Activation of Large Conductance Potassium Channels Inhibits the Afferent and Efferent Function of Airway Sensory Nerves in the Guinea Pig

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Abstract

Sensory nerves play an important role in airway disease by mediating central reflexes such as cough, and local axon reflexes resulting in the peripheral release of neuropeptides. We have tested whether the benzimidazolone compound, NS1619, an opener of large conductance calcium-activated potassium (BK_{Ca}) channels, inhibits the activity of sensory fibers, and central and local airway reflexes in guinea pig airways. In in vitro single fiber recording experiments, NS1619 applied to identified receptive fields in the trachea inhibited the firing of Aδ-fibers evoked by hypertonic saline and distilled water, and bradykinin-evoked firing of C-fibers. Electrically evoked nonadrenergic noncholinergic contractions of isolated bronchi mediated by the release of neurokinin A (NKA) from C-fibers, but not those elicited by exogenous NKA, were inhibited by NS1619. These effects of NS1619 were prevented by iberiotoxin, a selective blocker of BK_{Ca} channels. In conscious guinea pigs, cough evoked by aerosolized citric acid was also inhibited by NS1619. These data show that BK_{Ca} channel activation inhibits sensory nerve activity, resulting in a reduction of both afferent and efferent function. BK_{Ca} channel openers may therefore be of potential benefit in reducing neurogenic inflammation and central reflexes seen during inflammatory conditions of the airways, and may represent a new class of antitussive drug. (*J. Clin. Invest.* 1997. 99:513–519.) Key words: potassium channels • sensory nerves • airways • cough • neurogenic inflammation

Introduction

There is increasing evidence that sensory nerves play an important pathophysiological as well as regulatory role in the airways because their activation leads to a range of central and local reflex effects characteristic of inflammatory airway diseases such as asthma. These reflexes are under the control of two different classes of sensory fiber, namely the myelinated, rapidly adapting stretch receptors (RARs), and nonmyelinated C-fibers with bronchial or pulmonary endings (1, 2). Activation of RARs, also known as irritant receptors, elicits cough and bronchoconstriction via a central reflex pathway (3, 4). Recently, however, attention has focused on C-fibers, which are characterized by their sensitivity to the neurotoxin capsaicin as well as inflammatory mediators such as prostaglandins, 5-HT, and bradykinin (1). Activation of C-fiber endings leads to a variety of central reflex effects including bronchoconstriction, mucus secretion, and vasodilatation (5), and there is considerable evidence that they may also mediate cough (6, 7). It is now established that capsaicin-sensitive C-fibers also have an efferent function, mediating excitatory nonadrenergic noncholinergic (e-NANC) bronchoconstriction, mucus secretion, plasma exudation, and vasodilatation resulting from the peripheral release of sensory neuropeptides, principally the tachykinins substance P and neurokinin A (8).

The efferent function of capsaicin-sensitive sensory nerves may be inhibited at a presumed prejunctional level via the activation of several receptor types. Thus, agonists at μ-opioid (9), α_2-adrenergic (10), $GABA_B$ (11), histamine H_3 (12), neuropeptide Y_2 (13), and 5-$HT_{1\text{-like}}$ (14) receptors have all been shown to inhibit e-NANC bronchoconstriction in the guinea pig, implying an action on airway C-fibers. It has been proposed that the inhibition evoked by a number of these receptors (α_2, NPY-Y_2, μ-opioid, and 5-$HT_{1\text{-like}}$) is mediated via a common mechanism involving the opening of large-conductance, calcium-activated potassium channels (BK_{Ca}[1]; 14, 15). This hypothesis was based on the use of charybdotoxin, a relatively nonselective blocker of BK_{Ca} channels, and its ability to reverse the inhibitory action of these receptors. Such studies are necessarily complicated by the presence of several types of potassium channel, including BK_{Ca} channels, on airway smooth muscle (16), and the existence of these channels on airway nerves has not yet been demonstrated directly. Moreover, it is not known if the afferent activity of sensory fibers, and in particular that of myelinated RARs, is similarly modulated, and if this may be reflected in a corresponding inhibition of a central reflex pathway.

The recent development of novel compounds which activate BK_{Ca} channels has greatly facilitated their study in different tissues (17–19). In particular, the benzimidazolone compound, NS1619, has been shown to activate BK_{Ca} channels in airways and vascular smooth muscle (20–22), and in hippocampal neurones (23). We have used this compound in combination with iberiotoxin, a highly selective blocker of BK_{Ca} channels, to determine if the activation of these channels on sensory fiber endings in the guinea pig airways leads to an inhibition of fiber activity, using an in vitro model of single fiber

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1. *Abbreviations used in this paper:* BK_{Ca}, large conductance calcium-activated potassium channel; EFS, electrical field stimulation; e-NANC, excitatory nonadrenergic noncholinergic; K_{DR}, voltage-dependent delayed rectifier; NKA, nuerokinin A; RAR, rapidly adapting stretch receptors.

recording (24). In addition we have examined whether NS1619 may inhibit sensory neuropeptide release and central airway reflexes by studying its effect on e-NANC bronchoconstriction in isolated guinea pig bronchi, and on cough in the conscious guinea pig.

Methods

Single fiber recording. The procedure for in vitro recording from single vagal afferent fibers has previously been described in detail (24). Briefly, male Dunkin-Hartley guinea pigs (200–400 g) were killed by anesthetic overdose (100 mg/kg sodium pentabarbitone i.p.) and the trachea and main bronchi with the attached right vagus nerve were removed. The airways were opened longitudinally on their ventral surface and pinned epithelium side up in a chamber perfused at a rate of approximately 8 ml/min with Krebs' solution maintained at 37°C and bubbled with 5% CO_2/95% O_2. The vagus nerve was drawn through a small hole into a second chamber and overlaid with paraffin oil. Under a dissecting microscope the nerve was desheathed and divided into fine filaments until single unit activity could be detected via a silver wire recording electrode after electrical stimulation of the main nerve trunk. Single units were characterized as Aδ- or C-fibers according to their conduction velocity (Aδ-fibers 2–25 ms^{-1}, C-fibers < 1.5 ms^{-1}) and receptive fields of identified units located by gently probing the tissue surface with a blunt glass rod. Drugs were then applied directly onto the receptive field using a separate perfusion system via a small plastic tube (internal diameter 2.5 mm) held onto the tissue surface. During application of the drugs the perfusion of the whole tissue with Krebs' solution was maintained.

The effect of NS1619 on the activity of Aδ-fibers and C-fibers was assessed. We have previously found that Aδ-fibers respond to changes in osmolarity (25) but not to capsaicin or bradykinin (24). In the present study therefore control responses of individual Aδ-fibers were first obtained to hypertonic (4%) saline and distilled water applied for 30 s at 10 min intervals. NS1619 (30 μM) was then perfused onto the receptive field for 10 min and the saline and distilled water reapplied in the continued presence of NS1619. Responses to both stimuli were then retested after a 30-min washout period. With C-fibers control responses were first obtained to bradykinin (0.1 μM, 30 s) and then repeated 30 min later after prior perfusion of the receptive field with NS1619. Perfusion with NS1619 was maintained during this bradykinin application. After washout of NS1619, the tissue was again left for 30–40 min and then bradykinin was retested. In a separate series of experiments Aδ-fiber responses to hypertonic saline and distilled water were examined in the combined presence of NS1619 (30 μM) and iberiotoxin (100 nM). These concentrations of NS1619 and iberiotoxin were chosen as they have been shown to be effective on BK_{Ca} channels of vascular smooth muscle (22). To test for reproducibility of fiber responses to the different stimuli used here, Aδ-fibers were exposed twice to distilled water or hypertonic saline with a 10-min interval between each application. Similarly, C-fibers received two applications of bradykinin (0.1 M) with a 30-min interval.

Afferent impulses were amplified with an AC amplifier (Neurolog NL104), filtered and monitored using a loudspeaker amplifier and storage oscilloscope. All data were recorded and analyzed online using a personal computer with CED 1401 interface (Cambridge Electronic Design Ltd., Cambridge, UK). Impulses were captured and displayed with peristimulus-time histograms using Spike 2 CED software (Cambridge Electronic Design). Drug responses were measured as the number of impulses counted during the application period and are expressed as the mean impulses per second during this period. Where spontaneous activity was apparent the number of spikes counted in the 30 s before drug application was subtracted from the drug response.

e-NANC bronchoconstriction in vitro. Tissues were prepared as previously described (14, 15). Ring preparations of main and hilar bronchi were mounted under a resting tension of 500 mg in 10-ml organ baths containing Krebs' solution maintained at 37°C and gassed with 95% O_2/5% CO_2. Indomethacin (10 μM), propranolol (1 μM), and atropine (1 μM) were present throughout all experiments. Electrical field stimulation (EFS) was applied via two parallel platinum wire electrodes placed either side of the tissue and a Grass S88 (Grass Instruments, Inc., Quincy, MA) stimulator was used to deliver biphasic square wave pulses at supramaximal voltage (40 V), 0.5 ms pulse width, and 8 Hz frequency for 30 s. These parameters have been previously found to elicit reproducible submaximal e-NANC responses (14, 15). During an equilibration period tissues were stimulated two or three times to obtain stable e-NANC responses to EFS. Isometric changes in tension were measured using Grass FT.03 force-displacement transducers and recorded on a polygraph (Grass model 7D; Grass Instruments, Inc.).

After stable e-NANC responses had been obtained, NS1619 (1–30 μM) was added 10 min before two test responses to EFS. Each tissue received only one concentration of NS1619. In a separate series of experiments the inhibitory effect of NS1619 was tested in the same manner in the presence of iberiotoxin (100 nM). The e-NANC bronchoconstrictor response to nerve stimulation in guinea pig airways is mediated predominantly via NKA (26), and so to confirm a prejunctional site of action of NS1619 its effect on contractions elicited by exogenous NKA was examined. In paired tissues noncumulative concentration-response curves were established to NKA (1 nM–10 μM) in the presence of NS1619 (30 μM) or its vehicle (ethanol). All responses to EFS or NKA were calculated as mg tension and then the percent change calculated of the response before the drug compared to the response after the drug within the same tissue.

Cough. The procedure for measuring cough in conscious guinea pigs was as previously described (6). Briefly, animals were placed in a perspex box that allowed free movement during exposure to aerosols. Airflow into the box was supplied from a compressed air cylinder at 600 ml/min and changes in airflow induced by respiration and coughing were detected by a pneumotachograph (Fleisch, Zurich, Switzerland), amplified and recorded via a pressure transducer onto a pen recorder (Lectromed UK Ltd., Herts, UK). Cough sounds were amplified and recorded concurrently via a microphone placed inside the box. Solutions were delivered by aerosol via a miniultrasonic nebulizer (Pulmisonic DeVilbiss, Somerset, PA) with an output of 0.4 ml/min connected to the airflow port. Coughs were counted by a trained observer and recognized from the characteristic opening of the mouth and posture of the animal, the sound produced, and the sound and airflow recordings. Using these criteria together cough was easily distinguished from sneezes and augmented breaths.

The effect of NS1619 was assessed on the cough response to citric acid. All animals were treated with terbutaline sulphate (0.05 mg/kg, i.p.) 3 min before cough challenge to minimise respiratory distress. We have previously found that this pretreatment prolongs the time to respiratory distress seen with citric acid and capsaicin challenges without affecting the cough response (6). Animals were exposed to an aerosol of NS1619 (100 μM or 300 μM) or an equivalent volume of ethanol vehicle for 10 min, followed by exposure to 0.35 M citric acid for a further 10 min in the continued presence of drug or vehicle. Coughs were counted during the 10 min challenge period with citric acid, and each group contained eight animals.

Drugs. In all in vitro experiments Krebs' solution of the following composition was used (mM): NaCl, 118; KCl, 5.9; $MgSO_4$, 1.2; $CaCl_2$, 2.5; NaH_2PO_4, 1.2; $NaHCO_3$, 25.5; and glucose 5.6. Drugs were obtained from the following sources: NS1619 (1-(2′-hydroxy-5′-trifluoromethylphenyl)tripfluoro-methyl-2-[^{3}H]benzimidazolone), iberiotoxin (Research Biochemicals Inc., St. Albans, UK); NKA, citric acid, bradykinin (Sigma Chemical Co., Poole, UK). NS1619 was dissolved in ethanol, and NKA and bradykinin in distilled water, to stock solutions of 10 mM and frozen in small aliquots. Iberiotoxin was dissolved in 0.9% saline and 0.1% BSA to a stock solution of 10 μM. All drugs were diluted to the appropriate concentration in Krebs' solution on the day of the experiment, and citric acid was made each day in 0.9% saline.

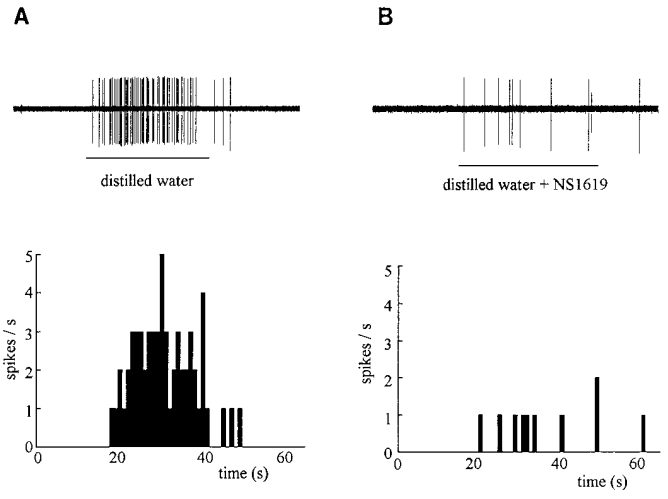


Figure 1. NS1619 inhibits the firing of Aδ-fibers in the guinea pig trachea. (*A*) Firing of a single Aδ-fiber to distilled water applied for 30 s onto its receptive field. (*B*) After a 10-min perfusion with NS1619 (30 μM) the response to distilled water, applied in the continued presence of NS1619, is greatly attenuated. (*C*) The response to distilled water recovers after a 30-min washout period. In each panel the discharge of the fiber is shown together with the peristimulus time histogram to illustrate the frequency of firing.

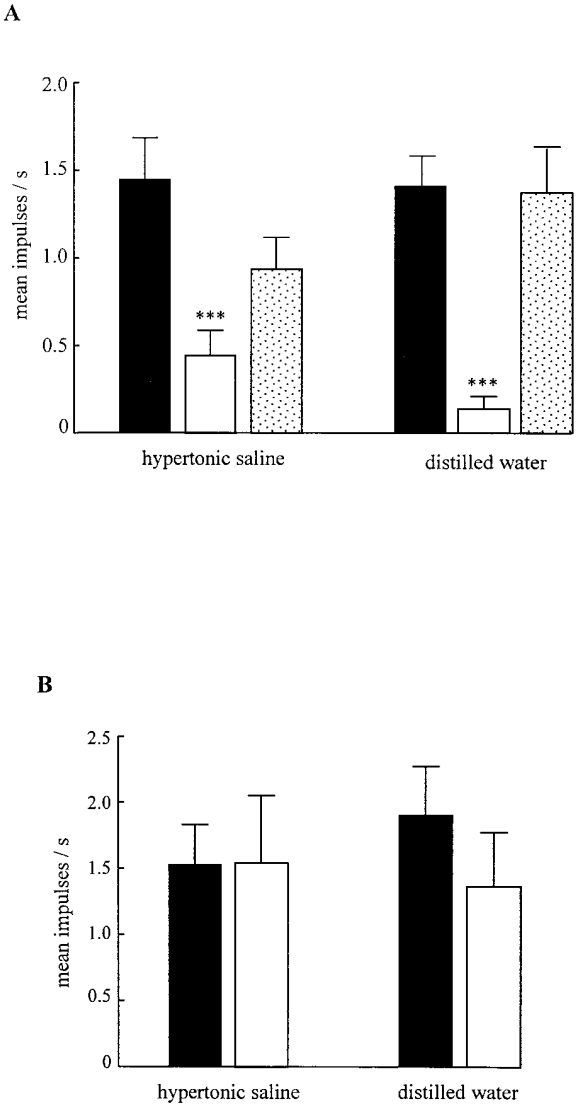


Figure 2. Summary of the effect of NS1619 on the activity of Aδ-fibers in guinea pig airways. (*A*) Control responses of Aδ-fibers to both hypertonic saline and distilled water (*solid columns*) were inhibited after perfusion of receptive fields with 30 μM NS1619 (*open columns*). In both cases the effect of NS1619 was fully reversible upon washout (*stippled columns*). (*B*) The inhibitory effect of NS1619 was abolished in the presence of iberiotoxin. In the combined presence of NS1619 and iberiotoxin (100 nM) responses of Aδ-fibers to hypertonic saline and distilled water (*open columns*) were not different from control (*solid columns*). In (*A*) and (*B*) each column represents the mean±SEM from six separate fibers. *** Significantly different from control ($P < 0.001$).

Statistics. Data are expressed as mean±SEM. Significance of difference was tested using Student's two-tailed *t* test for paired or unpaired data as appropriate, or Mann-Whitney two sample test for cough studies. $P < 0.05$ was considered significant.

Results

Single fiber recording. Recordings were made from 20 Aδ-fibers with mean conduction velocity of 6.0±1.6 ms^{-1}, and seven C-fibers (mean cv = 0.81±0.3 ms^{-1}). NS1619 produced a marked inhibition of firing of Aδ-fibers evoked by both hypertonic saline and distilled water. An example of the inhibitory effect of NS1619 on a single Aδ-fiber is shown in Fig. 1. Aδ-fiber responses to hypertonic saline were inhibited by 72.2±7.4% in the presence of NS1619, and those to distilled water by 90.9±4.3% ($n = 6$; $P < 0.001$ for both; Fig. 2 *A*). With both stimuli the inhibition was reversed upon washout of NS1619. Aδ-fibers using this preparation are not generally spontaneously active, and their basal activity, counted for 30 s before hypertonic saline or distilled water application (0.05±0.03 impulses s^{-1}), was not changed during NS1619 perfusion (0.02±0.01 impulses s^{-1}) showing that NS1619 does not have an excitatory effect alone.

The inhibitory action of NS1619 on Aδ-fiber firing was abolished after prior perfusion with iberiotoxin (100 nM; Fig. 3). Responses to hypertonic saline were unchanged when repeated in the combined presence of NS1619 and iberiotoxin (96.4±14.8% control; $n = 6$; $P > 0.05$; Fig. 2 *B*). Similarly, after perfusion with iberiotoxin and NS1619 responses to distilled water (1.38±0.4 impulses s^{-1}) were not significantly different from predrug control levels (1.91±0.36 impulses s^{-1}; $P >$

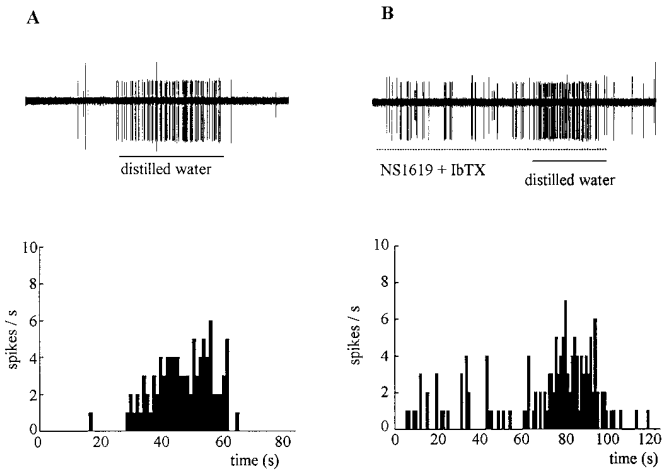


Figure 3. Iberiotoxin abolishes the inhibitory effect of NS1619 on Aδ-fiber firing. (*A*) Control response of a single Aδ-fiber to distilled water applied for 30 s onto its receptive field. (*B*) After perfusion for 10 min with NS1619 (30 μM) and iberiotoxin (100 nM) some spontaneous activity develops in this fiber. However, the response to distilled water is unchanged. (*C*) After a washout period of 30 min the response to distilled water remains unchanged.

0.05), representing 72.1±10.6% control. In three out of seven fibers tested, iberiotoxin elicited spontaneous firing when applied for the 10 min preincubation period (0.42±0.21 impulses s^{-1} compared to 0.06±0.03 impulses s^{-1} basal firing; Fig. 3).

A similar inhibitory effect of NS1619 was seen on C-fiber activity (Fig. 4). Thus, responses to bradykinin were inhibited by 93.0±7.2% (n = 3) in the presence of NS1619 (Fig. 5 *A*). Basal activity of C-fibers (0.04±0.03 impulses s^{-1}) was unchanged during NS1619 perfusion (0.05±0.03 impulses s^{-1}), and the inhibitory effect of NS1619 was reversed upon washout. Responses of both C-fibers and Aδ-fibers to their respective stimuli were highly reproducable, indicating that the inhibitory effect of NS1619 was not the result of tissue tachyphylaxis. Thus, C-fiber responses to a control application of bradykinin (2.47±0.55 impulses s^{-1}) were unchanged when repeated 30 min later (2.42±0.37 impulses s^{-1}; n = 4). Similarly, Aδ-fiber responses to hypertonic saline (1.65±0.27 impulses s^{-1}) and distilled water (2.19±0.27 impulses s^{-1}) were not different when repeated for a second time (1.44±0.29 and 1.89±0.29 impulses s^{-1}, respectively; n = 5 each).

Cough. NS1619 inhibited the cough reflex in conscious guinea pigs such that the cough response to citric acid was reduced by approximately 66% in animals previously exposed to NS1619 (300 μM; Fig. 5 *B*). Treatment with lower concentrations of NS1619 (100 μM) however did not significantly affect citric acid-evoked cough (1.03±0.18 coughs/min vs. 1.51±0.24 coughs/min control). Although higher concentrations of NS1619 were required in these experiments compared to the in vitro studies this was not unexpected since much lower airway concentrations of agents are achieved by aerosol delivery compared to intratracheal or systemic administration (27).

e-NANC bronchoconstriction. NS1619 produced a concentration-dependent inhibition of EFS-evoked e-NANC contractions of isolated bronchi, with the highest concentration tested (30 μM) eliciting a 55.3±5.9% inhibition of predrug control responses (Fig. 6 *A*). An equivalent volume of the vehicle for 30 μM NS1619 (ethanol) did not significantly inhibit contractile responses to EFS (12.9±4.6% inhibition, n = 4). The inhibitory effect of NS1619 was greatly attenuated in the presence of iberiotoxin (Fig. 6 *B*). Thus, in a separate series of experiments 30 μM NS1619 produced a 47.0±7.2% inhibition of e-NANC contractions, which was completely reversed when repeated in the presence of iberiotoxin (1.5±6.4% inhibition; n = 5; Fig. 6 *B*). In the presence of iberiotoxin bronchial preparations frequently developed increased tone, although there was no effect of iberiotoxin alone on e-NANC contractions (98.0±10.8% control; n = 3).

NS1619 had no apparent influence on the resting tone of preparations. In addition, there was no significant effect of NS1619 on contractions elicited by exogenously administered

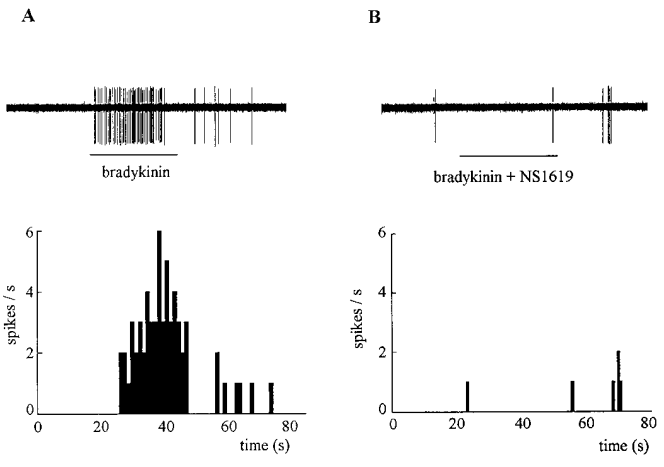
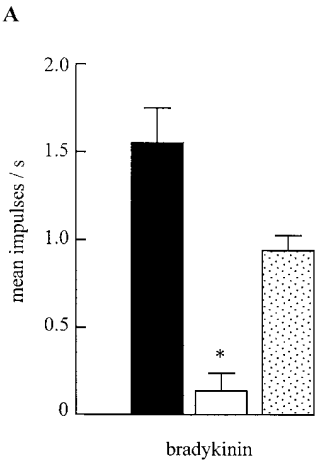


Figure 4. NS1619 inhibits the firing of C-fibers in guinea pig airways. (*A*) control response of a single C-fiber to bradykinin (0.1 μM) applied for 30 s onto its receptive field. (*B*) After perfusion for 10 min with NS1619 (30 μM) the response to bradykinin is abolished. (*C*) The response to bradykinin returns after a 30 min washout. In each case the discharge of the fiber is shown together with the peristimulus time histogram to illustrate the frequency of firing.



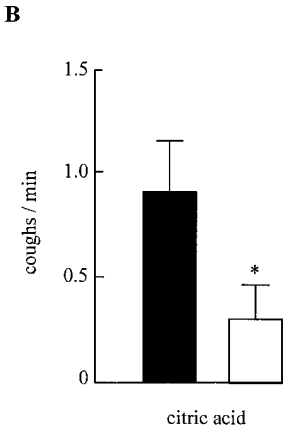
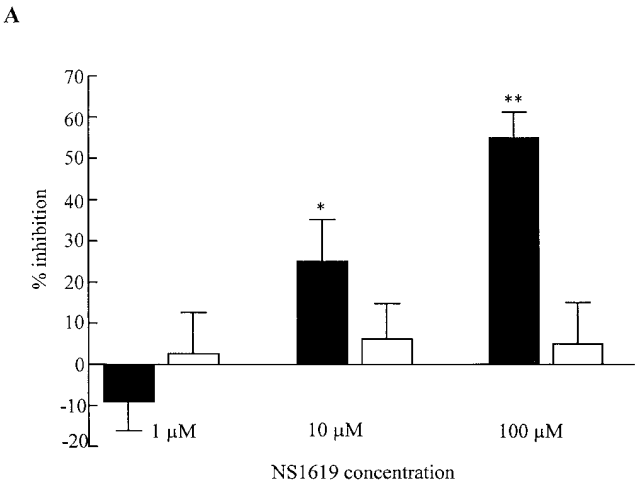


Figure 5. (*A*) Summary of the effect of NS1619 on the activity of C-fibers in guinea pig airways. Control responses of single C-fibers to 0.1 μM bradykinin (*solid columns*) were inhibited in the presence of 30 μM NS1619 (*open columns*). This effect was reversible upon washout (*stippled columns*). Data represents the mean± SEM from three separate C-fibers. * $P < 0.05$. (*B*) The effect of NS1619 on the cough reflex in conscious guinea pigs. Exposure of animals for 10 min to citric acid aerosol elicited a reproducible cough response (*solid columns*). After a prior exposure to aerosolized NS1619 (300 μM) the cough response to citric acid was greatly reduced (*open columns*). Each column represents mean± SEM number of coughs from eight animals. * $P < 0.05$.



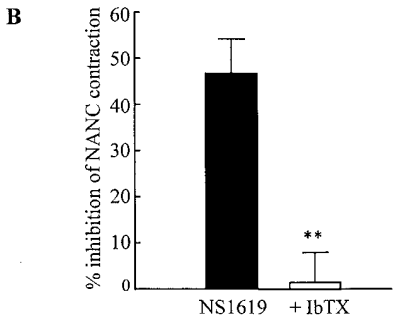


Figure 6. The effect of NS1619 on e-NANC bronchoconstriction. (*A*) NS1619 elicits a dose-dependant inhibition of electrically evoked e-NANC contractions in isolated guinea pig bronchi (*solid columns*), whilst contractions elicited by exogenous neurokinin A (1 μM) are unaffected (*open columns*). (*B*) The inhibition by NS1619 (30 μM) of e-NANC bronchoconstriction (*solid columns*) is completely reversed when repeated in the presence of iberiotoxin (100 nM). In (*A*) and (*B*) each column represents mean±SEM from four to six preparations. * $P < 0.05$, ** $P < 0.01$.

neurokinin A (NKA; Fig. 6 *A*). Responses to NKA in the presence of the highest concentration tested of NS1619 were 94.9±8.6% of predrug control levels ($n = 4$).

Discussion

Our previous studies examining the modulation of e-NANC bronchoconstriction in the guinea pig have provided indirect evidence for the presence of inhibitory BK_{Ca} channels on airway sensory nerve endings (14, 15). Consistent with this hypothesis, we show here that NS1619, a BK_{Ca} channel opener, inhibits e-NANC bronchoconstriction, as well vagal afferent fiber firing and cough in the guinea pig. This represents the first direct demonstration of an inhibitory effect of potassium channel modulators on airway sensory nerves, with a corresponding action on physiological reflexes.

Earlier evidence for the presence of BK_{Ca} channels on airway sensory nerves was based on the finding that charybdotoxin, a nonselective blocker of this channel type, prevented the inhibition of e-NANC contractions by the activation of a number of proposed prejunctional receptors. However, BK_{Ca} channels, as well as other potassium channels such as ATP-dependent (K_{ATP}) and voltage-dependent delayed rectifier (K_{DR}) potassium channels have also been shown to be present on airway smooth muscle from a number of species (16, 28). BK_{Ca} channels may be important regulators of the membrane potential and intrinsic tone of human and guinea pig airway smooth muscle (29, 30). Indeed, there is considerable evidence that these postjunctional channels are involved, at least in part, in mediating the relaxant action of β-adrenergic agonists on airway smooth muscle (31). The activation of BK_{Ca} channels present on the smooth muscle could therefore also result in a reduction of the bronchoconstrictor response to sensory nerve stimulation. However, in the present study we found that although NS1619 inhibited e-NANC bronchoconstriction, it did not affect contractile responses to exogenous NKA, implying that its primary site of action was on capsaicin-sensitive sensory nerve endings. In functional studies of this kind, the presence of prejunctional ion channels and receptors can only be inferred from the resulting smooth muscle response. However, the finding NS1619 also inhibited the firing of single C-fibers evoked by bradykinin indicates that the inhibition of e-NANC responses by NS1619 does indeed reflect an inhibitory effect on C-fiber endings.

We have previously shown that C-fibers and Aδ-fibers, corresponding to RARs described in vivo, innervating the guinea pig airways, are both highly mechanically sensitive, but differ in their response to applied agents. Thus, while C-fibers are excited by agents such as capsaicin, bradykinin, and protons (24, 32), Aδ-fibers do not appear to be chemosensitive but are excited by changes in osmolarity (25). In the present study we found that, in addition to C-fibers, NS1619 also evoked a marked inhibition of Aδ-fiber firing in response to hyper- and hypoosmolar solutions applied directly onto their receptive fields. Thus, NS1619 elicits a nonspecific suppression of sensory nerve activity in the guinea pig airways via a likely action on fiber endings.

Patch clamp studies have shown that in isolated smooth muscle cells from bovine trachea (21), rat portal vein (20), and basilar artery (22), NS1619 reversibly activates a hyperpolarising outward current resulting from the opening of BK_{Ca} channels. A similar increase in the opening of BK_{Ca} channels was seen in ventromedial hippocampal neurones (23). In all cases the effect of NS1619 was inhibited by iberiotoxin, a highly potent and selective blocker of BK_{Ca} channels (16, 33, 34). However, NS1619 also blocked voltage operated calcium channels and K_{DR} channels, and NS1619-evoked relaxation of both the basilar artery and portal vein was concluded to be due to a direct inhibition of calcium currents, rather than via BK_{Ca} channel mediated cellular hyperpolarisation, since the relaxation was not fully prevented by iberiotoxin or charybdotoxin. The precise cellular mechanism underlying the inhibitory effect of NS1619 in the present study cannot be elucidated from the techniques used here. However, the fact that the inhibition of both afferent fiber firing and e-NANC bronchoconstriction was fully prevented by iberiotoxin strongly argues for the activation of BK_{Ca} channels. Although an inhibition of voltage-sensitive calcium currents on the sensory nerve terminal could conceivably reduce transmitter release and consequently e-NANC bronchoconstriction, it would not affect the propagation of afferent impulses, and hence the firing of individual fibers recorded here or cough in the conscious animal. A block of K_{DR} channels is unlikely to account for the inhibitory effect of NS1619 since this would tend to increase, rather than decrease, the excitability of airway smooth muscle, and sensory fibers if present on their terminals. Similarly, the activation of K_{ATP} channels, which has also been shown to inhibit e-NANC responses (35) is unlikely to be involved since NS1619 does not have any activity at this channel type (23). The present findings therefore suggest that NS1619 activates BK_{Ca} channels on sensory nerve endings in the guinea pig airways. The resulting hyperpolarization would tend to reduce the excitability of the sensory neurones leading to a reduction in stimulus-evoked afferent impulses and transmitter release.

In addition to the inhibition of fiber activity and e-NANC bronchoconstriction, NS1619 also inhibited cough in conscious guinea pigs. Cough is a central reflex elicited by the activation of sensory receptors throughout the tracheobronchial tree. Although there is still some debate concerning the class of receptor primarily responsible for cough, it is likely that both RARs and C-fibers are involved. The most common tussive stimulus in man and animal models is citric acid, and we have previously shown that low pH is a selective stimulus for C-fibers in the guinea pig isolated trachea (32). Moreover, both fiber excitation and cough elicited by low pH and capsaicin are inhibited by the capsaicin antagonist capsazepine (6, 32), implying a common mechanism of action. The inhibition of citric acid-evoked cough by NS1619 would therefore appear to reflect an inhibitory action on C-fibers, although it is also possible that fibers outside the trachea could behave differently. Because Aδ-fibers were also potently inhibited, it is likely that cough elicited by the activation of these fibers would also be inhibited by NS1619, although this is difficult to test because there is no selective stimulus for these fibers. While the common inhibition of both afferent fiber firing and cough does suggest a cause–effect relationship, an indirect inhibition of cough by NS1619 via, for example, an effect on airway blood flow or mucus secretion cannot be ruled out. Similarly, it is possible that NS1619 may affect upper airway afferents which could influence the citric acid-evoked cough reflex.

The inhibition by BK_{Ca} channel activation of both local and central reflex pathways could have important implications for airway disease. As discussed earlier, tachykinins released from the peripheral endings of capsaicin-sensitive C-fibers in the airways can have a variety of inflammatory effects in the airways. Consequently much emphasis has been placed on the postjunctional receptors mediating this neurogenic inflammation, and how it may be modulated. However, C-fiber activation also elicits bronchoconstriction, mucus secretion, vasodilatation, and cough resulting from the initiation of central reflexes (5). In addition, RARs mediate cough as well as reflex bronchoconstriction, vasodilatation, and mucus secretion in animal models (4, 5, 36). Considerable evidence suggests that this central reflex pathway may be important in man. For example, inhalation of a variety of irritants or inflammatory mediators such as distilled water, hypertonic saline, bradykinin, and prostaglandins elicit cough or atropine-sensitive bronchoconstriction in man, indicative of a central reflex (37–40). All these agents have been shown to excite C-fibers or RARs in animal models (24, 25, 41, 42). In the normal airway these reflex effects are defensive, serving to limit the access of inhaled irritants into the lower airways. However, during conditions of inflammation such as asthma they may become exacerbated and harmful, contributing to the characteristic hypersecretion, edema, cough, and hyperreactivity. Indeed, an increased sensitivity of the cough reflex is a common feature of asthma, and other conditions such as viral infection (43, 44). Such exacerbated reflexes may result from a sensitizing effect of inflammatory mediators on airway sensory nerves, and in guinea pigs we have found that bradykinin elicits a profound sensitization of airway C-fibers that is accompanied by a corresponding enhancement of citric acid-evoked cough (45). Taken together, these considerations indicate that an inhibition of the activity of both C-fibers and RARs could be desirable.

In conclusion, we have shown that NS1619 inhibits the activity of myelinated and nonmyelinated sensory fibers innervating the guinea pig airways via activation of BK_{Ca} channels. These data suggest that selective BK_{Ca} channel openers could be of benefit in the treatment of airway disease by reducing both local and central airway reflexes resulting from the excitation and sensitization of sensory fibers by mediators released during inflammatory conditions.

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