BK channel β₁-subunit regulation of calcium handling and constriction in tracheal smooth muscle

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BK channels have a very large outward K⁺ conductance (200 pS) and, therefore, very effectively hyperpolarize the membrane. As such, they are important regulators of membrane voltage in a number of cell types (6, 15, 20).

BK channels bind Ca²⁺ at low affinity (~10 μM) (9, 28) and require colocalization with a Ca²⁺ source or the contribution of voltage to open the channel at physiological Ca²⁺ concentrations (8, 30, 31). In vascular smooth muscle, large voltage changes do not occur, and BK channels have an enhanced apparent Ca²⁺ sensitivity that is associated with a member of the accessory BK channel β₁-subunit family (β₁- to β₄-subunits), the β₁-subunit (23, 42). The important role of the β₁-subunit was previously demonstrated by targeted gene knockout of the β₁-subunit locus in mice. Knockout mice demonstrated BK channels with reduced opening in vascular smooth muscle and increased vascular tone and hypertension (4, 37). Similarly, the β₁-subunit has been shown to have an important role in vasodilator and colon smooth muscle tone (16, 36).

In airway smooth muscle, constriction generally occurs after receptor-coupled activation of inositol trisphosphate (IP₃) receptors, which releases Ca²⁺ from internal Ca²⁺ stores. This has been termed pharmacomechanical coupling (41), in contrast to electromechanical coupling, which is mediated through voltage-dependent Ca²⁺ channels. The predominant role of pharmacomechanical coupling in airway smooth muscle is strongly supported by the fact that voltage-dependent Ca²⁺ channel blockers such as nifedipine only partially relax after cholinergically evoked constriction (14) and are poor bronchial dilators themselves (1, 25). Yet, there is considerable evidence that the control of membrane voltage by K⁺ channels is an important factor affecting the relative constriction of airway smooth muscle. For example, depolarization of airway smooth muscle with KCl causes constriction (47), and hyperpolarization with K⁺ channel agonists can relax airway smooth muscle (33). In addition, most of the effects of the most common acute treatments for asthma, the β-adrenergic agonists, are due to activation of BK channels (24). Iberiotoxin, a highly specific BK channel blocker, reduces the bronchodilator effect of β-adrenergic agonists (7). It has frequently been hypothesized that BK channels may serve an important role in controlling bronchial constriction and are potential pharmaceutical targets for regulation of airway smooth muscle constriction during asthma (17). Yet the role of K⁺ channels in general, and BK channels in particular, in Ca²⁺ signaling in airway smooth muscle is poorly understood. Here, we have utilized the β₁-subunit knockout to evaluate the role of the BK channel β₁-subunit in tracheal smooth muscle constriction during cholinergic signaling. We show that modulation by the β₁-subunit has effects on airway constriction and that these effects are mediated by regulation of voltage-dependent Ca²⁺ channels.

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IN MANY SMOOTH MUSCLE CELLS, voltage-dependent Ca²⁺ channels are the major conduits for Ca²⁺-mediated constriction (21). K⁺ channels generally function to oppose constriction by hyperpolarizing membranes, which deactivates voltage-dependent Ca²⁺ channels and reduces Ca²⁺ influx. Among these, the large-conductance Ca²⁺-activated K⁺ (BK) channels are broadly expressed and have been studied in many cell types. These channels are composed of a pore-forming α-subunit and a tissue-specific β-subunit. Binding of intracellular Ca²⁺ and depolarizing voltage gate BK channels to open. When open, BK channels have a very large outward K⁺ conductance (>200 pS) and, therefore, very effectively hyperpolarize the membrane. As such, they are important regulators of membrane voltage in a number of cell types (6, 15, 20).

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C57BL/6 line of Jackson Labs (strain C57BL/6J) and maintained as homozygous lines. Control animals used in these studies also were the background C57BL/6 mice strain from Jackson Labs. All animal procedures were reviewed and approved by the University of Texas Health Science Center at San Antonio Institutional Animal Care and Use Committee. For tracheal constriction studies, animals were deeply anesthetized with isoflurane and then killed by cervical dislocation. The trachea was quickly removed and then dissected clean of surrounding tissues in ice-cold normal physiological saline solution (PSS). The tracheal tube was cut below the pharynx and above the primary bronchus bifurcation. Two metal wires (attached to a force transducer and a micrometer) were threaded into the lumen of the trachea, and the trachea was placed in an oxygenated (95% O2-5% CO2, pH 7.35 at 37°C) organ bath. Resting tension was continuously readjusted to 1 g for 1 h and then challenged with high-K+ PSS solution (KCl substituted for NaCl on an equimolar basis) until a reproducible constriction response was obtained. Subsequent experimental challenges with drugs were normalized to the constriction response in the high-K+ PSS solution. In high-K+ PSS, the K+ reversal potential is depolarized; therefore, K+ currents are unlikely to play a role in controlling membrane voltage and tone. This was consistent with our finding of no significant difference in the K+ evoked constriction between wild-type and knockout mice (n = 14).

Normal PSS consisted of (mM) 119 NaCl, 4.7 KCl, 1.18 KH2PO4, 1.17 MgSO4·7H2O, 18 NaHCO3, 0.026 EDTA, 11 glucose, and 12.5 sucrose. For the high-K+ PSS, 56.7 mM NaCl was replaced with equimolar KCl and all other salts were unchanged.

Isolation of tracheal myocytes. The trachea was isolated as described above. The dorsal muscle layer was cut away from the hyaline cartilage rings and minced into ~1-1 mm pieces in Ca2+-free HEPES-buffered Krebs-BSA solution (140 mM NaCl, 4.7 mM KCl, 1.13 mM MgCl2, 10 mM HEPES, 10 mM HEPES, and 1 mg/ml BSA fraction V, pH 7.3). After addition of 2.5 U/ml papain (MP Biomedicals) and 1 mg/ml dithiothreitol, the cells were dissociated at 37°C on a rocking platform for 20 min. The tissue was washed once with the Ca2+-free Krebs solution and digested with 12.5 U/ml of type VII collagenase (Sigma Chemical) for 10 min at 37°C. The tissue was washed three times in Ca2+-free Krebs-BSA solution and gently triturated to disperse single tracheal myocytes. Tracheal myocytes were stored on ice in Ca2+-free Krebs-BSA solution and used on the same day.

Measurement of BK channel activity. Isolated tracheal myocytes were loaded with fura 2-AM (Invitrogen, Carlsbad, CA) for assay of fluorescence intensity. Excitation wavelengths were selected with a high-speed filter changer (model DG5, Sutter Instrument, Novato, CA) using 100-ms windows for the 340- and 380-nm wavelength excitations and monitoring of the 510-nm emission at a frequency of 1 Hz during the experiment. For calibration of the fura 2 ratio to Ca2+ concentration, we used culture cells with β-escin to make the membrane permeable to Ca2+ and EGTA-buffered Ca2+ calibration solutions purchased from Molecular Probes (Invitrogen, Carlsbad, CA).

RESULTS

Expression of β1-subunit in airway smooth muscle. BK channel activity is readily detected in tracheal and bronchial smooth muscle cells by patch-clamp techniques (40). Biochemical copurification of the β1-subunit from bovine tracheal smooth muscle in a 1:1 ratio with the pore-forming α-subunit has provided direct evidence for the accessory β1-subunit (22, 23). We utilized the β-galactosidase reporter that was gene targeted to the β1-subunit translation initiation site in mice to directly determine the cell type that expresses the β1-subunit gene in airway tissues. Reporter activity is detected in the trachea of gene-targeted animals (Fig. 1, A, C, and E) but not control animals that lack the reporter (Fig. 1, B and D). Figure 1A shows a low-magnification image of β-galactosidase activity in the posterior wall of the trachea (region of muscle between hyaline cartilage rings). Consistent with previous reports showing that β1-subunit gene expression is smooth muscle specific (4, 46), expression in the trachea is observed only in the smooth muscle layers (arrows in Fig. 1, C and E).

Reduced opening of BK channels in β1-subunit-knockout smooth muscle cells. The β1-subunit confers an increased open probability and slow gating kinetics to BK channels expressed in heterologous expression systems (11, 27, 28). Knockout of the β1-subunit causes a dramatic reduction in BK channel openings and faster gating kinetics of a number of smooth muscle tissues (4, 10, 36). However, BK channel α-subunits in different smooth muscle tissues undergo alternative splicing (26, 44, 45), and the functional properties conferred by the β1-subunit can be dependent on the tissue-specific splice product of the α-subunit (12, 38). We investigated the functional consequences of the lack of β1-subunit in tracheal smooth muscle. Figure 2A shows BK single-channel activity after excision in 7 μM Ca2+-buffered internal solution. At −40 and +40 mV, wild-type BK channels showed increased activity compared with knockout BK channels. As shown by composite data in Fig. 2, B and C, wild-type BK channels at +40 mV have an approximately twofold increase in open probability and a twofold increase in open channel dwell time compared with BK channels lacking β1-subunits: 0.83 ± 0.06 vs. 0.44 ± 0.1 open probability and 5 ± 1.7 vs. 2.7 ± 0.7 ms open channel dwell time. In tracheal smooth muscle, membrane potentials generally do not overshoot 0 mV, and cholinergic signaling alters membrane potentials within negative voltage ranges (19). At −40 mV, open probability in the β1-subunit knockout was reduced >10-fold compared with wild-type cells: 2.4 ± 0.76e−2 vs. 0.17 ± 0.07e−2 (P < 0.05; Fig. 2B). These results indicate that BK channel opening is severely perturbed in the β1-subunit-knockout at physiological voltages. The mecha-
nism by which BK channels increase open probability is an increase in the open channel burst duration (30). As expected, channel burst durations are reduced in the β1-subunit knockout compared with wild-type BK channels: 2.1 ± 0.6 vs. 0.54 ± 0.08 ms at −40 mV (P < 0.05; Fig. 2C).

Physiological changes in BK channel activity can be assessed by measurement of spontaneous transient outward currents (STOCs) in nondialyzed cells by perforated patch-clamp recording (34). Transient outward currents are mediated by BK currents after Ca2+ -induced Ca2+ release events (“Ca2+ sparks”), which colocalize with, and activate, BK channels (18). Gene knockout of the pore-forming α-subunit has been shown to eliminate transient outward currents in bladder and vascular smooth muscle (29, 39), whereas knockout of the β1-subunit does not eliminate, but reduces the frequency of, spontaneous outward currents in vascular smooth muscle (4, 37). In tracheal smooth muscle, we see a similar effect. At −40 mV, BK current activity is significant in wild-type cells (6 of 6 cells at −40 mV; Fig. 3A), whereas β1-subunit-knockout smooth muscle cells show no or very little activity (0 of 5 cells at −20 mV; Fig. 3B). Nevertheless, as in the single-channel measurements (Fig. 2B), given sufficient depolarization, β1-subunit-knockout airway smooth muscle demonstrates spontaneous transient BK currents (Fig. 3A), although at a lower frequency. Composite data for frequency are shown in Fig. 3B: over the whole range of holding potentials, the knockout cells exhibited significantly lower transient BK currents: 487 ± 112 vs. 113 ± 29 STOCs/min at +20 mV (P < 0.01).

The effects of the β1-subunit knockout on STOC amplitudes and areas (Fig. 3, C and D) were less dramatic than the effects on frequency (Fig. 3B). Given that the STOC amplitude should represent the number of channels opening after a spark event (50), these data suggest that β1-subunit knockout causes a somewhat smaller recruitment of BK channels during a Ca2+ spark event (Fig. 3C). In addition, wild-type tracheal BK channels appear to have a tendency toward more sustained opening, as indicated by a greater STOC area (Fig. 3D). This is consistent with the longer open dwell times of wild-type BK channels (Fig. 2C). In summary, these results indicate that BK channel activity is significantly greater in wild-type than in β1-subunit-knockout trachea and, therefore, would be expected to play a greater role at physiological voltages in controlling Ca2+ influx and constriction.
Knockout of the β1-subunit increases resting Ca\(^{2+}\) and cholinergically evoked Ca\(^{2+}\) release. In airway smooth muscle, Ca\(^{2+}\) release via IP\(_3\) receptors initiates contraction. After Ca\(^{2+}\) release, contraction is maintained by Ca\(^{2+}\) influx channels (3). Acutely isolated tracheal smooth muscle cells were loaded with fura 2 for determination of the influence of the β1-subunit on Ca\(^{2+}\) influx. In tracheal smooth muscle, we found an ~50 nM increase in resting Ca\(^{2+}\) levels in the β1-subunit knockout: 177 ± 11 vs. 124 ± 0.8 nM (P < 0.01; Fig. 4A, inset). This indicates that BK channels contribute to regulation of basal Ca\(^{2+}\).

During cholinergically evoked excitation, Ca\(^{2+}\) increases from an early phase, which is mainly contributed by Ca\(^{2+}\) release from the sarcoplasmic reticulum (Ca\(^{2+}\) transient; Fig. 4A, component B), to a late sustained phase, which underlies sustained Ca\(^{2+}\) influx [Fig. 4A, components C (early sustained) and D (late sustained)]. Because the β1-subunit knockouts have an increased baseline Ca\(^{2+}\), we quantified the relative Ca\(^{2+}\) increases (subtracted from resting Ca\(^{2+}\)) at different phases of the Ca\(^{2+}\) transient to determine the changes in evoked Ca\(^{2+}\). In addition to the peak transient, we averaged the sustained component 15–17 s (component C) and 77–80 s (component D) after addition of carbachol (see METHODS) in anticipation that the β1-subunit knockout may have different effects on the components of the Ca\(^{2+}\) transients. The major effect of the β1-subunit knockout is an increase in the late sustained component of the Ca\(^{2+}\) influx (component D, 131 ± 16 and 77.2 ± 12 nM Ca\(^{2+}\) in knockout and wild-type cells, respectively, P < 0.005), with no significant effect on the Ca\(^{2+}\) transient and early sustained component (components B and C). In addition, although there was no statistical difference between early sustained components (component C), we saw a greater tendency for Ca\(^{2+}\) oscillations in the β1-subunit knockout than in wild-type cells (12 of 26 vs. 3 of 29 cells; Fig. 4B). Because very few wild-type cells exhibited Ca\(^{2+}\) oscillations, it would not be meaningful to quantify Ca\(^{2+}\) differences between knockout oscillating cells and wild-type cells. Therefore, the oscillating cells were not included in further analysis.

Because a major function of K\(^+\) channels is control of membrane voltage, a reasonable assumption is that increased Ca\(^{2+}\) in the β1-subunit knockouts arises from depolarization and recruitment of voltage-sensitive Ca\(^{2+}\) channels. Therefore, we utilized the L-type voltage-dependent Ca\(^{2+}\) channel blocker nifedipine (1 μM) to study the contribution of these channels to each component of cholinergically evoked Ca\(^{2+}\) transients. Indeed, nifedipine caused a substantial drop in all components of the Ca\(^{2+}\) transient (Fig. 5, A and B) in wild-type and knockout cells. Figure 5C shows that the average response of all components is similar between wild-type and knockout muscle blocked with nifedipine. Particularly important was the observation that nifedipine eliminated the difference in the late sustained Ca\(^{2+}\) influx (component D) between wild-type and β1-subunit-knockout cells (P = 0.22, wild-type vs. knockout; Figs. 4B and 5C). This can also be seen when the relative differences in Ca\(^{2+}\) evoked by carbachol before and after block with nifedipine are measured (Fig. 5, A and B; data are paired...
with the same tracheal cell). Consistent with these results, knockouts show a larger nifedipine-sensitive late sustained component than wild-type trachea cells: 48 ± 5 vs. 120 ± 33 nM (P < 0.02; Fig. 5). A similar result was obtained with perfusion of 0 extracellular Ca^{2+}, achieved with addition of the Ca^{2+} chelator EGTA at 1 mM (Fig. 6, A and B). As in nifedipine experiments, the significant difference between wild-type and knockout Ca^{2+} transients during the late sustained component in 2 mM Ca^{2+} solutions (Fig. 4B) was eliminated with 0 Ca^{2+}: 46 ± 13 and 41 ± 10 nM in wild-type and knockout, respectively (Fig. 6C). The largest change in Ca^{2+} mediated by the β1-subunit is seen during the late sustained component: 43 ± 11 and 93 ± 16 nM in wild-type and knockout, respectively (Fig. 6D). In summary, these results indicate that increased Ca^{2+} in the knockout trachea can be accounted for by increased activation of voltage-dependent Ca^{2+} channels. Also, although the β1-subunit knockout showed an increased frequency of cells with Ca^{2+} oscillations, block of voltage-dependent Ca^{2+} channels or use of 0 Ca^{2+} solutions did not eliminate Ca^{2+} oscillations (data not shown).

Fig. 4. Carbachol (CCh)-induced Ca^{2+} transients in WT and KO tracheal smooth muscle cells. A: fura 2-measured Ca^{2+} transients from WT (○) and KO (●) tracheal smooth muscle cells. Left inset: enlargement of non-baseline-subtracted resting Ca^{2+} (note nM concentration on left and right inset y-axes). Large trace and right inset KO traces are baseline subtracted to adjust KO to the same baseline as WT. Components of Ca^{2+} are labeled as follows: resting Ca^{2+} (A), transient phase (B), early sustained phase (C), and late sustained phase (D). B: fura 2-measured Ca^{2+} transients from KO tracheal smooth muscle cells with oscillatory Ca^{2+} changes. C: summary data comparing average values for different components in WT and KO. Significant difference is seen at resting Ca^{2+} (component A, **P = 0.0004, n = 28 WT and 24 KO) and during sustained phase (component D, P = 0.0062, n = 14 WT and 12 KO). Sustained Ca^{2+} phases (components C and D) were measured at 15–17 and 77–80 s, respectively, after addition of carbachol. Cells with oscillatory Ca^{2+} transients were excluded from these analysis.

Fig. 5. Effect of nifedipine on carbachol-induced Ca^{2+} transients. A and B: traces of carbachol-induced Ca^{2+} transients before (○) and after nifedipine block of voltage-gated Ca^{2+} channels (●) for WT and β1-subunit-KO cells. C: summary data comparing average values for different components in WT (open bars) and KO (gray bars) during Ca^{2+} channel block with nifedipine. No significant difference is seen with any component. D: summary of relative change of different components calculated as Ca^{2+} concentration after nifedipine subtracted from Ca^{2+} concentration before nifedipine (Δ[Ca^{2+}]). Values are means ± SE from paired experiments (n = 13 WT and 11 KO). *P = 0.025.
BK channel β1-subunit knockout increases tracheal constriction. Figure 7A shows cholinergically evoked constrictions normalized to tracheal responses in high-K⁺ conditions. Consistent with the expectation that BK channels control airway constriction, the β1-subunit-knockout mice have a larger carbachol-induced constriction than the wild-type animals. Summary dose-response curves (Fig. 7B) indicate that the main effect of the β1-subunit knockout is an increase in the maximal response, rather than a shift in the dose-response curve. Although some knockouts showed Ca²⁺ oscillations, we did not see oscillation in carbachol-induced constrictions.

Increased constriction of β1-subunit knockouts is accounted for by increased contribution of voltage-dependent Ca²⁺ channels during sustained contractions. The most likely role of BK channels is opposition of membrane depolarization during agonist-induced constriction. A reasonable hypothesis is that membrane depolarization recruits voltage-dependent Ca²⁺ channels and increases Ca²⁺ influx and constriction. Consistent with this hypothesis, we found that the β1-subunit-knockout mice relax to the same relative constriction as wild-type animals when treated with nifedipine [Fig. 7A, right, and 7B (dose-response + nifedipine)]. This indicates that the increased...
constriction of the knockout mice is largely accounted for by increased Ca\(^{2+}\) influx through L-type voltage-dependent Ca\(^{2+}\) channels.

It is fairly well established that two sources of Ca\(^{2+}\) underlie tracheal constriction: an early transient component, which is dependent on agonist-induced Ca\(^{2+}\) release from endoplasmic reticulum Ca\(^{2+}\) stores, and a subsequent sustained component, which is dependent on Ca\(^{2+}\) influx from plasma membrane channels. Consistent with the nifedipine experiments described above (Fig. 5), the \(\beta_1\)-subunit knockouts show the greatest effect on constriction during the sustained component, where voltage-dependent Ca\(^{2+}\) channels are expected to play a role (Fig. 7A). Nifedipine block of voltage-dependent Ca\(^{2+}\) channels results in similar constriction during the sustained component in knockout and wild-type cells (Fig. 7, A and B).

In vascular smooth muscle, block of BK channels results in similar levels of constriction in wild-type and \(\beta_1\)-subunit-knockout cells (4). This indicates that, without the \(\beta_1\)-subunit, BK channels cannot control constriction. In tracheal smooth muscle, the effect was similar. There was no significant difference in cholinergically evoked constriction in knockout trachea in the presence or absence of paxilline block (Fig. 7, D and E), indicating that BK channel activity does not significantly contribute to relaxation in the absence of the \(\beta_1\)-subunit. In contrast, wild-type trachea shows a significant difference with and without paxilline block (Fig. 7, C and E).

**DISCUSSION**

Airway smooth muscle presents a medically important model for studying the role of ion channels during receptor-coupled activation of Ca\(^{2+}\) transients. In contrast to vascular smooth muscle, where the voltage-dependent Ca\(^{2+}\) channel is a major Ca\(^{2+}\) conduit mediating contraction, the role of voltage-dependent Ca\(^{2+}\) channels and other Ca\(^{2+}\) influx pathways in airway smooth muscle is poorly defined. Therefore, pharmaceuticals targeted to ion channels in the airway have not been utilized to treat asthma.

These data demonstrate that BK channels in \(\beta_1\)-subunit-knockout airway smooth muscle exhibit a large reduction in channel openings as indicated by single-channel activity and STOC frequency. As a consequence, the BK channel contribution to relaxation is perturbed sufficiently that pharmacological block of BK channels shows that same relative constriction as the \(\beta_1\)-subunit-knockout trachea (Fig. 7, C–E). This is similar to vascular smooth muscle, in which iberiotoxin-induced block of wild-type vessels induced constriction but had no further contractile effect on \(\beta_1\)-subunit-knockout vessels (4). This is in contrast to bladder smooth muscle, where the \(\beta_1\)-subunit knockout shows a partial effect on constriction compared with iberiotoxin block (36). A distinction between tonic smooth muscles, such as vascular and tracheal muscle, and bladder smooth muscle, which is phasic in excitation, is that large depolarizations in bladder smooth muscle are likely to complement BK activation in the \(\beta_1\)-subunit knockout. Indeed, we see much smaller differences in single-channel and STOC activity of wild-type and knockout cells at depolarizing voltages.

Tao et al. (43) demonstrated that cholinergically evoked constriction in bovine airway is largely independent of voltage-activated Ca\(^{2+}\) channels unless sarcoplasmic reticulum Ca\(^{2+}\) stores are depleted or BK channels are blocked. In the latter case, the mode of contraction coupling switches to an excitation, voltage-dependent coupling. Our findings utilizing the \(\beta_1\)-subunit knockout in mouse trachea indicate more of a partial contribution, rather than a switch in coupling, between pharmacologically evoked and excitation-evoked contraction.

In wild-type mice, voltage-dependent Ca\(^{2+}\) channels appear to contribute \(\sim 17\%\) of the carbachol-induced constriction at saturating carbachol doses (Fig. 7B). In the absence of the \(\beta_1\)-subunit, BK channel activity decreases and the contribution of voltage-dependent Ca\(^{2+}\) channels increases to \(\sim 30\%\) of the constriction (Fig. 7B), which is smaller than the contribution of \(\beta_1\)-subunits in aortic smooth muscle. Pluger et al. (37) reported that maximal contraction is increased \(\sim 65\%\) by the \(\beta_1\)-subunit knockout. Thus BK channels make a significant, but smaller, contribution to agonist-induced relaxation in tracheal smooth muscle than in vascular muscle.

The Ca\(^{2+}\) pathways downstream of voltage-dependent Ca\(^{2+}\) channels that are regulated by BK channels are likely to be complex. The \(\beta_1\)-subunit knockout increased resting Ca\(^{2+}\) levels and increased the sustained component of Ca\(^{2+}\) influx during cholinergic evoked signaling. These consequences were translated to increased cholinergically evoked constriction. A conclusion that may be drawn is that the regulation of membrane voltage by the BK channel is important, although less apparent, until these channels are blocked by deletion of the \(\beta_1\)-subunit. The hyperpolarizing activity of BK channels is likely to hold membrane voltage during cholinergic contractions below the operational voltages at which voltage-dependent Ca\(^{2+}\) channels and excitation-contraction coupling contribute to airway constriction (3). This may reconcile the fact that voltage-dependent Ca\(^{2+}\) channels are regarded to provide a minority of the Ca\(^{2+}\) for contraction (19), yet the K\(^+\) channels would have an important role such as that observed during \(\beta\)-adrenergic-mediated relaxation.

An interesting finding was the high frequency of oscillatory Ca\(^{2+}\) changes after cholinergic activation in cells from \(\beta_1\)-subunit-knockout mice (Fig. 4B). However, we did not see a similar effect on constriction, which was entirely tonic in response to high K\(^+\) or cholinergically evoked constriction. Thus it is unclear how the \(\beta_1\)-subunit knockout-induced Ca\(^{2+}\) oscillations influence the muscle contractile properties in the trachea. In other examples, perturbing BK channels promote oscillations in the trachea. Oscillatory contractions have been described for guinea pig tracheal smooth muscle cells treated with the BK channel blockers charybdoxin and iberiotoxin (48, 49). However, these studies did not investigate Ca\(^{2+}\) changes that may underlie the oscillatory contractions. In more recent studies in bronchioles, slow oscillatory Ca\(^{2+}\) transients have been observed in response to depolarization with K\(^+\) (35). This may be considered analogous to BK channel block or the \(\beta_1\)-subunit knockout, because blocking of K\(^+\) channels presumably depolarizes the cell. However, slow Ca\(^{2+}\) oscillations were correlated with twitching, rather than constriction, of the airway (35).

Because the Ca\(^{2+}\) oscillations are not affected by nifedipine or 0 external Ca\(^{2+}\), the \(\beta_1\)-subunit may have other effects in addition to their actions on membrane voltage or Ca\(^{2+}\) influx through plasmalemma channels. The increase in resting Ca\(^{2+}\) concentration in the \(\beta_1\)-subunit knockout could play a role. Increases in global Ca\(^{2+}\) have been shown to promote sarco-
plasmic reticulum Ca$^{2+}$ loading and, thereby, sustain Ca$^{2+}$ release (5). As well, increases in cytosolic Ca$^{2+}$ concentrations and sarcoplasmic reticulum Ca$^{2+}$ stores promote IP$_3$ receptor activation and Ca$^{2+}$ oscillations (2, 13). Indeed, the mechanism by which knockout of the $\beta_1$-subunit causes Ca$^{2+}$ oscillations and perhaps affects other currents requires further study.

In summary, these findings show that tracheal smooth muscle requires the accessory $\beta_1$-subunit for promotion of normal BK channel activation. Despite the predominant role of agonist-induced signaling in the trachea, BK channels have a significant role in controlling resting Ca$^{2+}$ and cholinergically evoked Ca$^{2+}$ influx contributed by voltage-dependent Ca$^{2+}$ channels.

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