

Effect of nedocromil sodium on neurogenic mechanisms in vitro

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We have developed a guinea pig model that allows monitoring of single afferent nerve fiber activity after stimulation of tracheal nerve endings with various stimuli. Action potentials from nodose or jugular neurons are recorded extracellularly. Previous experiments have shown that the excitability of the nerve endings to, for example, mechanical stimuli can be increased by antigen challenge of presensitized guinea pigs. Nedocromil sodium 10^{-4} mol/L significantly reduced by more than 50% the generation of action potentials in C fibers in response to stimulation by capsaicin. The precise mechanism is not known, although the membrane depolarization induced by capsaicin was reduced in the presence of nedocromil sodium. This agent did not have a generalized inhibitory effect on afferent excitability because it had no effect on sensitivity to electrical or mechanical stimuli. Nedocromil sodium did not affect the generation of action potentials in A δ and C fibers in the presence of hypertonic saline solution. (J Allergy Clin Immunol 1996;98:S107-11.)

Key words: Afferent, airways, axon-reflex, efferent, nodose ganglia, parasympathetic ganglia, sensory nerve, vagus nerve

The vast majority of nerve fibers innervating the airways are carried by the vagus nerves and consist primarily of preganglionic efferent parasympathetic fibers and afferent fibers. Much of our information on the function of airway nerves is based on in vivo physiologic studies and in vitro studies with use of electrical field stimulation of extrinsically denervated airway segments. During the past several years we have attempted to obtain a more detailed understanding of the function and modulation of airway innervation by use of in vitro preparations in which the extrinsic innervation is kept intact. More specifically, we have isolated the vagus nerves, trachea, and mainstem bronchi from guinea pigs to study the physiologic and pharmacologic features of the sensory and parasympathetic innervation with use of conventional electrophysiologic techniques (Fig. 1). These techniques are ideally suited for the study of the neuropharmacologic features of airway innervation. In this report we describe these experimental models and discuss some preliminary studies on the effect of

Abbreviation used

fEPSP: Fast excitatory postsynaptic potential

nedocromil sodium on sensory and parasympathetic activity in the guinea pig airway in vitro.

SENSORY INNERVATION

The vagal sensory innervation to the guinea pig trachea and bronchi is derived mainly from cell bodies located in the jugular and nodose ganglia (Fig. 1). Each nodose ganglion can be visualized as a small swelling along either vagus nerve adjacent to the carotid sinus; the jugular ganglion is rostral to the nodose ganglion at a plexus of cranial nerves just inside the jugular foramen. Both of these ganglia send myelinated and unmyelinated axons to the trachea and bronchi in the vagus, recurrent, and superior laryngeal nerves. These fibers are referred to as A fibers (myelinated fibers, fast conducting) and C fibers (unmyelinated, slow conducting). The A fibers are further subdivided on the basis of their conduction velocity. The A fibers innervating the trachea and bronchial wall are for the most part relatively slow-conducting A fibers, referred to as A δ fibers. Many of the C fibers are associated with capsaicin sensitivity and contain

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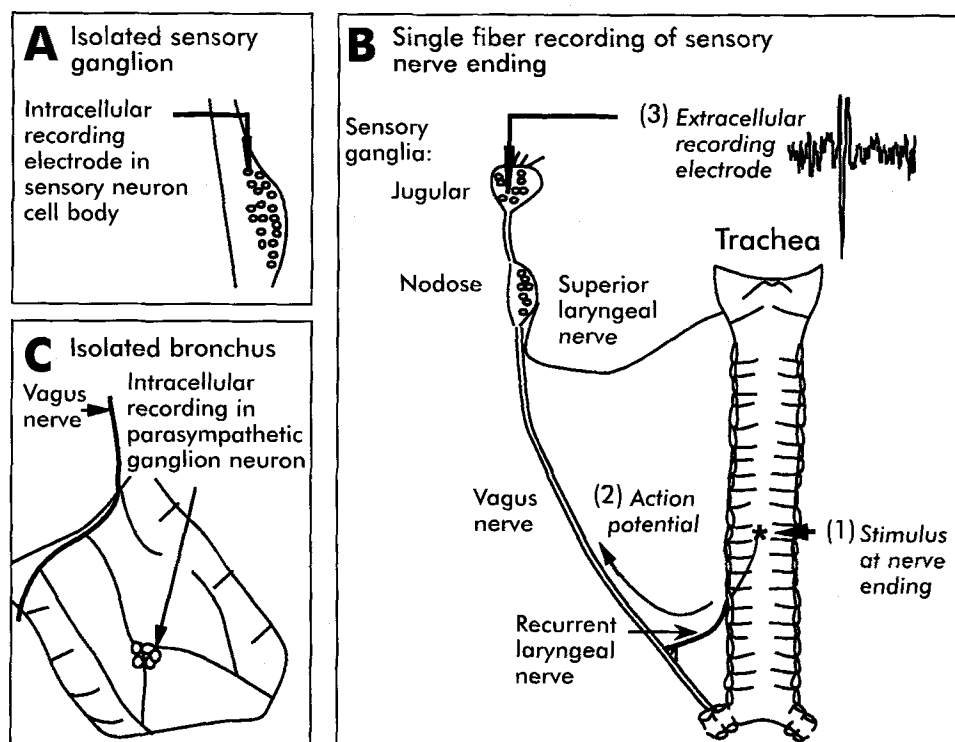


FIG. 1. Different preparations used in the study of airway nerves in vitro. **A**, A sensory nerve cell body (somata) located in the jugular or nodose ganglion is impaled with a recording microelectrode. Active and passive membrane properties and receptors affecting these properties can be analyzed. **B**, Stimuli (1) (such as pressure, osmolality, and pH) at nerve endings evoke action potentials (2) that are recorded with an extracellular electrode near cell bodies in sensory ganglia (3). **C**, A cell body located in a bronchial parasympathetic ganglion is impaled with a recording microelectrode. Active and passive membrane properties and vagus nerve-stimulated synaptic potentials are monitored before and after pharmacologic or immunologic stimulation.

neuropeptides such as substance P and calcitonin gene-related peptide.

We have developed two in vitro preparations to study sensory nerve physiologic and pharmacologic features: (1) intracellular recording of active and passive membrane properties of individual neurons located in the sensory ganglia (Fig. 1, *A*), and (2) extracellular recording of action potentials from nodose or jugular neurons elicited by various stimuli at a single nerve ending in the airway wall (Fig. 1, *B*).

Our knowledge of the cellular mechanisms underlying afferent nerve activation is limited primarily by the relative inaccessibility of the nerve terminals to direct intracellular recording. For this reason, insights into the ability of various stimuli to influence membrane function at the nerve endings have been obtained by inference from studies on the cell body. We have used conventional intracellular recording techniques to study the electrophysiologic features of somal membranes of vagal

afferent neurons.^{1,2} The sensory ganglion is isolated from the animal, pinned out in a recording chamber, and superfused with oxygenated buffer solution (Fig. 1, *A*). With this technique we have characterized the passive and active electrophysiologic properties of vagal sensory neurons. This technique is also useful to evaluate mechanistically the influence of exogenously applied¹ and endogenously released² inflammatory mediators on neuronal excitability.

Intracellular electrophysiologic studies of the membrane properties of sensory cell bodies provide insights into how mediators and drugs can influence neural excitability. The limitation of this model lies in the assumption that the cell body in the vagal ganglion accurately reflects properties of the nerve endings in the airway wall. To begin to address this limitation, we have recently used an in vitro preparation to assess the excitability of the sensory nerve endings in the airway wall. In this experimental design, which is a modification of

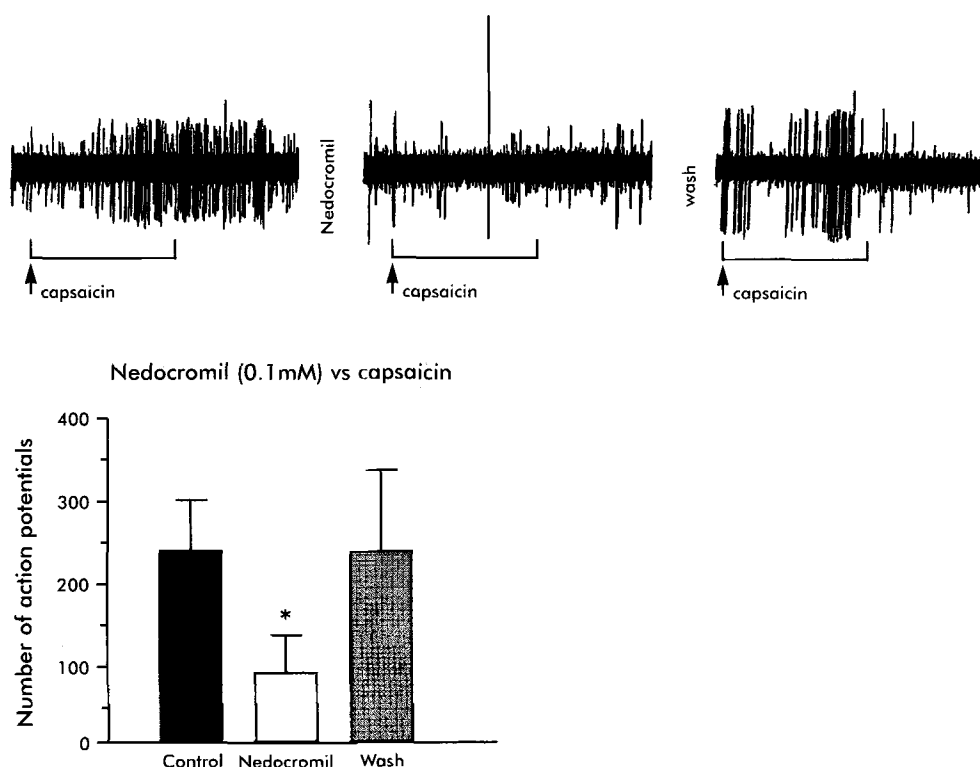


FIG. 2. Representative recording of a C fiber unit potential with a receptive field in the guinea pig-isolated superfused (8 ml/min) trachea. The tissue was exposed to capsaicin, 0.3 $\mu\text{mol/L}$ for 5 minutes (denoted by horizontal bar). The number of action potentials for 10 minutes beginning with the application of capsaicin was quantified with use of offline data analysis (note: in this example wave form analysis revealed that there were actually two distinct units stimulated by capsaicin, but only the larger of the units was quantified). After washing the tissue for 15 minutes, nedocromil sodium (100 $\mu\text{mol/L}$) was added beginning 10 minutes before the second application of capsaicin (in data not shown we have found that two consecutive capsaicin applications result in similar numbers of action potentials). The tissue was again superfused with drug-free buffer for an additional 15 minutes, and then capsaicin was applied for the third time. The histogram shows the average ($\pm$ SEM; $n = 4$) number of action potentials elicited by capsaicin before, during, and after nedocromil sodium treatment (as previously described).

that reported by Fox et al.,³ the guinea pig trachea and mainstem bronchi are isolated with the vagus nerves and sensory ganglia intact (Fig. 1, B). Action potentials arriving from the nerve endings in the airway wall are monitored by means of an extracellular electrode placed near the cell bodies in the nodose or jugular ganglion. With use of this technique, the activity of single afferent fibers can be quantified.

To evaluate the "excitability" of nerve endings, we have quantified the amount of current (with use of small concentric electrodes placed over the receptive field in the airway) and mechanical force (with use of calibrated von Frey's hairs) required to stimulate action potentials in mechanosensitive nerve endings in the trachea.⁴ We noted that release of mediators, by means of antigen challenge of previously sensitized airways, results in a

reproducible increase in the excitability of the nerve endings such that the amount of force necessary to stimulate the ending is reduced approximately threefold after antigen challenge.⁴

PARASYMPATHETIC ACTIVITY

Parasympathetic neurons innervate airway glands, vasculature, and smooth muscle. In this efferent pathway, preganglionic axons arise from neurons located in midbrain dorsal nuclei and travel in the vagus nerve to synapse on small clusters of parasympathetic neurons located in ganglia dispersed throughout the dorsal surface of the trachea and bronchi (Fig. 1, C). The predominant excitatory neurotransmitter released from preganglionic terminals is acetylcholine. Synaptic transmission involves the release of acetylcholine, which diffuses across the synaptic cleft to bind to nicotinic cholinergic recep-

TABLE I. Effect of nedocromil sodium on vagus nerve-evoked synaptic events in bronchial ganglion neurons

	Nedocromil sodium*			
	Control	10 $\mu\text{mol/L}$	50 $\mu\text{mol/L}$	100 $\mu\text{mol/L}$
% Failures†	8 $\pm$ 7	14 $\pm$ 8	41 $\pm$ 12‡	40 $\pm$ 10‡
% Threshold fEPSPs†	67 $\pm$ 29	47 $\pm$ 19	47 $\pm$ 24	23 $\pm$ 11‡
fEPSP amplitude (mV)	10 $\pm$ 2	7.5 $\pm$ 3	5.8 $\pm$ 2‡	5.6 $\pm$ 2‡

*Nedocromil sodium was bath applied to intrinsic ganglion neurons ($n = 3$ to 6 for each value) on the guinea pig bronchus in vitro for 1 to 5 minutes before the event was quantified.

†Vagus nerve stimulation evokes either no response (failure) or an fEPSP that is either subthreshold (no action potential elicited) or threshold for generating an action potential. Fifty vagus nerve stimuli are averaged for each n value for control and treated groups.

‡ $p < 0.05$ compared with control value.

tors located predominantly on the dendrites of principal ganglion neurons. The released acetylcholine causes a passive transient depolarization, referred to as the fast excitatory postsynaptic potential (fEPSP). It has been demonstrated that tracheal and bronchial intrinsic ganglion neurons act as filters, that is, they do not transmit every signal emanating from the central nervous system into a postganglionic action potential.⁵ Thus slight changes in the resting membrane potential or membrane resistance of the neurons can greatly affect the net output from the ganglia.

With use of conventional intracellular recording techniques, we have characterized active and passive membrane properties and synaptic transmission in guinea pig bronchial parasympathetic ganglia⁵ (Fig. 1, C). Bronchial ganglion neurons exhibit two distinct action potential profiles during a 500 msec depolarizing step: 60% of the neurons fire a single or phasic burst of action potentials and accommodate, whereas the remaining cells fire tonically throughout the step. A neuron displaying the accommodating action potential pattern is very limited in its ability to fire high-frequency action potentials.⁵ With use of the same preparation, electrical stimulation of the vagus nerve results in measurable fEPSPs; thus this model is ideally suited to study the pharmacologic and physiologic features of synaptic neurotransmission. As reported for sensory neurons, this preparation allows the study of the effect of endogenously released and exogenously applied mediators on the excit-

ability of bronchial parasympathetic neurons and changes in synaptic efficacy.^{6,7}

Changes in the excitability of sensory fibers can indirectly lead to an increase in parasympathetic activity by means of the activation of central reflex pathways. Stimulation of tachykinin-containing afferent C fibers in the airways may also influence transmission through parasympathetic ganglia by means of peripheral reflex activity. Anatomic evidence indicates that tachykinin-containing sensory nerve fibers are localized within airway parasympathetic ganglia in many species, including guinea pigs and humans.^{8,9} With use of electrophysiologic and functional studies, we recently noted that stimulation of these tachykinin-containing fibers results in activation of the local parasympathetic ganglion neurons.^{10,11} This local stimulation of autonomic neurons by transmitters released from afferent terminals is referred to as a peripheral reflex.

EFFECT OF NEDOCROMIL SODIUM

We have begun an investigation into the effects of nedocromil sodium (100 $\mu\text{mol/L}$) on A δ and C fibers in the guinea pig isolated trachea preparation. Nedocromil sodium significantly reduced the response of C fibers to capsaicin. In four experiments the response to capsaicin (0.3 $\mu\text{mol/L}$) was reversibly reduced by more than 50% by nedocromil sodium (Fig. 2). Similar effects on canine airway C fibers in vivo have been reported.¹² The mechanism of this inhibitory effect of nedocromil sodium has not yet been determined. However, in our intracellular electrophysiologic studies on sensory cell somata, we noted that capsaicin caused a membrane depolarization and a decrease in input impedance. In two experiments the membrane depolarization induced by capsaicin (0.5 $\mu\text{mol/L}$) was 6 and 5 mV in the absence of nedocromil sodium and 1 and 0 mV in the presence (100 $\mu\text{mol/L}$) of the drug.

Nedocromil sodium did not have a generalized inhibitory effect on afferent excitability. Bath-applied nedocromil sodium (100 $\mu\text{mol/L}$) for 15 minutes had no effect on the voltage sensitivity of afferent fibers in the guinea pig-isolated trachea. In five fibers, for example, the average voltage applied to the receptive field with a concentric stimulating electrode was 13.2 $\pm$ 6.8 V and 12.7 $\pm$ 6.7 V in the absence and presence, respectively, of nedocromil sodium. The mechanical sensitivity of the afferent fibers was also unaffected by nedocromil sodium treatment. For example, it required an average force of 0.35 $\pm$ 0.12 g and 0.41 $\pm$ 0.10 g to stimulate C fiber endings in the

absence and presence of nedocromil sodium, respectively ($n = 5$, $p > 0.1$). Both A δ and C fibers respond with action potential formation to a hypertonic stimulus (7% saline solution). The number of action potentials elicited by the hypertonic challenge was not different in the absence or presence of nedocromil sodium (100 μ mol/L; $n = 3$ A δ fibers and $n = 3$ C fibers).

Nedocromil sodium may also have multiple effects on the excitability of airway parasympathetic ganglion neurons. The most dramatic effect of nedocromil sodium was on synaptic transmission: nedocromil sodium, 50 μ mol/L, decreased the amplitude of fEPSPs by 42% ($n = 4$), and 100 μ mol/L caused a 44% decrease ($n = 6$; Table I).

CONCLUSION

With use of conventional electrophysiologic recording techniques, we are able to study the mechanisms by which autacoids, drugs, and other stimuli influence the afferent and efferent limb of airway innervation. Our preliminary studies with nedocromil sodium demonstrate that this drug does not have a generalized inhibitory effect on airway afferent nerve ending excitability. Rather, nedocromil sodium had a selective inhibitory effect; of the stimuli tested, only the response to capsaicin was notably inhibited. In addition, we provide evidence that an inhibition of the efficacy of transmission through the bronchial parasympathetic ganglia may contribute to the inhibitory effect of nedocromil sodium on reflex activity in vivo.

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