Airway smooth muscle electrophysiology in a state of flux?

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A recent study (4) published in the American Journal of Physiology described a rather surprising physiological response in airway smooth muscle (ASM): GABA activating a Cl\(^-\) flux in ASM cells and yet directly relaxing isolated ASM tissues and augmenting β-agonist-evoked relaxation. This is puzzling; but to explain why this is so, a quick summary of ASM electrophysiology and excitation-contraction (EC) coupling is needed (see Fig. 1).

A wide variety of bronchoconstrictors release Ca\(^{2+}\) sequestered within the sarcoplasmic reticulum (SR), which in turn activates very small-conductance Ca\(^{2+}\)-dependent Cl\(^-\) channels (Fig. 1B) (6, 9). The latter are generally taken to be responsible for the depolarization that accompanies excitation, but concurrent suppression of K\(^+\) currents and activation of other nonselective cation conductances may also contribute. There is a long-standing dispute about the degree to which these electrophysiological changes, and the consequent voltage-dependent Ca\(^{2+}\) influx, are required for contraction [reviewed in greater detail elsewhere (10)]. Bronchodilators, on the other hand, generally activate K\(^+\) channels and hyperpolarize the membrane; however, given the uncertainty pertaining to the dispute just mentioned, this may be more of an epiphenomenon (Fig. 1A), and other cellular events may be more important for the relaxation [e.g., Ca\(^{2+}\) homeostatic mechanisms that decrease intracellular calcium concentration ([Ca\(^{2+}\)]\(_i\)) (Fig. 1A); stimulation of myosin light chain phosphatase; inhibition of myosin light chain kinase].

In this context, it is puzzling that GABA activates a Cl\(^-\) conductance and yet elicits bronchodilation (4). This brings to mind another high-profile and equally puzzling study published elsewhere (3) that showed that agonists of bitter taste receptors evoke substantial relaxation through a mechanism that is dependent on release of internal Ca\(^{2+}\). Opening of Cl\(^-\) channels in ASM is expected to cause depolarization (which is associated with contraction rather than relaxation); likewise, elevation of [Ca\(^{2+}\)]\(_i\) is expected to lead to contraction, not relaxation. Are these expectations unfounded, or are there gaps in our current understanding of ASM electrophysiology and EC coupling that do not accommodate these novel ionic data? Sometimes, it’s when the pieces of the puzzle don’t quite fit well together that one is forced to go back a few steps, rethink the whole problem, and hopefully come up with a better explanation.

First, how is Cl\(^-\) handled within the ASM cell? More specifically, what are the actual physiological concentrations of Cl\(^-\) in ASM at rest as well as during excitation or relaxation? This is not a trivial question. The majority of patch-clamp studies use chloride salts of the major cations, fixing Cl\(^-\) concentration at ~120–160 mM on either side of the membrane. This artificially sets the Cl\(^-\) equilibrium potential (E\(_{Cl}\)) at ~0 mV; under those conditions, opening of Cl\(^-\) channels can only provide a depolarizing influence. In the native cell, however, proteins and other molecules account for a substan-

Fig. 1. A: bronchodilators stimulate the sarcoplasmic reticulum (SR) to both take up Ca\(^{2+}\) from the deep cytosol and also to release it toward the plasmalemma where it is extruded; Ca\(^{2+}\)-dependent K\(^+\) channels may also be activated. B: bronchoconstrictors cause Ca\(^{2+}\) release from the SR, leading to depolarization (via Ca\(^{2+}\)-dependent Cl\(^-\) channels) and contraction. C: large Cl\(^-\) fluxes at the membrane can draw Cl\(^-\) out of the SR, which in turn would enhance release of Ca\(^{2+}\). D: changes in Cl\(^-\) concentration, membrane potential (E\(_{mem}\)), and/or intracellular Ca\(^{2+}\) concentration ([Ca\(^{2+}\)]\(_i\)) may also modulate RhoA activity. E: accumulation of Na\(^+\) in the subplasmalemmal space (via nonselective cation channels) can stimulate Ca\(^{2+}\) influx via reverse-mode Na\(^+\)/Ca\(^{2+}\) exchange activity. Ryanodine receptors on the SR normally serve to release stored Ca\(^{2+}\) (F), but when the SR becomes depleted [e.g., by abolition of Ca\(^{2+}\) uptake; cyan lines (G)], those same channels can serve as a pathway for Ca\(^{2+}\) entry into the depleted SR.
tial portion of the negative charges in the cytosol, and Cl\(^{-}\) concentration must of necessity be much lower. As such, the physiological value for \(E_{Cl}\) must be more negative, possibly falling within the range of potentials circumscribed by electrical slow waves orchestrated by alternating Ca\(^{2+}\)-dependent Cl\(^{-}\) currents, voltage-dependent Ca\(^{2+}\) currents, and various K\(^{+}\) currents [oscillating \(-10\) mV on either side of \(-35\) mV (12, 13)]. If so, opening of Cl\(^{-}\) channels can either depolarize or hyperpolarize, depending on the continuously changing membrane potential (\(E_m\)). In addition to modulating \(E_m\), Cl\(^{-}\) efflux may also modulate Ca\(^{2+}\) release from the SR (6, 8). That is, Cl\(^{-}\) channels are also present on the SR membrane and serve to short-circuit the accumulation of charge across the SR membrane during Ca\(^{2+}\) release or Ca\(^{2+}\) uptake (8, 17); as such, a sudden and profound Cl\(^{-}\) flux out of the cell could cause a transient shift in \(E_m\) across the membrane of the internal Ca\(^{2+}\) store, thereby briefly increasing the driving force on Ca\(^{2+}\) out of the SR (a form of “turbocharging” Ca\(^{2+}\) release; Fig. 1C).

Second, what exactly is the relationship between \(E_m\) and mechanical activity? This relationship is direct in cardiac muscle and several types of smooth muscle (gastrointestinal, vascular, lymphatic); depolarization promotes opening of voltage-dependent Ca\(^{2+}\) channels, Ca\(^{2+}\) influx, and elevation of [Ca\(^{2+}\)]i, (possibly with some amplification of the latter via Ca\(^{2+}\)-induced Ca\(^{2+}\) release from the SR), which in turn activates the contractile apparatus. This explains why, in those diverse muscle preparations, constrictor and relaxant stimuli act in large part through depolarization or hyperpolarization, respectively, and why blockers of voltage-dependent Ca\(^{2+}\) channels are so useful in pathological conditions that involve those muscle types. Contraction in ASM under otherwise normal conditions (please see next paragraph), however, is relatively unaffected during voltage clamp at negative potentials (14), or in the presence of blockers of voltage-dependent Ca\(^{2+}\) channels (2) or even outright removal of external Ca\(^{2+}\) (at least until the internal Ca\(^{2+}\) store becomes depleted). Instead, bronchoconstriction is mediated in large part by 1) increased [Ca\(^{2+}\)]i (brackets denote concentration) via the generation of a series of Ca\(^{2+}\) waves from the SR, the frequency of which determines the degree of contraction (16); and 2) increased Ca\(^{2+}\) sensitivity of the contractile apparatus via RhoA/ROCK- and/or PKC-mediated suppression of myosin light chain phosphatase activity (19).

It is important to emphasize the caveat “under otherwise normal conditions” in the paragraph above: ASM tissues that have been pretreated with cyclopiazonic acid or ryanodine (to deplete the internal Ca\(^{2+}\) store) subsequently act like the other muscle types in that they become exquisitely sensitive to inhibitors of voltage-dependent Ca\(^{2+}\) channels (20). It seems, then, that those channels somehow modulate changes in Ca\(^{2+}\) sensitivity (the Ca\(^{2+}\) release pathway being obviated under these conditions), a suggestion we have made in regards to RhoA/ROCK: it may be that translocation of RhoA to the membrane (an essential step in its activation) is facilitated by depolarization, ionic changes occurring just under the membrane, and/or direct interactions between RhoA and voltage-dependent ion channels (Fig. 1D) (15). Another voltage-dependent Ca\(^{2+}\) influx pathway involves Na\(^{+}\)/Ca\(^{2+}\) exchange (NCX). The latter is fully reversible and the direction in which it translocates Ca\(^{2+}\) is determined by the transmembrane gradients for [Na\(^{+}\)], [Ca\(^{2+}\)], and voltage (6). Ca\(^{2+}\) influx is favored at more depolarized potentials and/or when cytosolic [Na\(^{+}\)] rises (18). Both of those changes occur during excitation of ASM as a result of opening of nonselective cation channels (6, 7, 18) (Fig. 1E). Slow wave activity sweeps \(E_m\) back and forth across the reversal potential for NCX, allowing the latter to alternate between the forward and reverse modes and to be exquisitely modulated by subtle changes in cytosolic [Na\(^{+}\)]i and [Ca\(^{2+}\)]i (6). There may be many other cellular events related to EC coupling that are sensitive to changes in \(E_m\) and/or \(E_{Cl}\).

Third, what exactly is the relationship between changes in [Ca\(^{2+}\)]i and mechanical activity? It has long been recognized that elevation of [Ca\(^{2+}\)]i, is a key event underlying excitation and contraction. Methodological advances, particularly confocal fluorimetry, have steered us away from the misleading preoccupation with amplitudes (height of the initial spike or of the ensuing “plateau” seen in large populations of cells) and toward the real issue: Ca\(^{2+}\) wave frequency in individual cells (16). Those same advances helped explain earlier confusing reports that [Ca\(^{2+}\)]i can also be elevated by bronchodilators (3, 22). It is now known that the SR forms sheets near the plasmalemma, thereby creating a narrow, diffusionally constrained space between the two types of membrane and dividing the cytosol into two functionally distinct spaces and separating electrical and mechanical activities (Fig. 1) (11). As such, bronchoconstrictors release stored Ca\(^{2+}\) toward the deep cytosol [via inositol 1,4,5-trisphosphate (IP\(_3\)) receptors] and activate the contractile apparatus (Fig. 1B), whereas bronchodilators might release Ca\(^{2+}\) toward the plasmalemma (via ryanodine receptors) and Ca\(^{2+}\) extrusion mechanisms (the plasmalemmal Ca\(^{2+}\) pump and NCX) (21), in the process also activating membrane conductances such as Ca\(^{2+}\)-dependent K\(^{+}\) channels (Fig. 1A). This structural arrangement may also explain the puzzling finding that activation of voltage-dependent Ca\(^{2+}\) channels can mediate SR refilling in ASM tissues pretreated with cyclopiazonic acid to inhibit the SR-Ca\(^{2+}\) pump (SERCA) (1, 14). Innumerable studies of Ca\(^{2+}\) uptake in a wide variety of cell types have all shown SERCA to be the only type of Ca\(^{2+}\) pump on the SR, and no study has identified a SERCA that is insensitive to cyclopiazonic acid. As such, it seemed that Ca\(^{2+}\) somehow transitions from the extracellular space directly into the SR, bypassing SERCA altogether, through some unknown SR entry pathway. Our laboratory is now considering the possibility that this entry pathway comprises ryanodine receptors, which have otherwise traditionally been viewed simply as a Ca\(^{2+}\) release pathway. It may be that entry of external Ca\(^{2+}\) into the diffusionally constrained space formed by the “superficial buffer barrier” would raise [Ca\(^{2+}\)]i, to a level well above that within the depleted SR. With the Ca\(^{2+}\) concentration gradient across the SR membrane reversed in this way, opening of any kind of Ca\(^{2+}\)-permeable pathway on the SR would provide a route for that subplasmalemmal Ca\(^{2+}\) to enter the depleted SR in retrograde fashion (compare Fig. 1F and 1G). Ryanodine receptors are activated by cytosolic Ca\(^{2+}\) and highly conductive to Ca\(^{2+}\) (10-fold more so than IP\(_3\)-gated ones) and thus could represent such a SERCA-independent Ca\(^{2+}\) entry pathway into the SR.

Where do we go from here? Clearly, we need to reconsider the role(s) of Cl\(^{-}\) channels, \(E_{Cl}\), voltage-dependent Ca\(^{2+}\) channels and release of internal Ca\(^{2+}\) in ASM EC coupling. A more informed approach will require a knowledge of the normal,
physiological concentrations of Cl\textsuperscript{−} in ASM at rest and during excitation, the distribution of Cl\textsuperscript{−} channels on the SR vs. the plasmalemma, and the relative distributions of ryanodine receptors and IP\textsubscript{3}-gated channels on the inward and outward faces of the SR sheets. That more informed approach should also take into account the degree to which there can be cytosolic compartmentalization of Cl\textsuperscript{−}; regional heterogeneity has certainly been shown for Ca\textsuperscript{2+} [e.g., sparks and puffs; the superficial buffer barrier (22)], for Na\textsuperscript{+} and H\textsuperscript{+} with use of ion-selective dyes, but not for Cl\textsuperscript{−} (although Cl\textsuperscript{−}-sensitive dyes are available). Another gap in our knowledge is whether intracellular effectors such as the monomeric G protein RhoA or myosin light chain phospha...