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 ([Tyr₁,D-Trp₆,₈,₉,Lys₁₀]-NKA(4–10)) (10⁻⁶ M) and SR48968 (N-[25]-4-(4-acetamido-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, 1985). 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 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 ([Tyr₁,D-Trp₆,₈,₉,Lys₁₀]-NKA(4–10)) (10⁻⁶ M) and SR48968 (N-[25]-4-(4-acetamido-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.
1987; Salome et al., 2003). 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 remodeling 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., 1998; Khangure et al., 2004). The ligated bronchial segment was then mounted horizontally in a 30-ml Mayflower organ bath (Hugo 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 delivery of different lengths to be mounted. The cannula 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 obtained (at least 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 H₂O (by opening and closing the three-way tap) (Fig. 1, A and B, vi) before reassessing airway contractile responses to stretch (Rstretch) (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 L-type Ca²⁺ channels was assessed by pretreatment for 30 min with nifedipine (2 × 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 NK₁ receptor antagonist, L-732,138 (10⁻⁵ M (data not shown); 10⁻⁴ M; 30 min); or the NK₂ receptor antagonists, MEN 10376 (10⁻⁷ M (data not shown), 10⁻⁶ M (data not shown), 10⁻⁵ M; 30 min) or SR48968 (3 × 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, Gd³⁺), 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 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 pretreatment for 30 min with Gd³⁺ (10⁻³ M), whereas a role for L-type Ca²⁺ channels was assessed by pretreatment for 30 min with nifedipine (2 × 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 NK₁ receptor antagonist, L-732,138 (10⁻⁵ M (data not shown); 10⁻⁴ M; 30 min); or the NK₂ receptor antagonists, MEN 10376 (10⁻⁷ M (data not shown), 10⁻⁶ M (data not shown), 10⁻⁵ M; 30 min) or SR48968 (3 × 10⁻⁶ M; 30 min).

**Figure 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 H₂O). ↓, restoration of transmural pressure to 5 cm H₂O. Rstretch 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 concentrations of agonists and the resulting R\(_{\text{stretch,30}}\) was measured. The results are shown in Fig. 2B and C. The significance of the differences between the groups were determined using Student’s \(t\) test. A value of \(p < 0.05\) was considered statistically significant.
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 mechano-sensitive 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. 3. Relationship between contractile agonist concentration and Rstretch](https://example.com/fig3.png)
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 constrict 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
that Rstretch is only seen in the presence of an agonist, it
ical properties and elicit a prolonged Rstretch. Given the fact
that after priming of the contractile apparatus with an ago-
nist, stretch caused fluidization of the cytoskeleton, and dur-
ning redevelopment of force, the contractile apparatus was
able to regenerate force above and beyond prestretch levels.
However, further experiments suggest the possibility that the
Rstretch phenomenon we observe in bovine bronchial seg-
ments may possess a neurogenic component.

DI-induced bronchoconstriction is abnormal in humans,
given that it is only seen in moderate to severe asthmatics.
Our bovine bronchial segments were not inflamed nor exhib-
ited spontaneous tone and did not manifest a stretch-induced
contraction until they were pretreated with a contractile
agonist (CCh or 5-HT), which we used to mimic the increased
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
pressures in the human lung at functional residual capacity
and TLC, respectively (Noble et al., 2007). In fact, we have
demonstrated that pretreatment with CCh or 5-HT, at con-
centrations that produce relatively little change in basal
tension, can produce a fundamental change in ASM biophys-
ical properties and elicit a prolonged Rstretch. Given the fact
that Rstretch is only seen in the presence of an agonist, it
would appear to be nonmyogenic in nature. Others have also
demonstrated an Rstretch in ASM that required pretreatment
with a pharmacological agent to prime the contractile appa-
ratus, such as tetraethylammonium chloride or a cholinergic
agonist (Stephens et al., 1975; Thulesius and Mustafa, 1994).
The authors of these studies interpreted this phenomenon as
a functional transformation of multiunit 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 stretches. The authors con-
cluded 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 our
setup, the airway stretch is static; as such, 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). There-
fore, to investigate whether airway Rstretch,30 is also medi-
ated by neuronal input, we treated the isolated airway seg-
ments with the sensory neurotoxin capsaicin (Geber et al.,
2006). In this study, capsaicin-induced depletion of sensory
nerve endings abolished the Rstretch,30 which was unmasked
by CCh, suggesting the involvement of sensory neurons. Sur-
prisingly, 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 Na1,8 and Na1,6, which are ex-
pressed 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 Rstretch,30, 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 aff irming
a central role for those receptors (and for NKA) in airway
Rstretch,30. These results are supported by a recent study that
showed an NK2 selectivity pertaining to bronchial hyperre-
activity and suggested an importance for capsaicin-sensitive
nerves in bronchoconstriction in mice (Elekes et al., 2007).
Another study found that NK2 receptors played a predomi-
nant role in a guinea pig model of mechanically induced
bronchoconstriction (Corboz et al., 2008). In contrast, pro-
tease-activated receptor-2-mediated, TTX- and capsaicin-
sensitive neurons in murine small intestine did not reveal
differences in NK1 versus NK2 selectivity (Zhao and Shea-
donhue, 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 Rstretch,30. On the one
hand, a significant inhibitory effect of Gd3+ implicated a
central role for nonselective cation channels in these contrac-
tions; 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 Rstretch,30. 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 Rstretch,30.
L-type Ca2+ channels have been well characterized in ASM
(Green et al., 1993; Janssen, 1997); however, the electrophys-
iological and pharmacological properties of those channels
are not consistent with an involvement in agonist-evoked
responses (Janssen, 2002). Nonetheless, our data clearly sug-
gest that airway stretch-activated contractions may signal
through a different pathway than agonist-evoked contrac-
tions because of their dependence on L-type Ca2+ channels.

In conclusion, our data suggest that airway Rstretch may
occur through a nonmyogenic pathway (because pretreat-
ment with a contractile agonist is required), and airway
sensory C-fibers are involved in mediating Rstretch in bron-
chial segments. Moreover, it seems that this mechanosensi-
tivity 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 re-
results highlight an alternative pathway for potential thera-
peutic targeting in asthmatic patients where a bronchoconstrictory 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 providing the gadolinium, a stretch-sensitive channel blocker, on diaphragm muscle. We thank Tracy Tazzeo for technical assistance and for obtaining the bovine lungs used for the study. We also thank sanofi-aventis for providing the gadolinium, a stretch-sensitive channel blocker, on diaphragm muscle.

References

Corbuz MR, Fernandez X, and Hey JA (2008) Increased blocking activity of combined the bovine lungs used for the study. We also thank sanofi-aventis for providing the gadolinium, a stretch-sensitive channel blocker, on diaphragm muscle.

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