Inhibition of Cholinergic Neurotransmission in Guinea Pig Trachea by NS1619, a Putative Activator of Large-Conductance, Calcium-Activated Potassium Channels\(^1,2\)

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**ABSTRACT**

Indirect functional studies suggest that large-conductance calcium-activated potassium channels (BK\(_{\text{ca}}\) channels) are involved in the control of ACh release from postganglionic, parasympathetic nerve terminals in the airways. The role of BK\(_{\text{ca}}\) channels in regulating cholinergic neurotransmission was assessed by 1) investigating the effect of the putative BK\(_{\text{ca}}\) channel opener NS1619 on cholinergic contractile responses and ACh output evoked by electrical field stimulation (EFS; 40 V, 0.5 ms, 4 Hz for 15 s every 4 min) and comparing the effect obtained with the inhibition of EFS-evoked ACh release by oxotremorine M, a muscarinic agonist, and 2) evaluating the sensitivity of these responses to the BK\(_{\text{ca}}\) channel blocker iberiotoxin (IbTX). NS1619 (30 \(\mu\)M) inhibited cholinergic contractile responses by 60.0%. In contrast, NS1619 had no effect on contractile responses evoked by exogenous ACh (1 \(\mu\)M), which indicated that it was acting prejunctionally. NS1619 (30 \(\mu\)M) significantly inhibited EFS-induced ACh release by 33.9%. Oxotremorine M suppressed EFS-evoked ACh release in a concentration-dependent manner (at 1 \(\mu\)M, 77.4% inhibition). In neither case was the inhibition reversed by IbTX (100 nM).

Collectively, the mechanical data suggest that NS1619 inhibits cholinergic contractile responses by interacting prejunctionally. The failure of IbTX to reverse the inhibitory action of NS1619 and oxotremorine M on ACh release indicates that activation of muscarinic autoinhibitory receptors is not coupled to the opening of IbTX-sensitive BK\(_{\text{ca}}\) channels. Therefore, we propose that caution be exercised when using NS1619 as an activator of BK\(_{\text{ca}}\) channels.

The cholinergic parasympathetic nervous system plays a dominant role in the control and regulation of airway tone in animals and humans (Barnes, 1993). A number of functional studies measuring changes in airways smooth muscle contractility suggest that cholinergic neurotransmission is modulated by a variety of receptors that are located prejunctionally on cholinergic nerve terminals (Barnes, 1994) and that, on activation, inhibit or facilitate neurotransmitter release. In particular, it has been reported that activation of prejunctional receptors with alpha-2 adrenoceptor agonists (Grundstrom et al., 1981), beta-2 agonists (Rhoden et al., 1988), neuropeptide Y (NPY; Strетton and Barnes, 1988), \(\mu\)-opioid agonists (Belvisi et al., 1990, 1992) and vasoactive intestinal peptide (VIP; Ellis and Farmer, 1989) inhibits ACh release in the airways. It is noteworthy, however, that this conclusion is derived entirely on indirect evidence obtained from experiments where the agonist in question more effectively suppressed changes in airways smooth muscle tone elicited by EFS than contractions evoked by exogenous ACh. We have recently reported that this indirect method of measuring cholinergic neurotransmission is, in fact, not predictive of changes in ACh release per se and have concluded that neurotransmitter output can be unambiguously monitored only if it is measured directly (Belvisi et al., 1996).

By measuring ACh output directly, we and others have confirmed the presence of prejunctional muscarinic autoinhibitory receptors in guinea pig (Kilbinger et al., 1991; Patel et al., 1995) and human trachea (Patel et al., 1995; Wessler et al., 1995; Ten Berge et al., 1996). In the CNS, inhibition of neurotransmitter output is thought to require the opening of prejunctional K\(^+\) channels, membrane hyperpolarization and a reduction in Ca\(^{++}\) influx via voltage-activated Ca\(^{++}\) channels (North et al., 1987; Miller, 1990). Several K\(^+\) channels have been described on nerve cells, including BK\(_{\text{ca}}\) channels (Reinhart et al., 1989) which in airways smooth muscle are...
important regulators of membrane potential and intrinsic tone (Murray et al., 1991; Kotlikoff, 1993). Indeed, there is evidence that postjunctural BK_{Ca} channels are involved, at least in part, in mediating the relaxant action of beta adrenergic agonists on airways smooth muscle (Jones et al., 1990; Miura et al., 1992a). In contrast, relatively few studies have investigated the role of BK_{Ca} channels in prejunctional neuromodulation. Indirect evidence from our laboratory suggests that in guinea pig and human airways, inhibition of EFS-induced cholinergic contractile responses after activation of a number of prejunctional receptors is mediated by a common charybdoxin-sensitive mechanism; this suggests that BK_{Ca} channels might play an important role (Miura et al., 1992b). However, charybdoxin is not selective for BK_{Ca} channels and has been reported to inhibit voltage-gated K+ channels (K_{v1.3} type) in lymphocytes (Sands et al., 1989) and brain (Vazquez et al., 1990) and BK_{Ca} channels in Aplysia neurons (Hermann and Erxleben, 1987).

Recently, Sellers and Ashford (1994) described a novel compound, NS1619 (Olesen et al., 1994), that increased the open-state probability of BK_{Ca} channels in hypothalamic neurons dissociated from rat coronal brain slices. The effect of NS1619 was concentration-dependent and was reversed by the selective BK_{Ca} channel antagonist IbTX (Gelvez et al., 1989). Moreover, NS1619 was selective for the BK_{Ca} channels over the ATP-sensitive K+ channels in the same preparation (Sellers and Ashford, 1994). Given that indirect functional studies suggest that BK_{Ca} channels play a role in regulating ACh output from cholinergic neurons innervating airways smooth muscle, experiments were designed 1) to examine the effect of the putative BK_{Ca} channel activator NS1619 on EFS-induced cholinergic contractile responses and ACh output, and compare them with the inhibition of EFS-evoked ACh release by oxo M, an agonist at prejuncrional muscarinic autoinhibitory receptors (Patel et al., 1995), and 2) to evaluate the sensitivity of NS1619- and oxo M-induced responses to IbTX.

Materials and Methods

Preparation of guinea pig trachea. Male Dunkin-Hartley guinea pigs (Harlan-Olac; 300–500 g) were killed by cervical dislocation. The lungs, with trachea and bronchi attached, were rapidly removed and placed in oxygenated KHS of the following composition (in mM): NaCl 118, KCl 5.9, MgSO4 1.2, CaCl2 2.5, NaH2PO4 1.2, NaHCO3 25.5 and glucose 5.6. The trachea was dissected away from the lungs and main bronchi and opened longitudinally by cutting through the cartilage; the epithelium was subsequently removed by careful dissection, minimizing damage to the smooth muscle. Parasympathetic ganglia are located in the airway wall, external to smooth muscle and cartilage, so care was taken in obtaining a clean tracheal strip preparation. Even so, these ganglia may still be present and could represent another site of prejunctional modulation of EFS-evoked cholinergic contractile responses and ACh release. In our experiments this site is indistinguishable from prejunctional neuromodulation at the neuro-effector junction. However, under similar stimulation parameters described in our experiments, the ganglionic blocking drug hexamethonium has no effect on EFS-evoked cholinergic contractile responses (D’Agostino et al., 1990) or on EFS-evoked ACh release experiments in the isolated guinea pig tracheal preparation (Wessler et al., 1981). Iodomethacin (10 μM) was present throughout all experiments to prevent the formation of endogenous prostaglandins, which are known to affect cholinergic neurotransmission to the airways (Walters et al., 1984).

Measurement of EFS-induced cholinergic contractile responses. Eight transverse segments of trachea, each containing 3 to 4 cartilaginous rings, were prepared and suspended between parallel platinum-wire electrodes in 10-ml organ baths containing KHS at 37°C that was continually gassed with a 95% O2/5% CO2 mixture. The tissues were allowed to equilibrate for 1 h with frequent washing under a resting tension of 1 g, which was optimal for determining changes in muscle tone. Isometric contractile responses were measured with force-displacement transducers (model FT-03; Grass Instruments, Quincy, MA) connected to a polygraph (Model 7D; Grass Instruments). EFS was delivered by two platinum-wire field electrodes inserted in parallel (10 mm apart) with the tissue suspended between them. A stimulator (model D445; Digitimer Ltd., Welwyn Garden City, Hertfordshire, U.K.) provided monophasic square-wave pulses of supramaximal voltage (40 V) at source of 0.5-ms duration.

For experiments involving cholinergic contractile responses, EFS was applied for 15 s every 4 min at a frequency of 4 Hz, which evoked rapid increases in tone that were approximately 50% of the maximal EFS-induced contraction. After at least four stable responses of equal magnitude were obtained, NS1619 (1–30 μM) or its vehicle (0.1% ethanol) was added, and EFS-induced cholinergic contractile responses were elicited until the maximal effect of the drug was observed. One concentration of NS1619 was tested per tissue. In some tracheal strips, the effect of IbTX (100 nM) was studied on EFS-induced cholinergic contractile responses in the absence and presence of NS1619 (30 μM). In the latter experiments, IbTX (100 nM) was added at a time when NS1619 had produced a maximal inhibitory effect. This was after approximately 50 min. Measurements for the effect of IbTX were taken when the maximal effect of this drug was observed. The effect of NS1619 (30 μM) on EFS-evoked cholinergic contractile responses was also investigated after pretreatment of the tissues with IbTX (100 nM) for 30 min. Contractile responses obtained after EFS under the aforementioned conditions were abolished by atropine (1 μM) and tetrodotoxin (3 μM), which indicates that the generation of tension was due solely to the release of ACh from parasympathetic nerves.

Measurement of contractile responses evoked by exogenous ACh. In a separate series of experiments, we investigated the effect of NS1619 (1–30 μM) on contractile responses evoked by the application of 1 μM ACh, which elicited contractions that were similar in magnitude to those evoked by EFS (40 V, 0.5-ms pulse width, 4 Hz for 15 s). After three control responses to 1 μM ACh had been obtained, NS1619 was added for 50 min (the time it took NS1619 (30 μM) to produce maximal inhibition of EFS-evoked contractile responses), and the contractile response to a further addition of 1 μM ACh was analyzed. In a separate series of experiments, cumulative concentration-response curves to ACh (1 nM–10 mM) were compared before and after incubation of the tissues with NS1619 (1–30 μM) for 50 min. One concentration of drug was tested per tissue.

Measurement of ACh release from parasympathetic nerves. The release of ACh from cholinergic nerves was measured as previously described (Ward et al., 1993). Briefly, eight strips of smooth muscle with the cartilage and epithelium removed were studied in parallel. Each tissue was connected top and bottom with silver wire and mounted in a jacketed chamber. Tissues were superfused (Watson-Marlow model 503S; Smith and Nephew, Falmouth, U.K.) at a rate of 1 ml/min throughout the experiment with oxygenated KHS (pH 7.4) maintained at 37°C. The tissues were allowed to equilibrate for 30 min, during which time they were continuously superfused with KHS solution. EFS (40 V, 0.5-ms pulse width, 4 Hz) was delivered continuously, for the last 10 min, via the silver-wire electrodes. Tissues were then placed into vials containing 1.5 ml of oxygenated KHS supplemented with [3H]-choline (67 nM; specific radioactivity: 3.15 Tbq/nmol), and EFS was applied (40 V, 0.5-ms pulse width, 4 Hz) for 45 min to facilitate uptake of [3H]-choline into cholinergic nerve terminals. At the end of this period, tissues were superfused with KHS containing hemicholinium-3 (10 μM) to pre-
vent the reuptake of unlabeled choline into the nerves. Preparations were washed for 2 h before the beginning of the experiment to achieve a stable base line of tritium release. During this period the superfusate was discarded. It has been shown previously that most of the tritium outflow evoked by EFS of epithelium-intact trachea is \(^{3} \text{H}\)-phosphorylcholine in addition to \(^{3} \text{H}\)-ACh, whereas EFS of epithelium-denuded tracheal preparations does not elicit significant release of \(^{3} \text{H}\)-phosphorylcholine (Wessler et al., 1990). Furthermore, the release of ACh after EFS of guinea pig trachea is better maintained in the presence of indomethacin (D’Agnostino et al., 1990). Accordingly, in the studies described herein, epithelium-denuded tissue preparations were used, and indomethacin (10 \(\mu\)M) was present throughout (Ward et al., 1993; Patel et al., 1995).

EFS (40 V, 0.5-ms pulse width, 4 Hz for 1 min) was applied to each tissue, and 1-ml samples were collected every minute for 3 min before, 1 min during and 3 min after stimulation and at 5-min intervals outside these times. Previous studies in the authors’ laboratory have confirmed that the tritium released during EFS under the aforementioned conditions is frequency-dependent and tetrodotoxin-sensitive and is, therefore, neuronal in origin (Ward et al., 1993). Furthermore, ACh, at a concentration that evoked a contrac-

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Effects of NS1619 on contractile responses evoked by EFS and exogenous ACh. EFS (40 V, 0.5-ms pulse width, 4 Hz for 15 s every 4 min) of guinea pig trachea evoked rapid cholinergic contractile responses that were stable and highly reproducible. Addition of NS1619 (1–30 \(\mu\)M) to tissues produced a concentration-dependent inhibition of EFS-induced contractile responses that amounted to 12.6 \pm 4.0\% (\(n = 5\), N.S.), 40.3 \pm 8.1\% (\(n = 4\), P < .05) and 60.0 \pm 7.3\% (\(n = 7\), P < .05) at 1, 10 and 30 \(\mu\)M, respectively, when compared with control responses elicited before the addition of NS1619 (figs. 1A and 2). In contrast, addition of NS1619 (1–30 \(\mu\)M) for 50 min had no significant effect on the contraction evoked by an equi-effective concentration of exogenous ACh (1 \(\mu\)M) when compared with responses elicited before the addition of NS1619 (figs. 1C and 2). NS1619 (1–30 \(\mu\)M) had no effect on the cumulative concentration-response curves to ACh (table 1).

Effect of IbTX on EFS-induced cholinergic contractile responses in the absence and presence of NS1619. In a separate series of experiments, IbTX (100 \(\mu\)M) significantly, but not completely, reversed the inhibitory action of NS1619 (30 \(\mu\)M) on EFS-induced cholinergic contractile responses (40.6 \pm 7.4\% reversal, \(n = 5\), P < .05; figs. 1A and 3). However, IbTX alone (100 \(\mu\)M) consistently caused a transient increase in basal tone (70.7 \pm 125.5 mg tension, \(n = 5\); fig. 1B), and so EFS-evoked contractile responses were evaluated when the contraction had returned to base line. However, the immediate changes in tone produced by IbTX may make interpretation of the data difficult. For these reasons we performed experiments where tissues were pretreated with IbTX (100 \(\mu\)M) before the addition of NS1619. In these experiments the inhibitory action of NS1619 (30 \(\mu\)M) on EFS-evoked cholinergic contractile responses was not attenuated (618.8 \pm 126.6 mg before treatment; 731.8 \pm 144.5 mg after IbTX and 95.8 \pm 43.0 mg upon the addition of NS1619, \(n = 6\), P < .05). In time-matched, vehicle control experiments, there was no change in EFS-evoked cholinergic contractile responses.

Effect of NS1619 and oxo M on EFS-induced ACh output from cholinergic nerves. Addition of NS1619 (1–30 \(\mu\)M) to guinea pig trachea for 15 min elicited a concentration-dependent inhibition of ACh output evoked by EFS (40 V, 0.5-ms pulse width, 4 Hz for 15 s) greater than 80.0 \% while IbTX (100 \(\mu\)M) had no effect on ACh output (fig. 3A). NS1619 (30 \(\mu\)M) and IbTX (100 \(\mu\)M) together caused a further significant inhibition of ACh output (fig. 3B). This inhibition was not attenuated by preincubation of the tissues with NS1619 (30 \(\mu\)M) and IbTX (100 \(\mu\)M) (fig. 3C).

**Results**

**Effect of NS1619 on cholinergic contractile responses evoked by EFS and exogenous ACh.** EFS (40 V, 0.5-ms pulse width, 4 Hz for 15 s every 4 min) of guinea pig trachea evoked rapid cholinergic contractile responses that were stable and highly reproducible. Addition of NS1619 (1–30 \(\mu\)M) to tissues produced a concentration-dependent inhibition of EFS-induced contractile responses that amounted to 12.6 \pm 4.0\% (\(n = 5\), N.S.), 40.3 \pm 8.1\% (\(n = 4\), P < .05) and 60.0 \pm 7.3\% (\(n = 7\), P < .05) at 1, 10 and 30 \(\mu\)M, respectively, when compared with control responses elicited before the addition of NS1619 (figs. 1A and 2). In contrast, addition of NS1619 (1–30 \(\mu\)M) for 50 min had no significant effect on the contraction evoked by an equi-effective concentration of exogenous ACh (1 \(\mu\)M) when compared with responses elicited before the addition of NS1619 (figs. 1C and 2). NS1619 (1–30 \(\mu\)M) had no effect on the cumulative concentration-response curves to ACh (table 1).

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that amounted to 33.9 ± 9.1% (P < .05, n = 8) at the highest concentration (30 μM) examined (figs. 4A and 5A). Oxo M (0.01–10 μM) also inhibited ACh release in a concentration-dependent manner with a maximal effect (77.4 ± 8.9% inhibition, n = 56, P < .05) observed at 1 μM (figs. 4B and 5B).

Furthermore, the inhibitory action of NS1619 (30 μM) and oxo M (30 nM) at the concentrations used for reversal experiments, did not diminish over the time course of the experiments (24.5 ± 5.1% and 29.5 ± 6.0% inhibition for NS1619 (30 μM) at 15 and 50 min, respectively, n = 6; 41.4 ± 8.2% and 41.1 ± 16.4% inhibition for oxo M (30 nM) at 15 and 50 min, respectively, n = 6). Separate time-control experiments showed that ACh release did not diminish over the three EFS periods used in ACh-release protocols (second EFS: 4.5 ± 14.8% inhibition, n = 8, N.S.; third EFS: 4.4 ± 11.8% inhibition, n = 8, N.S. compared to the first control EFS).

**Effect of IbTX on the inhibitory action of NS1619 and oxo M on EFS-induced ACh output from cholinergic nerves.** The inhibitory action of NS1619 seemed to be mediated by a mechanism(s) unrelated to the opening of BK Ca channels, because treatment of tracheal strips with IbTX (100 nM) failed to reverse significantly the inhibitory action of NS1619 (30 μM) on cholinergic transmission (before IbTX: 33.4 ± 3.4% inhibition, n = 8, P < .01; after IbTX: 17.2 ± 18.5% inhibition, n = 8, N.S.; fig. 6). In those experiments the inhibitory action of NS1619 on EFS-evoked ACh release was consistent, but the ability of IbTX to reverse this inhibitory action was variable. In 5 out of 8 tissues, addition of IbTX in the presence of NS1619 inhibited ACh release (values ranging from 41.4% to 73.1% inhibition), whereas in the remain-

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**Table 1**

Effect of NS1619 on cumulative concentration-response curves evoked by ACh in guinea pig trachea

<table>
<thead>
<tr>
<th>Treatment</th>
<th>pD2 Before</th>
<th>pD2 After</th>
<th>Maximal Contraction to ACh (mg tension) Before</th>
<th>Maximal Contraction to ACh (mg tension) After</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle control</td>
<td>5.47 ± 0.25</td>
<td>5.37 ± 0.2</td>
<td>898.6 ± 158.5</td>
<td>819.7 ± 160.8</td>
</tr>
<tr>
<td>1 μM NS1619</td>
<td>5.12 ± 0.25</td>
<td>5.107 ± 0.11</td>
<td>1425 ± 280.2</td>
<td>1446 ± 226.5</td>
</tr>
<tr>
<td>10 μM NS1619</td>
<td>5.46 ± 0.23</td>
<td>5.17 ± 0.15</td>
<td>1762 ± 135.1</td>
<td>1654 ± 135.5</td>
</tr>
<tr>
<td>30 μM NS1619</td>
<td>5.18 ± 0.15</td>
<td>5.02 ± 0.2</td>
<td>1371 ± 181.5</td>
<td>1496 ± 174.1</td>
</tr>
</tbody>
</table>

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*Fig. 2.* The effect of NS1619 (1–30 μM) on cholinergic contractile responses evoked by EFS (40 V, 0.5-ms pulse width, 4 Hz for 15 s; filled bars) and on contractile responses evoked by exogenous ACh (1 μM; open bars) epithelium-denuded tracheal strips. Data-points represent the percentage change from control responses preceding drug administration and are expressed as mean ± S.E.M. of 4 to 7 independent observations. *P < .05 compared with contractile responses preceding drug administration by Student’s paired t test.

*Fig. 3.* The effect of IbTX (100 nM) on the inhibitory action of NS1619 (30 μM) on EFS-induced cholinergic contractile responses (40 V, 0.5-ms pulse width, 4 Hz for 15 s) in epithelium-denuded guinea pig tracheal strips. Data-points represent the mean ± S.E.M. of five independent observations. *P < .05, **P < .01 compared using ANOVA, followed by the Bonferroni correction test.

*Fig. 4.* Profile of the effect of NS1619 (30 μM; panel a) and oxo M (30 nM; panel b) on ACh release evoked by EFS (40 V, 0.5-ms pulse width, 4 Hz for 1 min) in a single guinea pig tracheal strip. The results are expressed as rate coefficient (×10³), which is a measure of the fractional [³H] release plotted against time (minutes).
ing 3 of the 8 tissues, ACh release was facilitated (values ranging from 3.9% to 29% facilitation compared with the first control stimulation). IbTX alone (100 nM) had no statistically significant effect on ACh output, although marked variability was noted (fig. 6).

Compared with respective control tissues, IbTX (100 nM) did not reverse the inhibitory action of oxo M (30 nM) on EFS-induced ACh release (before IbTX: 50 ± 4.1% inhibition, n = 9, P < .01; after IbTX, 48.5 ± 6.2% inhibition, n = 9, P < .01; fig. 6).

**Discussion**

Two experimental observations provide persuasive evidence that the putative BKCa channel opener NS1619 inhibited ACh release from cholinergic nerve terminals innervating guinea pig trachea by a prejunctional mechanism. First, NS1619 suppressed EFS-induced cholinergic contractile responses without affecting tension generation elicited by exogenous ACh. Second, NS1619 significantly inhibited the tetrodotoxin-sensitive release of [3H]-ACh from tracheal smooth muscle preparations in response to EFS. A direct measurement of ACh output was considered prudent because EFS-induced changes in smooth muscle tone are not always predictive of events purported to occur prejunctionally (Belvisi et al., 1996).

The involvement, at least in part, of neuronal BKCa channels in the inhibition of cholinergic neurotransmission effected by NS1619 was suggested by the finding that the highly selectively inhibitor of BKCa channel activation IbTX reversed NS1619-induced suppression of EFS-induced cholinergic contractile responses by ~40%. However, this interpretation must be advanced cautiously, because a consistent finding of these studies was that IbTX increased basal airways smooth muscle tone, which is in complete agreement with previous reports by other investigators (Baker et al., 1994). Indeed, it was considered possible that the interaction of IbTX with postjunctional BKCa channels on the airways smooth muscle (Jones et al., 1990; Miura et al., 1992a) might complicate the interpretation of the data obtained from functional studies and could, in fact, be responsible for an apparent reversal of the inhibitory effects of NS1619 on cholinergic contractile responses. For these reasons we performed experiments in which the effects of NS1619 on cholinergic contractile responses were analyzed in IbTX-pretreated tissues. In these experiments, the inhibitory action of 30 μM NS1619 on EFS-evoked cholinergic contractile responses was not attenuated. These data suggest that the apparent reversal of the inhibitory effects of NS1619 may be due to some postjunctional action of IbTX on BKCa channels on smooth muscle and that the inhibitory action of NS1619 on cholinergic responses was not due to the opening of BKCa channels.

Given these potential limitations of functional measurements as indicators of ACh release, we performed further studies to determine unequivocally whether BKCa channels regulate cholinergic neurotransmission by measuring ACh release directly.

Using this technique, we obtained evidence that NS1619 inhibited EFS-induced cholinergic neurotransmission, which is consistent with the results obtained from the functional studies described above. However, in contrast to airway sen-
sory nerves, where NS1619 activates BK$_{Ca}$ channels and inhibits nerve function in an IbTX-reversible manner (Fox et al., 1997). IbTX failed to reverse significantly the inhibitory effect of NS1619. This difference may be attributed to the different nerve types investigated, which may have different channel populations present that are also activated by NS1619, and/or to different subtypes of BK$_{Ca}$ channels. Indeed, two types of BK$_{Ca}$ channels (type I and type II) have been demonstrated electrophysiologically in rat brain plasma membrane vesicles incorporated into planar lipid bilayers (Reinhart et al., 1989, 1991) and in nerve terminals of the rat neurohypophysis (Wang et al., 1992). Type I BK$_{Ca}$ channels are readily blocked by IbTX, whereas type II channels are resistant to IbTX and charybdotoxin. Therefore, it is possible that inhibition of cholinergic neurotransmission in guinea pig airways is mediated via type II BK$_{Ca}$ channels.

However, a more likely scenario based on these data is that NS1619 suppresses EFS-induced ACh output by interacting with other types of prejunctional channels or other effectors of exocytosis that ultimately govern neurotransmission. Indeed, in electrophysiological and functional experiments, NS1619 inhibits the voltage-dependent delayed rectifier (K$_{s}$) and t-type calcium channels in rat portal vein (Edwards et al., 1994) and rat cerebral artery smooth muscle (Holland et al., 1996). NS1619 similarly prevents levocromakalim-induced activation of K$_{s}$ channels in rat portal vein smooth muscle (Edwards et al., 1994). It is conceivable, therefore, that an action on NS1619 at one or more of these channels could account for the suppression of EFS-induced cholinergic neurotransmission.

One of the possible mechanisms may include an inhibitory action of NS1619 on Ca$^{2+}$ channels. The influx of Ca$^{2+}$ through voltage-dependent Ca$^{2+}$ channels is the central stimulus for transmitter release in central and peripheral neurons (Miller, 1990). N-type Ca$^{2+}$ channels have been found to regulate stimulus-induced release of ACh and noradrenaline from central neurons and from neuroeffector junctions within the peripheral nervous system (Wessler et al., 1990). Similarly, t-type Ca$^{2+}$ channels are involved in the regulation of catecholamine release from chromaffin cells (Cena et al., 1983) and in the control of EFS-evoked ACh release from enteric neurons of the guinea pig colon (Marino et al., 1993). Therefore, the possibility that NS1619 inhibits EFS-induced ACh release by directly blocking Ca$^{2+}$ channels clearly warrants investigation.

In a parallel set of experiments, we assessed the role of BK$_{Ca}$ channels in the inhibition of ACh release via activation of M$_{2}$-muscarinic autoinhibitory receptors. Activation of the M$_{2}$-receptor by the nonselective agonist oxo-M inhibited EFS-evoked ACh release in a concentration-dependent manner. We reasoned that if this receptor subtype mediates the inhibitory effects on ACh release solely via the opening of BK$_{Ca}$ channels, then the effects of oxo M should be abolished by IbTX. The inability of IbTX to reverse the inhibitory effect of oxo M suggests that activation of BK$_{Ca}$ channels is not necessary for the inhibition of ACh release from cholinergic nerve terminals. This conclusion is consistent with a previous study demonstrating that activation of BK$_{Ca}$ channels is not required for the suppression of ACh release in guinea pig trachea by alpha-2 adrenergic receptor and muscarinic agonists (Baker et al., 1994).

In conclusion, our data suggest that the inhibitory action of NS1619 on EFS-evoked ACh release is not mediated by activation of IbTX-sensitive BK$_{Ca}$ channels. Moreover, oxo M suppresses ACh output by interacting at the level of the cholinergic nerve terminals via a mechanism that does not involve the opening of IbTX-sensitive channels.

References


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