chloride channel present on the mucosal surface of airway epithelial cells.<sup>15</sup> These agents were effective only when applied to the presumed extracellular surface of the channel and acted in a dose-related and reversible manner. It should be noted, however, that the biophysical properties of this channel differ from each of the others discussed earlier in this article.

The physiologic roles of airway epithelial chloride transport are not fully understood, but one possible function relates to volume regulation. In response to a hypotonic stimulus, cells swell and subsequently undergo a regulatory volume decrease to return to normal volume. This regulatory volume decrease is mediated in part by the efflux of chloride, which is osmotically linked to water transport from the cell. A hypotonicity-activated small-conductance chloride channel has been identified in several cell types, including endothelial cells,<sup>16</sup> fibroblasts,<sup>17</sup> epithelial cells,<sup>18</sup> and Jurkat cells.<sup>19</sup> Of particular interest is the cloning of a protein, ICIn, which is involved in cell volume regulation.<sup>20</sup> The protein appears to be linked to the cytoskeletal element actin, thus providing a mechanism in which changes in cell volume can activate chloride transport. Of importance, recent studies have shown that the hypotonicity-activated chloride channels in 3T3 fibroblasts and pulmonary endothelial cells associated with ICIn are blocked by both nedocromil sodium and cromolyn sodium.<sup>21,22</sup> Again, cyclic nucleotides feature prominently in these studies of epithelial chloride channels as they are potent blockers of both ICIn (cyclic guanosine monophosphate [cGMP] is the most potent)<sup>20</sup> and of the chloride channel we have described.<sup>15</sup> Whether there is a link between these two proteins is the subject of current investigation.

## **AFFERENT AND EFFERENT NERVES**

In vitro studies suggest an effect of nedocromil sodium and cromolyn sodium on neuronal chloride transport. Chloride efflux from sensory nerves is recognized to produce depolarization and hence the generation of action potentials.<sup>23</sup> Nedocromil sodium prevents the contraction of the guinea pig bronchus that is induced by electrical field stimulation in the presence of atropine.<sup>24</sup> Bronchospasm is likely to be mediated by the release of sensory neuropeptides from C fiber terminals. Furthermore, nedocromil sodium reduces substance P-mediated up-regulation of preganglionic stimulation of cholinergic responses in the rabbit bronchus.<sup>25</sup> It has been shown that nedocromil sodium induces a slow and long-lasting depolarization of the rabbit vagus nerve,<sup>26</sup> presum-

ably preventing repeated firing of action potentials. This effect was not seen in the presence of a low-chloride solution or chloride channel blockers, suggesting that chloride efflux is prevented by nedocromil sodium. Of note, in the same preparation, sodium cromoglycate did not depolarize the nerve but was capable of preventing the response to nedocromil sodium. In addition, inhalation of low-chloride solutions in human beings is well recognized to produce coughing, and this effect is blocked by nedocromil sodium.<sup>27</sup>

### OTHER OBSERVATIONS ON THE MECHANISM OF ACTION OF CHROMONES

Numerous studies are beginning to implicate the action of chromones on chloride transport. In addition, G protein-related regulation features prominently in many of the studies.<sup>3,4</sup> Cromolyn sodium was shown in the 1970s and early 1980s to inhibit agonist-induced influx of calcium ions<sup>11</sup> and to phosphorylate a 78 kDa protein in rat peritoneal mast cells.<sup>28</sup> These findings were associated with an inhibition of histamine release after antigen challenge.<sup>28,29</sup> Similar findings have since been reported for nedocromil sodium.<sup>30</sup> The time course for the appearance of the phosphorylated 78 kDa protein was reported to be consistent with the activation of a mechanism that switches off the secretory response. Research by Wells and Mann<sup>29</sup> showed that cGMP causes a phosphorylation reaction identical to that produced by cromolyn sodium, indicating that the 78 kDa protein may be a substrate for cGMP-dependent phosphorylation. In addition, a cross-tachyphylaxis has been observed between the inhibitory effects of cromolyn sodium and cGMP on histamine release from rat peritoneal mast cells.<sup>31</sup> It will be important to link the observations on the effect of these agents on chloride channels with their action on G proteins.

### CLINICAL RELEVANCE OF CHLORIDE TRANSPORT

An important area of inquiry is the relation of these laboratory studies to the clinical effects of nedocromil sodium and cromolyn sodium. It is well known that changes in the osmolarity of the periciliary fluid are capable of increasing airway resistance and that this increased resistance can provoke an attack of asthma.<sup>32</sup> Such changes in tonicity induce compensatory changes in cell volume. In vitro studies have shown a reduction in volume of cultured human nasal epithelial cells after exposure of the luminal but not serosal side to hypertonic saline solution.<sup>33</sup> This type of challenge probably reproduces the effects of exercise on airway surface fluid tonicity. The net

result of these alterations in cell size may be to alter either the access to or the firing of afferent nerve endings situated within the epithelial layer. The inhibitory effects of nedocromil sodium and cromolyn sodium on chloride transport may thus be envisaged as acting at several key points to prevent the bronchospasm<sup>34,35</sup> and cough<sup>36</sup> in persons with asthma induced by inhalation of nonisotonic solutions. By reducing alterations in cell volume these agents may indirectly alter mechanical irritation of sensory fibers or alter the access of chemical irritants to these afferent nerves. More direct effects on the sensory nerve endings may reduce firing of these nerves, whereas effects on inflammatory cells such as mast cells and eosinophils prevent the release of mediators.

### CONCLUSIONS

The nonspecific chloride channel-blocking activity of the chromones, demonstrated in a large range of cells, is consistent with their similarly large range of inhibitory effects in the clinical laboratory. These findings suggest that alterations in chloride transport may be a component of the asthmatic response, but whether this represents a primary abnormality or an effect on compensatory mechanisms is unclear.

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## DISCUSSION

**P. J. Barnes.** Does frusemide block this chloride channel?

**M. Paulmichl.** Eric Alton has produced some data showing that frusemide is directly blocking the current. We have some similar early studies that also show that this type of ion channel can be blocked by furosemide. One can also indirectly block the chloride channels by blocking the frusemide-sensitive transporters, thus reducing intracellular chloride, and eventually produce the same result as with a direct block of the channels.

**S. E. Dahlén.** When you block this chloride shift, do you affect bicarbonate transport and pH in the cell?

**M. Paulmichl.** We see acidification after swelling the cell, which is probably caused by bicarbonate's leaving the cell by way of chloride channels. However, the bicarbonate concentration and also the proton concentration are regulated by other mechanisms, such as the chloride-bicarbonate exchange and the sodium-proton exchange, so I would expect largely to change intracellular pH simply by adding furosemide.

**J. P. Seale.** How does Markus Paulmichl's picture of the complete channel with its molecular structure relate to what you, Eric Alton, reconstitute in your membrane preparation?

**E. W. F. W. Alton.** They are probably the same. We are looking at the single-channel level, whereas Markus Paulmichl is looking at the whole-current level, but within that whole current is the effect of many channels. Markus Paulmichl showed the structure of a single channel and, with a bit of luck, it will also be the structure that we are observing.

**T. H. Lee.** How are these chloride ions regulated by mediators such as histamine?

**E. W. F. W. Alton.** The only work that I know of was done some time ago by Nadel in the dog tracheal epithelium, and he showed that histamine up-regulates chloride secretion, probably through an increase in intracellular calcium (Nadel JA, Dairs B. Regulation of  $\text{Na}^+$  and  $\text{Cl}^-$  transport and mucous gland secretion in airway epithelium. Ciba Foundation Symposium 1978;54:133-47). I do not know whether there are any direct effects on this sort of channel.

**P. J. Barnes.** Was Alan Norris suggesting that nedocromil sodium is having effects that are not specific to the volume-dependent channel, but that may be more "promiscuous"?

**A. A. Norris.** From the data that we have, yes; there is probably a common theme. In all of the models and channels that we have discussed so far, the drug, by blocking chloride ion movement, can in turn block calcium entry. That surely is one of the most important mechanisms of all, because it initiates several second-messenger triggers.

**P. J. Barnes.** The potency of the drugs in blocking these channels seems to be very low. Is that consistent with the effects on inhibition of mediators?

**A. A. Norris.** It is very difficult to reconcile some of the electrophysiologic findings with the functional studies. What we would like to do, ideally, is to carry out both a functional and an electrophysiologic study in the same cell type.

**M. Paulmichl.** It is not surprising that these substances act on more than just the volume-dependent chloride channel because, from the biophysical considerations of the kinetics of the current, we would put the calcium-dependent and the swelling-dependent chloride current, both of which are outwardly rectifying, into the same family. As you saw from the mutation experiments, we can somehow convert the swelling-dependent current into the calcium-dependent current simply by doing the mutation. On the question of potency, I doubt whether the use of a highly specific, highly potent blocker is desirable because of possible interference with a mechanism that is essential for cell life. We know from our current experiments that during the cell cycle these proteins are regulated. I would say that a specificity and a "low" potency are exactly what is needed.

**E. W. F. W. Alton.** On the subject of promiscuity, we see six or eight different chloride channels in our bilayer work, and this is the only channel that the drug did block. Undoubtedly, though, it has other effects, for instance in nerves. Nebulized

uridine triphosphate is being used in a clinical trial by Knowles MR to open the calcium-activated chloride channel in cystic fibrosis. It will be interesting to see what happens clinically—whether this channel is important and is blocked by uridine triphosphate.

**P. J. Barnes.** Early data from this nebulized uridine triphosphate trial show that it is nontoxic and seems to have a significant effect on mucociliary clearance.

**E. W. F. W. Alton.** It would be expected that because this channel is not constitutively active, we are talking about episodic activity in the presence of a hypotonic or hypertonic stimulus.

**M. Paulmichl.** If nucleotides such as adenosine triphosphate are added from the extracellular side, P-2 purinergic receptors are also activated, and the intracellular calcium is increased and many other channels are switched on. The whole scenario becomes quite complicated.

**S. E. Dahlén.** Can you clarify whether nedocromil sodium and cromolyn sodium are binding close to this nucleotide site, or are they actually flowing through the channel?

**E. W. F. W. Alton.** The flickering block just means that the drug goes into a part of the channel and then simply moves out again back on to the extracellular surface. It does not actually go through the channel.

**S. E. Dahlén.** Is the drug acting by itself, or is it inducing the synthesis of an effector protein? The antiexudative effect of these compounds can be inhibited by blocking de novo protein synthesis (Rand et al. Eur J Pharmacol 1995;282:207-11).

**E. W. F. W. Alton.** In the bilayer there is nothing except that single channel; that feature is one of the few advantages of using bilayers.

**S. T. Holgate.** Adenosine-induced bronchoconstriction is very effectively inhibited by frusemide and bumetanide as well as by nedocromil sodium and cromolyn sodium. The receptor for that effect has been shown, at least in a mouse line, to be an A3 adenosine receptor that has been linked to chloride channels. Can anyone tell us about that relation? It may explain how adenosine-induced bronchoconstriction is so potently inhibited by these drugs.

**E. W. F. W. Alton.** Adenosine can increase intracellular calcium and produce chloride secretion very effectively in these airway epithelial cells, and it can easily be seen that nedocromil sodium could be blocking that effect. Adenosine is a routine stimulus that we use to produce chloride secretion in the human bronchus.