Chloride in airway smooth muscle: the ignored anion no longer?

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Gallos G, Yim P, Emala CW. Chloride in airway smooth muscle: the ignored anion no longer? Am J Physiol Lung Cell Mol Physiol 302: L733–L735, 2012. First published February 17, 2012; doi:10.1152/ajplung.00053.2012.—This Perspectives accompanies an Editorial Focus that summarizes new developments concerning the role of chloride in airway smooth muscle physiology. We provide several observations and mechanistic insights to reconcile recent experimental evidence with existing paradigms concerning chloride channel-mediated effects on airway smooth muscle tone. In addition, we highlight the potentially complex and dynamic nature that chloride currents and membrane potential have on calcium handling and airway smooth muscle contractility.

D.J. JANSSEN’S EDITORIAL FOCUS IN THIS ISSUE OF AMERICAN JOURNAL OF PHYSIOLOGY LUNG CELLULAR AND MOLECULAR PHYSIOLOGY (12) ILLUMINATES MANY UNANSWERED QUESTIONS CONCERNING THE ROLE OF MEMBRANE POTENTIAL AND CHLORIDE ION FLUXES IN THE CONTROL OF AIRWAY SMOOTH MUSCLE CONTRACTION AND RELAXATION. DR. JANSEN HAS MADE SEMINAL CONTRIBUTIONS TO THE UNDERSTANDING OF THE IMPORTANCE OF CHLORIDE FLUX IN AIRWAY SMOOTH MUSCLE AND RefERS TO A RECENT PUBLICATION OF OURS (5), IN WHICH WE DESCRIBE A PRORELAXANT EFFECT OF LIGANDS FOR THE GABA A CL CHANNEL.

As Dr. Janssen’s elegant studies have shown us (7, 8, 11, 14, 15), opening of another class of chloride channels on airway smooth muscle [namely calcium-activated chloride channels (CaCCs)] has been associated with chloride efflux, membrane depolarization, and cell contraction. The question arises: what possible mechanism could explain these apparent discrepancies?

To begin, it is critical to consider both the preparation (intact tissue vs. single cells) and the context (resting tension vs. contracted tissue) under which electrophysiological data are being obtained and analyzed. The reason that this is so important is because under various conditions the direction of a given ionic flux may change depending on both the electrochemical potential and specific ionic gradients involved. For example, we know from Dr. Janssen’s work that the resting membrane potential of an airway smooth muscle cell is approximately −60 mV. Opening a chloride channel at this membrane potential should cause chloride efflux and depolarization and theoretically contribute to contraction of the intact airway smooth muscle. However, assuming an intracellular chloride concentration of 30–50 mM in an airway smooth muscle cell (based on vascular and gut smooth muscle), this would put the chloride reversal potential in the region of −30 mV as predicted by the Nernst equation. Interestingly, Dr. Janssen has demonstrated that acetylcholine can depolarize an airway smooth muscle cell above this predicted threshold (−30 mV) (14), and in our hands sharp electrode recordings of intact guinea pig airway smooth muscle exposed to acetylcholine recorded the membrane potential at −20 mV. Therefore, in this context, it is feasible that once a contraction or depolarization event is achieved and sustained in a tissue above the chloride reversal potential, subsequent opening of a chloride channel will now favor chloride influx and hyperpolarization and theoretically promote relaxation. Our data are consistent with this as follows: application of a GABA A agonist at resting tension induces minimal change in tension (sometimes resulting in a very small contraction). However, if airway smooth muscle is first contracted, GABA A agonists induce relaxation in all of our studies [similarly, treatment with a GABA A antagonist (gabazine) on top of a contraction further augments the contraction]. Although the contractile state of the tissue offers an explanation for the apparent discrepancy that opening a CaCC channel can promote contraction one moment but opening a GABA A Cl− channel may promote relaxation at another point, it does not account for the dynamic nature of these currents and changes in membrane potential. Namely, that although opening a GABA A channel may indeed allow cytosolic chloride to accumulate (if the reversal potential has been surpassed), doing so at some point should hyperpolarize the cell membrane enough to reach the chloride equilibrium point and prevent further accumulation of cytosolic chloride. The more pressing question then becomes how does chloride actually impact tone, and by what mechanism can intracellular chloride affect other cation and anion states?

Dr. Janssen eloquently summarizes the relationship between intracellular/extracellular chloride concentrations, slow waves (13, 26), and resting membrane potential as well as the possible role of chloride in “charge balancing” calcium release across the SR membrane (8). In this body of work, an intriguing argument is proposed that relatively higher cytosolic chloride may “impede” efflux of chloride from the SR upon sarcoplasmic calcium release. Since chloride cannot adequately flow out of the SR, the calcium released from the SR creates a charge imbalance within the SR. Thus, by not balancing the flow of positive charge leaving the SR with a concomitant egress of chloride anions from the SR, an electrogenic debt is created by excess chloride being retained within the SR and this impedes further SR calcium release. Yet there remain other experimental observations we have made that suggest things are a bit more complex than the above explanations.

In unpublished observations, we noted that the CaCC antagonists niflumic acid and 5-nitro-2-(3-phenylpropylamino)-benzoic acid both relax contractions induced by potassium channel blockade with tetraethylammonium chloride (TEA Cl). In fact, GABA A agonists or CaCC antagonists both induce relaxation at the maintenance phase of a TEA-induced contraction. TEA Cl has long been known to enhance slow wave activity in intact airway smooth muscle tissue, which differs from its effect seen in single myocytes where TEA-induced depolarization is not accompanied by slow waves (27). Clearly, an oscillating mem-
brane potential allows the tissue to rhythmically transition through a range of membrane potentials likely below and above the chloride equilibrium potential, making the behavior of chloride ions more complex and difficult to predict. It is also clear that membrane potentials are not static throughout the tissue and these oscillations are amplified when treated with TEA Cl. In this regard, the contractile agonist, as well as the fluctuating membrane potential during slow wave oscillation, influences ionic flux, which may vary both in magnitude and direction throughout a given slow wave.

Furthermore, chloride handling may not be uniform throughout the cytosol. As has been described for calcium, perhaps chloride channels in the plasma membrane (as well as the SR membrane) form distinct microdomains with individual responses to a local chloride electrochemical gradient. Of particular note is the finding in neuronal cells of GABA<sub>A</sub> channel clustering within distinct membrane regions (3, 24). Perhaps this is analogous to the proximity of Orai1 on the plasma membrane to STIM1 on the SR explaining mechanisms of store-operated calcium entry (2).

An alternative and independent hypothesis to explain the apparent discrepancy involving chloride movement and its potential effects on airway smooth muscle tone stem from the observations that the phenotypes of airway smooth muscle cells are neither homogenous nor static. Over a decade ago, Dr. Julian Solway noticed two distinct subpopulations of airway smooth muscle cells and related them as having distinct contractility profiles (6). Today his findings are proving pertinent as emerging evidence suggests that mesenchymal stem cells exist in the lung that can differentiate into a variety of cell types including smooth muscle and whose phenotypic expression of a classic airway smooth muscle protein marker (smooth muscle myosin heavy chain) is temporally variable (28). It is also known that in the embryonic lung a subset of smooth muscle cells are responsible for a depolarization-induced peristaltic wave that is critical for normal airway branching (18). It is unknown whether this cell type persists in the adult lung but, if present, it could contribute an underlying propagating depolarization through gap junctions that results in alterations in cell membrane potential in adjoining airway smooth muscle cells or across a tissue. Thus we speculate that heterogeneity of electrophysiological properties (i.e., chloride equilibrium potentials, resting membrane potentials, and the magnitude of depolarization by different agonists) of smooth muscle cell types could account for the differential effects of chloride currents mediated by calcium-activated chloride channels vs. ligand-gated chloride channels such as GABA<sub>A</sub>. In fact, Dr. Janssen’s published work also suggests such heterogeneity in that not all airway smooth muscle cells demonstrate Cl<sup>-</sup>-dependent spontaneous transient inward currents (85 of more than 200 cells) (16). In further support of the hypothesis of multiple phenotypes of airway smooth muscle cells we offer the observation that punctuate heterogeneous immunohistochemical staining of GABA<sub>A</sub> channels occurs across an airway smooth muscle layer as opposed to homogeneous staining over all airway smooth muscle cells. There is evidence suggestive of heterogeneous staining of β2/β3 subunit containing GABA<sub>A</sub> channels in the airway smooth muscle layer when closely examining the higher resolution immunohistochemical images we have already published (Fig. 5 in Ref. 23).

Although the relative importance of plasma membrane potential in the initiation and maintenance of contraction of an airway smooth muscle cell has been controversial (11, 19), we believe this has occurred in large part because of the lack of efficacy of clinical trials of L-type calcium channel antagonists (1). Given recent data, however, one has to question whether the baby has been thrown away with the bath water, perhaps dismissing the importance of membrane potential because of the limitations of L-type calcium channel blockade may be overly premature.

Good evidence exists that membrane potential plays an important modulatory role in airway smooth muscle contractility including 1) contraction induced by blockade of potassium channels (e.g., tetraethylammonium chloride, iberiotoxin), 2) voltage-gated T-type calcium channels contribution to increased tension (10, 29), and 3) traditional G protein-activated calcium signaling pathways are regulated by membrane potential. This exciting last observation follows from several recent studies that demonstrate membrane potential modulates both M3 muscarinic (G<sub>q</sub>-coupled) receptors (21) and M2 muscarinic (G<sub>i</sub>-coupled) receptors (4) independent of receptor occupancy by their endogenous ligands (4, 20) and that the G<sub>q</sub>-PLC-inositol phosphate calcium signaling pathway is coupled to membrane potential (9, 22). Additionally, membrane potential may also play a role in calcium sensitization by regulation of Rho kinase activity (20). Depolarization induced by electric field stimulation (25) or pharmacologically (i.e., potassium chloride) (17) have both demonstrated increases in Rho kinase activity and increases in muscle force generation. Thus it is clear that we have much to learn about chloride handling in smooth muscle cells, the possible heterogeneity within smooth muscle subtypes, and the myriad of signaling events beyond ion channels that are regulated by membrane potential.

REFERENCES


