Involvement of the Neurokinin-2 Receptor in Airway Smooth Muscle Stretch-Activated Contractions Assessed in Perfused Intact Bovine Bronchial Segments


Firestone Institute for Respiratory Health, Father Sean O’Sullivan Research Centre, and Department of Medicine, McMaster University, St. Joseph’s Hospital, Hamilton, Ontario, Canada

Received May 16, 2008; accepted August 20, 2008

ABSTRACT

The airway response to deep inspirations (DIs) in asthmatics has been shown to be ineffective in producing bronchodilation and can even cause bronchoconstriction. However, the manner by which a DI is able to cause bronchoconstriction remains ambiguous. We sought to investigate the pathway involved in this stretch-activated contraction and whether this contraction is intrinsic to airway smooth muscle (ASM). In brief, intact bovine bronchial segments were dissected, and side branches were ligated and then mounted horizontally in an organ bath. Intraluminal pressure was measured under isovolumic conditions. Instantaneously opening and then closing the tap on a column of fluid 5 to 30 cm high evoked a sudden increase in intraluminal pressure (equivalent to the height of the column of fluid) followed by a stress relaxation response of the ASM. When tissues were stimulated with carbachol (10⁻⁸ M) or serotonin (10⁻⁷ M) for 10 min, and the consequent agonist-evoked pressure response was dissipated manually, the response to the same transmural stretch was accompanied by a slowly developing and prolonged increase in intraluminal pressure. This stretch-activated response was significantly diminished by the stretch-activated cation channel blocker gadolinium (10⁻³ M), the L-type Ca²⁺ channel blockers nifedipine (2 × 10⁻⁶ M), diltiazem (10⁻⁵ M), and verapamil (10⁻⁵ M), the sensory neurotoxin capsaicin (10⁻⁶ M), and the neurokinin (NK₂) receptor antagonists MEN 10376 ([Tyrᵦ,ᵦ-Trp₆,₈,₉,Lys₁₀]-NKA(4–10)) (10⁻⁵ M) and SR48968 (N-[25]-4-(4-acetamido-4-phenylpiperidin-1-yl)-2-(3,4-dichlorophenyl)butyl]-N-methylbenzamide) (3 × 10⁻⁶ M). These results show the ability of isolated airways to exhibit stretch-activated contractions and suggest a role for stretch-activated cation channels, sensory afferent neurons, the neurotransmitter NKA, and L-type Ca²⁺ channels in these isolated airway responses.

Mechanotransduction, defined as the conversion of mechanical stress into biochemical information, is essential to the proper functioning of cells and organ systems (Hallworth, 1995). In the vasculature, blood pressure is strictly regulated by signaling pathways that respond to mechanical stress to ensure the precise control of blood flow under physiological conditions ranging from vigorous exercise to complete rest (Folkow, 1990). In 1902, Bayliss performed experiments using dog hindlimb, which showed blood vessels responding to increased transmural pressure by constricting. This phenomenon was later termed a myogenic response because it was an intrinsic property of the vascular smooth muscle independent of neural, metabolic, or hormonal input (Davis and Hill, 1999). Similar to the vasculature, the airways are also constantly subjected to mechanical stress because of the inflation and deflation of the lungs. This stress produces both relaxant and constrictor responses in airway smooth muscle (ASM) (Maksym et al., 2005). Thus, airway stretch is suggested to be either beneficial (bronchodilatory) in healthy individuals or harmful (leading to airway hyperresponsiveness) in asthmatics.

A deep inspiration (DI) is clinically measured as a breath taken from functional residual capacity to total lung capacity (TLC). DIs produce bronchodilation in nonasthmatic individuals, whereas in asthmatics, they do not convey this protective effect and can even cause bronchoconstriction (Lim et al., 2008).
The mechanisms by which a DI is able to cause bronchoconstriction remain ambiguous. One suggestion is that smooth muscle activation and tension generation cause an increase in ASM stiffness to the point where it stretches little during a DI. This can subsequently cause the ASM to enter a frozen state, where it stays in a high-stiffness, low-hysteresis latch state (An et al., 2007). Another theory suggests that a DI-induced bronchoconstriction is a peripheral parenchymal hysteresis-associated event, related to the lung pressure-volume hysteresis curve. After lung inflation to TLC during a DI, the lower recoil pressures during deflation at any given volume can lead to smaller airways than before the DI was performed because of unloading of the ASM, which narrows the airways more than it would have otherwise (Lim et al., 1987). Because the failure of bronchodilation after a DI depends on the degree of airway obstruction in asthma, severe asthmatics lose more than they gain when performing a DI. However, the airway inflammation and remodelling present in asthmatic airways may also add to the increased ASM contractility after stretch, by the release of stimuli that can prime the contractile apparatus to react excessively in the presence of stretch. Passive sensitization to IgE has been shown to unmask stretch-activated contractions in human airways in vitro (Mitchell et al., 1997), suggesting a role for inflammatory mediators.

In vascular smooth muscle, stretch-activated contractions are mediated in part through the release of substance P (SP) from sensory neurons (Scotland et al., 2004). ASM tone is regulated in part by a subset of myelinated and unmyelinated sensory nerves, such as slowly adapting and rapidly adapting pulmonary stretch receptors and C-fiber receptors. C-Fibers terminate in the airway epithelium and in proximity to the ASM deep within the submucosa. These are nociceptive and respond to many of the mediators released by tissue damage. They are also polymodal and respond to both mechanical and chemical stimuli such as the sensory neurotoxin capsaicin (Widdicombe, 2003). Capsaicin mediates its excitatory effects by binding to the vanilloid receptor, transient receptor potential vanilloid 1 (Guo et al., 1999; Gunthorpe et al., 2002). When activated, C-fiber receptors release sensory neuropeptides, including SP and neurokinin (NK) A, both of which can exert a bronchoconstrictor response (Joos et al., 2000).

Recent single cell and tissue bath studies have shown that ASM per se can contract in response to stretch (Noble et al., 2004; Maksym et al., 2005). These responses are mediated by the opening of mechanically gated stretch-activated cation channels (Hamill and Martinac, 2001). Pretreatment of guinea pig tracheal ASM strips with the stretch-activated cation channel blocker gadolinium (Gd³⁺) significantly decreased isometric force generation after stretch (Ito et al., 2006).

In this study, we set out to investigate the effect of acute airway stretch on agonist-induced contraction in bovine bronchial segments and the possibility that SP and NKA release can mediate stretch-activated contractions in these tissues. Moreover, we assessed the potential involvement of stretch-activated cation channels using the isolated bronchial segment technique previously described by Mitchell et al. (1989).

Materials and Methods

Animals. All experimental procedures were approved by the McMaster University Animal Care Committee (McMaster University, Hamilton, ON, Canada) and conform to the guidelines set by the Canadian Council on Animal Care (Ottawa, ON, Canada). Lower lobes of lung were obtained from cows (200–500 kg) euthanized at a local abattoir and transported to the laboratory in ice-cold modified Krebs buffer solution (116 mM NaCl, 4.6 mM KCl, 1.2 mM MgSO₄, 2.5 mM CaCl₂, 1.3 mM NaH₂PO₄, 23 mM NaHCO₃, 11 mM d-glucose, and 0.01 mM indomethacin), saturated with 95% oxygen-5% carbon dioxide to maintain pH at 7.4. Upon receipt of the lobes of lung, intact bovine bronchial segments (2-mm diameter, 20-mm length) were carefully dissected free from surrounding parenchyma, excised, and immediately used or stored in modified Krebs solution at 4°C for up to 24 h.

Bronchial Segment Preparation. After the dissection and excision of the bronchial segment, side branches were tightly ligated with surgical silk (4-0) as previously mentioned (Mitchell et al., 1998; Khangure et al., 2004). The ligated bronchial segment was then mounted horizontally in a 30-ml Mayflower organ bath (Hugh Sachs Elektronik-Harvard Apparatus GmbH, March-Hugstetten, Germany) containing warmed modified Krebs buffer solution (37°C) gassed with carbogen (95% O₂-5% CO₂) as previously mentioned by Mitchell and Sparrow (1994) with modifications. In brief, both luminal ends of the airway were mounted on adjustable cannulae that allowed airways of different lengths to be mounted. The tissue lumen was filled with warmed modified Krebs solution gassed with carbogen via a jacketed reservoir, the height of which set the baseline transmural pressure (~5 cm H₂O). This baseline pressure was selected to simulate the transmural pressure found in relaxed airways (Noble et al., 2007). The connectors at each end of the airway possessed three-way taps, which could be opened to flush the airway with modified Krebs solution or closed to make the airway lumen isovolumic. The intraluminal pressure was recorded with a pressure transducer (Hewlett-Packard Medical Products, Andover, MA) attached proximally to the airway. The pressure transducer output was fed through a pressure amplifier (Hewlett-Packard Medical Products Group) and data were digitally recorded using WinDaq DI-720 recording software (DataQ Instruments, Akron, OH). Manual transmural pressure variation was induced by varying the height of perfusate in a column manometer attached distally to the cannulated airway.

The airway segment was mounted at 115% of its resting length (the latter being the length of the segment when dissected free from parenchyma at zero transmural pressure). This coaxial stretch has been shown previously to produce increased contractile responses compared with an airway segment mounted at resting length (Khangure et al., 2004). Subsequently, a pressure test was performed to ensure that there were no leaks in the airway. The segment was then left to equilibrate for ~2 h, during which the lumen and adventitia were regularly washed with fresh modified Krebs solution. After tissue equilibration, transmural pressure was set to 5 cm H₂O by manually opening the three-way tap, which communicated with the reservoir of Krebs buffer 5 cm higher than the bronchial segment; equilibration of pressures between the two compartments was essentially instantaneous. With the three-way tap now closed (isovolumic condition), tissues were treated with 60 mM KCl, and the contractile response (isovolumic increase in intraluminal pressure) was recorded to test viability. After washing four times, baseline pressure was then reset to ~5 cm H₂O by opening/closing the tap.

Tissue Baths. After the tissue viability test, the airway was allowed 20 min of recovery time under isovolumic conditions. Subsequently, electric field stimulation (EFS) responses were evoked at 5-min intervals until a uniform response was observed (after approximately three to four repetitions) under isovolumic conditions. EFS was delivered by a train of pulses (60 V, 2-ms pulse duration,
and frequency of 20 pulses/second), evoked via circular electrodes placed above and below the airway in the organ bath, which were connected to a Grass S48 stimulator (Grass Instruments, Quincy, MA). The airway was then stretched by opening the three-way tap, which now communicated with a column of fluid 10 to 30 cm in height, allowing the pressure between the two to equilibrate instantaneously and then closing the tap (Fig. 1, A and B, ii and iii); this increased intraluminal pressure was maintained isovolumically for 3 min. Intraluminal pressure was then restored to baseline by opening/closing the tap and allowing equilibration with the reservoir 5 cm higher than the tissue (Fig. 1, A and B, iv). The tissue was allowed 5 min of recovery time. To mimic the increased airway tone seen in asthmatic airways, this process was repeated after pretreatment with \(10^{-8}\) M carbachol (CCh) or \(10^{-7}\) M serotonin (5-HT) added to the bath solution to induce submaximal ASM tone under isovolumic conditions (Fig. 1, A and B, v). Ten minutes later, at which point agonist-induced tone had reached a plateau, transmural pressure was reset to \(-5\) cm H\(_2\)O (by opening and closing the three-way tap) (Fig. 1, A and B, vi) before reassessing airway contractile responses to stretch (\(R_{\text{stretch}}\)) (Fig. 1, A and B, vii–ix). This protocol enabled the investigation of the effects of mechanical stretch on intraluminal pressure generation in the perfused isolated bronchial segment.

**Pharmacological Interventions.** To investigate the pathway involved in airway stretch-activated contractile responses, tissues were treated with a range of different antagonists after assessment of stretch-activated contractions under control conditions (where tissues were pretreated with \(10^{-8}\) M CCh). The possible role for stretch-activated cation channels was tested by pretreating for 30 min with Gd\(^{3+}\) (\(10^{-3}\) M), whereas a role for L-type Ca\(^{2+}\) channels was assessed by pretreatment for 30 min with nifedipine (\(2 \times 10^{-6}\) M), verapamil (\(10^{-5}\) M), or diltiazem (\(10^{-5}\) M). To assess any potential neurogenic component of airway constriction (Joos et al., 2000; Canning and Fischer, 2001; Widdicombe, 2003), we tested the effect of pretreating with the Na\(^{+}\)-channel blocker, tetrodotoxin (TTX) (\(10^{-6}\) M; 10 min); the sensory excitatory neurotoxin, capsaicin (\(10^{-5}\) M; 20 min); the NK\(_1\) receptor antagonist, L-732,138 (\(10^{-5}\) M (data not shown); \(10^{-4}\) M; 30 min); or the NK\(_2\) receptor antagonists, MEN 10376 (\(10^{-7}\) M (data not shown), \(10^{-6}\) M (data not shown), \(10^{-5}\) M; 30 min) or SR48968 (\(3 \times 10^{-6}\) M; 30 min).

**Chemicals and Solvents.** L-732,138 was obtained from BIO-MOL Research Laboratories (Plymouth Meeting, PA). MEN 10376 was obtained from LKT Labs (St. Paul, MN). SR48968 was kindly donated by sanofi-aventis (Bridgewater, NJ). All other pharmacological agents were obtained from Sigma Diagnostics Canada (Mississauga, ON, Canada). The 10 mM stock solutions were prepared in distilled water (CCh, 5-HT, diltiazem, Gd\(^{3+}\)), dilute acetic acid (TTX), absolute ethanol (nifedipine, verapamil, L-732,138), or dimethyl sulfoxide (MEN 10376, SR48968). Dilutions of these were made in physiological medium; the maximal bath concentration of

---

**Fig. 1.** Experimental protocols for this study. A, pressure recording during the various manipulations used in our experimental protocol; details are given under Materials and Methods, Results, and Discussion. ↑, response to a pressure pulse stretch (30 cm H\(_2\)O). ▼, restoration of transmural pressure to 5 cm H\(_2\)O. \(R_{\text{stretch}}\) was quantified as illustrated. Italicized labels refer to the cartoon drawings of airway cross-sections given in B, summarizing (in a nonquantitative fashion) the changes in pressure (P), volume (V), and airway diameter (L) during the various steps in our experimental protocol.
solvents did not exceed 0.1%, which we have found elsewhere to have little or no effect on mechanical activity.

**Statistical Analysis.** Stretch-activated contractions ($R_{\text{stretch}}$) were quantified as the difference between the minima and the maxima observed in the transmural pressure recordings after a sudden isovolumic stretch (Fig. 1A). All responses were reported as means ± S.E.M.; $n$ refers to the number of animals. Statistical analyses comparing multiple groups were done using one-way analysis of variance followed by the Bonferroni’s multiple comparison post hoc test. Statistical comparisons between paired groups were made using the paired Student’s $t$ test. A value of $p < 0.05$ was considered statistically significant.

**Results**

**Airway Stretch-Activated Contractions.** In resting tissues at a baseline transmural pressure of 5 cm H$_2$O, instantaneously opening and closing the tap communicating with a column of Krebs solution 10 to 30 cm high led to a sudden increase in transmural pressure (presumably accompanied by a small increase in luminal volume, although this was not measured) followed by a prolonged isovolumic stress relaxation response (Fig. 1, A and B, i–iii). After restoring transmural pressure to baseline (by opening/closing the tap communicating with a 5-cm column of fluid and allowing some fluid to escape) (Fig. 1, A and B, iv), the tissue was challenged with CCh (10$^{-8}$ M) under isovolumic conditions (Fig. 1, A and B, v); there was an increase in airway tone shown by a rise in active transmural pressure. When this cholinergic tone had stabilized, we reset transmural pressure to 5 cm H$_2$O (by opening/closing the tap and allowing fluid to exit the airway; Fig. 1, A and B, vi) and allowed 5 min for the tissue to re-equilibrate under those new isovolumic conditions before reassessing the response to a sudden pressure pulse (10–30 cm H$_2$O, as described above; Fig. 1, A and B, vii–ix). In contrast to what was seen in the absence of any underlying cholinergic stimulation (above), the instantaneous spike and transient decrease in transmural pressure (stress relaxation) were now followed by a slowly developing and prolonged contraction ($R_{\text{stretch}}$), the magnitude of which increased with increasing pressure pulse amplitude (Fig. 2B). To determine whether that third component of the mechanical response was a uniquely cholinergic phenomenon, we repeated this experiment using 10$^{-7}$ M 5-HT and obtained the same relationship between test pressure pulse amplitude and magnitude of $R_{\text{stretch}}$ (Fig. 2C). To characterize the mechanisms underlying $R_{\text{stretch}}$, all subsequent experiments used a standard test pulse of 30 cm H$_2$O because the contractile response ($R_{\text{stretch,30}}$) was maximal at this point (Fig. 2, B and C) and because this mirrors the transmural pressure seen during a deep inspiration to TLC in humans (Scichilone et al., 2004; Allen et al., 2005).

**Relationship between Agonist Concentration and $R_{\text{stretch,30}}$.** Next, we investigated the dependence of $R_{\text{stretch,30}}$ upon the degree of excitation produced by agonist stimulation. Tissues were stimulated with varying concentration of agonists (CCh or 5-HT) under isovolumic conditions and the $R_{\text{stretch,30}}$ was measured. The results showed that the magnitude of $R_{\text{stretch,30}}$ increased with increasing concentration of agonists. The relationship was linear and could be described by the equation $R_{\text{stretch,30}} = a + bC$, where $C$ is the concentration of agonist and $a$ and $b$ are constants. The values of $a$ and $b$ were determined from the experimental data and were found to be statistically significant ($p < 0.05$).

![Fig. 2](image-url) Effects of CCh and 5-HT pretreatment on bronchial responsiveness to stretch. Experiments were performed under isovolumic conditions. Agonists were added to the bath 10 min before the experimental protocol. A, instantaneous transmural stretch to 30 cm H$_2$O (from a baseline of 5 cm of H$_2$O) elicited a contraction in airways pretreated with a contractile agonist (10$^{-8}$ M CCh or 10$^{-7}$ M 5-HT) but not in unpretreated tissues. Mean magnitudes of $R_{\text{stretch}}$ evoked by transmural pressures of 10 to 30 cm H$_2$O in the absence or presence of 10$^{-8}$ M CCh (B) or 10$^{-7}$ M 5-HT (C). $n = 6$ for both. *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$. 
trations of CCh or 5-HT for 10 min, after which transmural pressure was returned to 5 cm H₂O by allowing fluid to exit the lumen of the airway, and 5 min was given before evaluating the response to a transmural pressure pulse of 30 cm H₂O. Even when tissues were stimulated with CCh or 5-HT at concentrations that evoked little or no contractile response of their own (Fig. 3, B and D), there was a substantial Rstretch,30 (Fig. 3, A and C). The latter increased in magnitude with increasing degrees of excitatory stimulation, reaching a peak at 10⁻⁸ M CCh and 10⁻⁷ M 5-HT; these agonist concentrations were submaximally effective with respect to evoking a direct bronchoconstrictor response.

**Effect of Stretch-Activated Cation Channel Blockade on Rstretch,30** To investigate whether stretch-activated cation channels are involved in Rstretch,30, we used a mechanosensitive cation channel blocker, Gd³⁺ (Coirault et al., 1999). Control responses were established upon pretreatment with 10⁻⁸ M CCh at a transmural pressure load of 30 cm H₂O before antagonist treatment. Gd³⁺ (10⁻³ M) significantly reduced airway Rstretch,30 compared with control (Fig. 4).

**Effect of L-Type Ca²⁺ Channel Blockade on Rstretch,30** To investigate whether L-type Ca²⁺ channel blockade would affect Rstretch,30, we established our control responses upon pretreatment with 10⁻⁸ M CCh at a transmural pressure load of 30 cm H₂O before treating the airway segments with a variety of L-type Ca²⁺ channel blockers for 20 min and then re-evaluating Rstretch,30. Blockers included the dihydropyridine nifedipine, the phenylalkylamine verapamil, and the benzothiazepine diltiazem (Fleckenstein, 1977; Triggle and Swamy, 1980; Middleton, 1984). Nifedipine (2 × 10⁻⁶ M), verapamil (10⁻⁵ M), and diltiazem (10⁻⁵ M) all abolished Rstretch,30 (Fig. 5).

**Effect of Capsaicin and TTX on Rstretch,30** To determine whether neurogenic mechanisms contributed to this Rstretch,30, we treated airway segments with the sensory excitatory neurotoxin capsaicin (10⁻⁵ M; 20 min) to induce desensitization of sensory afferents by causing depletion of neurotransmitters contained within their nerve terminals. Control responses were established upon pretreatment with 10⁻⁸ M CCh at a transmural pressure load of 30 cm H₂O before treating the airway segments with neurotoxin. Treatment with 10⁻⁵ M capsaicin abolished Rstretch,30 (Fig. 6), thus showing a vital role for sensory afferent neurons in this phenomenon. It is interesting to note that TTX (10⁻⁶ M) did not significantly affect Rstretch,30 (although we did find it to be sufficient to abolish EFS-evoked responses; data not shown), suggesting that a TTX-resistant neural component might be involved in mediating these contractions (Fig. 6).

**Effect of NK₁ and NK₂ Receptor Antagonists on Rstretch,30** Our results above suggest the involvement of a TTX-resistant sensory neural mechanism in Rstretch,30. Thus, we sought to determine the neurotransmitter implicated in these contractions by blocking either NK₁ or NK₂ receptors to assess the role of SP or NKA, respectively. The NK₁ receptor antagonist L-732,138 (10⁻⁴ M) showed no significant effect on Rstretch,30, suggesting that airway stretch-activated contractions are not mediated by SP release from sensory neurons. A 10-fold lower concentration of L-732,138 (10⁻⁵ M) elicited no significant effect on Rstretch,30 (data not shown). It is interesting to note that blockade of NK₂ receptors by either MEN 10376 (10⁻⁵ M) or SR48968 (3 × 10⁻⁶ M) caused a significant reduction in Rstretch,30 suggesting an essential role for NKA (Fig. 7). Concentrations of 10⁻⁷ and 10⁻⁶ M
Fig. 4. Effects of mechanically gated cation channel blockade on $R_{\text{stretch,30}}$. Mean values of $R_{\text{stretch,30}}$ measured before (open bars) and during (closed bars) treatment with Gd$^{3+}$ (10^{-5} M; n = 6). *, $p < 0.05$.

Fig. 5. Effect of L-type Ca$^{2+}$ channel blockers on $R_{\text{stretch,30}}$. Mean values of $R_{\text{stretch,30}}$ measured before (open bars) and during (closed bars) treatment with nifedipine (2 $\times$ 10^{-6} M; n = 6), verapamil (10^{-5} M; n = 6), or diltiazem (10^{-5} M; n = 6). ***, $p < 0.001$.

Fig. 6. Effect of neurotoxin treatment (capsaicin and TTX) on $R_{\text{stretch,30}}$. Mean values of $R_{\text{stretch,30}}$ measured before (open bars) and during (closed bars) treatment with capsaicin (10^{-5} M; n = 6) or TTX (10^{-5} M; n = 6). ***, $p < 0.01$.

Fig. 7. Effects of NK$_{1}$ and NK$_{2}$ receptor blockade on $R_{\text{stretch,30}}$. Mean values of $R_{\text{stretch,30}}$ measured before (open bars) and during (closed bars) treatment with the NK$_{1}$ receptor antagonist L-732,138 (10^{-5} M; n = 6) or the NK2 receptor antagonists MEN 10376 (10^{-5} M; n = 6) or SR48968 (10^{-5} M; n = 6). ***, $p < 0.01$; ***, $p < 0.001$.

MEN 10376 caused a dose-dependent but nonsignificant decrease in $R_{\text{stretch,30}}$ (data not shown).

Discussion

There have been numerous reports of stretch eliciting a contractile response in ASM; however, most of these studies have used ASM cells or strips (Gunst and Russell, 1982; Mitchell et al., 1997; Maksym et al., 2005). Previous studies have also deemed ASM $R_{\text{stretch}}$ as a myogenic event (Stephens et al., 1975; Thulesius and Mustafa, 1994), suggesting an intrinsic property of ASM itself. However, an examination of the pathway involved in airway $R_{\text{stretch}}$ has not been addressed previously using perfused intact bronchial segments.

Here, we describe the ability of perfused bovine bronchial segments to contract in response to stretch, but only when pretreated with submaximally effective, or even subthreshold, concentrations of a contractile agonist (CCh and 5-HT). In Fig. 1B, we summarize the changes in pressure, volume, and muscle length at different points in our experimental protocol. Because of the incompressibility of the liquid within the airway lumen, airway volume and muscle length remain constant during isovolumic conditions. The increase in pressure and muscle length seen in Fig. 1B, ii, is attributed to a change in volume of fluid within the airway. The subsequent isovolumic loss of pressure immediately after a test pulse (Fig. 1B, iii), on the other hand, is attributed to “stress relaxation” in the passive tissues; this may include processes such as fluidization of the cytoskeleton during the stretch and equilibration of the series and parallel elastic elements, although our experimental approach is not able to resolve these. Moreover, we hypothesize that after agonist pretreatment (Fig. 1, A and B, v), the loss of pressure and decrease in muscle length seen in Fig. 1B, vi, are related to the volume of fluid expelled from the airway lumen upon opening/closing of the three-way tap; clearly, the change in muscle length would be proportional to the concentration of agonist used. As such, the changes in airway volume (and muscle length) upon eliciting an $R_{\text{stretch,30}}$ in Fig. 1B, vii, would have been less than that seen in Fig. 1B, ii. At this smaller muscle length, the isovolumic stress relaxation is now followed by substantial force generation (Fig. 1B, ix), which we attribute, in part, to reorganization of the contractile apparatus and changes occurring in the contractile signaling pathway because of the presence of stretch and agonist activation. However, we do not view this $R_{\text{stretch}}$ as being solely related to a change in the muscle’s position on the length-tension curve because it was seen even at concentrations of agonists that did not generate any tone on their own (and, therefore, there would be no change in airway diameter/muscle length during the transition from v to vi in Fig. 1, A and B).

Discrepancies between stretch applied to intact airways versus isolated ASM bundles have been noted, where in intact airways, stretch promoted increased muscle contractility, and the opposite effect is seen in ASM bundles (Khangure et al., 2004; Noble et al., 2004). These discrepancies may be species-related and/or attributed to different properties of different regions in the airway tree, where $R_{\text{stretch}}$ may be a more significant phenomenon in small resistance airways compared with larger airways. A possible
Despite the fact that R-stretch is only seen in the presence of an agonist, it was evident that concentrations that produce relatively little change in basal ASM tone seen in asthmatic airways. For our experimental setup, we chose to use a baseline transmural pressure of 5 cm H2O and maximal pressure pulse of 30 cm H2O to mimic slow-onset, slow-offset stimulation that resembles realistic physiologic and pharmacologic conditions. The authors of these studies interpreted this phenomenon as a functional transformation of multitunit smooth muscle into a single unit, mediated by a contractile agonist. Although others have observed a stretch-induced relaxation in bovine tracheal strips in the presence of an agonist (acetylcholine), this was done using oscillatory stretch. The authors concluded that these tidal changes in length can cause an excess rate of detachment that is faster than the rate of attachment, thus causing a net decrease in ASM force production. In the setup, the airway stretch is static; such as, the myosin and actin interactions should have been able to return to a latch state. Thus, the discrepancies between our data and those presented by Fredberg et al. (1997) seem to be because of differences in experimental protocols.

Mechanotransduction is sometimes mediated in part through activation of sensory neurons (Scotland et al., 2004). In addition, neurogenic mechanisms can contribute to airway responsiveness (Joos et al., 2000; Widdicombe, 2003). Therefore, to investigate whether airway R-stretch is also mediated by neuronal input, we treated the isolated airway segments with the sensory neurotoxin capsaicin (Geber et al., 2006). In this study, capsaicin-induced depletion of sensory nerve endings abolished the R-stretch, which was unmasked by CCh, suggesting the involvement of sensory neurons. Surprisingly, we found this sensory neuronal component to be unaffected by TTX. TTX-resistant channels have previously been characterized on neurons controlling many different organ systems, including Na+, L and Na+, which are expressed on sensory C-fibers and neurons in the peripheral nervous system with nerve endings in proximity of smooth muscle, respectively (Ogata and Ohishi, 2002).

Upon demonstrating a TTX-resistant sensory neuronal pathway involvement in R-stretch, we sought to characterize the neuronal pathway that mediates this response. Of the numerous neurotransmitters found within airway sensory nerve terminals, SP and NKA have been shown to contribute to bronchoconstriction in asthmatics. The receptors for these neurotransmitters, NK1 and NK2, respectively, have been well characterized in ASM (Joos et al., 2000). Using the NK1 receptor antagonist, L-732,138, the peptide NK1 receptor antagonist, MEN 10376, and the nonpeptide NK2 receptor antagonist, SR48968, we found no significant difference upon blockade of NK1 receptors, whereas NK2 receptor blockade significantly decreased contractile responses, thus affirming a central role for those receptors (and for NKA) in airway R-stretch. These results are supported by a recent study that showed an NK2 selectivity pertaining to bronchial hyper-reactivity and suggested an importance for capsaicin-sensitive nerves in bronchoconstriction in mice (Elekes et al., 2007). Another study found that NK2 receptors played a predominant role in a guinea pig model of mechanically induced bronchoconstriction (Corboz et al., 2009). In contrast, protease-activated receptor-2-mediated, TTX- and capsaicin-sensitive neurons in murine small intestine did not reveal differences in NK1 versus NK2 selectivity (Zhao and Shepard, 2003), as we observed, which could possibly be explained by species differences between bovine and murine or tissue differences between bronchi and small intestine.

Given the fact that mechanotransduction often involves stretch-sensitive ion channels, we also probed the effect of various cation channel blockers on R-stretch. On the one hand, a significant inhibitory effect of Gd3+ implicated a central role for nonspecific cation channels in these contractions; it is as yet unclear whether these are the same set of Gd3+-sensitive channels that we have shown previously are activated by intracellular Ca2+ store depletion in bovine ASM cells (Helli et al., 2005). Nifedipine, a dihydropyridine class of L-type Ca2+-channel blocker, was also tested, albeit originally as a negative control for Gd3+. We were surprised to find that nifedipine also abolished R-stretch. To determine whether this was a nonspecific effect of nifedipine, we then employed two other structural classes of L-type Ca2+-channel blocker, verapamil (a phenylalkylamine) and diltiazem (a benzothiazepine), and found these too abolished R-stretch. L-type Ca2+ channels have been well characterized in ASM (Green et al., 1993; Janssen, 1997); however, the electrophysiological and pharmacological properties of those channels are not consistent with an involvement in agonist-evoked responses (Janssen, 2002). Nonetheless, our data clearly suggest that airway stretch-activated contractions may signal through a different pathway than agonist-evoked contractions because of their dependence on L-type Ca2+ channels.

In conclusion, our data suggest that airway R-stretch may occur through a nonmyogenic pathway (because pretreatment with a contractile agonist is required), and airway sensory C-fibers are involved in mediating R-stretch in bronchial segments. Moreover, it seems that this mechanosensitivity is sensed by stretch-activated cation channels. After an elevation in transmural pressure, we propose that stretch-activated cation channels located on C-fibers penetrating the airway wall are activated, resulting in the release of NKA from these nerve endings. This NKA, in turn, binds to postjunctional NK2 receptors located on the smooth muscle to mediate an airway stretch-activated contraction. These results highlight an alternative pathway for potential thera-
pulmonary response to a DI may play a role in airway hyperresponsiveness.

Acknowledgments

We thank Tracy Tazzeo for technical assistance and for obtaining the bovine lungs used for the study. We also thank sanofi-aventis for the generous sample of the NK2 receptor antagonist SR48968.

References


Mitchell HW and Sparrow MP (1994) Increased responsiveness to cholinergic stimulation of small compared to large diameter cartilaginous bronchi. Eur Respir J 7:298–305.


Address correspondence to: Dr. Luke Jeffrey Janssen, Firestone Institute for Respiratory Health, St. Joseph’s Hospital, Room L-314, 50 Charlton Avenue East, Hamilton, ON, Canada L8N 4A6. E-mail: janssen@mcmaster.ca