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Influence of mexiletine on action potential discharge and conduction in nodose Aδ afferent neurons innervating guinea pig isolated trachea

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Abstract

Local anesthetics are among the most effective peripherally acting antitussives. A complete understanding of their pharmacological properties in airway afferent neurons associated with the cough reflex has been hampered by an incomplete understanding of the contribution of various classes of afferent neuron to cough. The aim of this study was to evaluate the influence of the antitussive local anesthetic mexiletine on nodose ganglion-derived vagal afferent Aδ-fibers innervating guinea pig trachea. This distinct subtype of airway sensory neuron was recently shown to be involved in evoking cough in anaesthetized guinea pigs. The current findings demonstrate that a concentration of mexiletine sufficient to inhibit citric acid- or mechanically-induced action potential initiation at the nerve ending did not block action potential conduction along axons. These findings are indicative of differences in sensitivity to local anesthetics of highly specialized regions of afferent neurons involved in initiation or conduction of impulses.
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1. Introduction

Local anesthetics such as mexiletine and lidocaine are effective at inhibiting cough evoked by irritants and mechanical or chemical stimuli [1–5]. Multiple subtypes of airway afferent neurons are associated with the cough reflex. The fiber described in the classical studies of Widdicombe and co-workers conducts in the Aδ range, adapts rapidly to maintained mechanical stimuli and is commonly referred to as a Rapidly Adapting Receptor (RAR). The inhibitory influence of the local anesthetic procaine on cat tracheaobronchial RARs has been described [6].

More recently, a subtype of tracheal and laryngeal afferent neuron responsible for initiating cough in anaesthetized guinea pigs was identified as a population distinct from classical RARs. These fibers are acid-sensitive, low threshold mechanosensors arising from the nodose ganglia. They differ from RARs in several important ways including their slower conduction velocity, insensitivity to changes in transpulmonary pressures and stretch and their insensitivity to bronchoconstrictors [7]. In addition, the receptive fields of these fibers are primarily localized to the extrapulmonary airways whereas RARs, at least in guinea pigs are localized to the intrapulmonary airways [7]. Their sensitivity to local anesthetics has not been described.

The aim of the current study was to evaluate the influence of the antitussive local anesthetic mexiletine [1] on action potential discharge in the recently described distinct subtype of tracheal afferent fiber involved in evoking cough in guinea pigs. The isolated, innervated tracheal preparation used allowed a comparison of the inhibitory influence of mexiletine on impulse generation at the receptive field and impulse conduction along the axon. The findings of this study are indicative of regional differences in susceptibility of nodose Aδ-fibers associated with the cough reflex to local anesthetics. In particular, mexiletine had an inhibitory effect on the generation of action potentials at the nerve ending at a concentration below that required to block conduction of action potentials in axons.

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2. Materials and methods

2.1. Tissue preparation

Male Hartley guinea-pigs (200–400 g) were killed by asphyxiation with CO_2 and exsanguinated. The trachea and primary bronchi with intact right-side extrinsic innervation (including nodose ganglia) were removed and placed in a dissecting dish containing Krebs bicarbonate buffer solution (KBS). Connective tissue was carefully trimmed away from the trachea, larynx and right bronchus leaving the vagus, superior laryngeal, and recurrent nerves, including nodose ganglia intact. A longitudinal cut was made to open the larynx, trachea and bronchus along the ventral surface. Airways were then pinned, mucosal surface up, to a Sylgard-lined Perspex chamber. The right nodose ganglia, along with the rostralmost vagus and superior laryngeal nerves, were gently pulled through a small hole into an adjacent compartment of the same chamber for recording of single fiber activity. Both compartments were superfused with KBS gassed with 95% O_2–5% CO_2. The temperature was maintained at 35 °C with a flow rate of 6–8 ml min^{-1}.

2.2. Extracellular electrophysiological recording

Extracellular recordings were performed as previously described [8]. Briefly, a fine aluminosilicate glass microelectrode pulled using a Flaming/Brown micropipette puller (Model P97, Sutter Instrument Company, Novato, CA, USA) and filled with 3 M sodium chloride was placed into an electrode holder and connected directly to a headstage (A-M Systems, Everett, WA, USA). A return electrode of silver–silver chloride wire and a silver–silver chloride pellet ground were placed in the perfusion fluid of the recording chamber and attached to the headstage. The recorded signal was amplified (A-M Systems) and filtered (low cut-off = 0.3 kHz; high cut-off = 1 kHz) and the resultant activity was displayed on an oscilloscope (TDS 430, Tektronix, Beaverton, OR, USA) and digitized (CED 1401, Cambridge Electronic Design, Cambridge, UK). The data were stored for off-line analysis on a PC computer using the software program Spike 2 for Windows version 4 (Cambridge Electronic Design).

2.3. Discrimination of single fiber activity, location of receptive field and estimation of conduction velocity

Single fiber activity in the airway was discriminated by placing a concentric electrical stimulating electrode (Model RH- NE-100, David Kopf Instruments, Tujunga, CA, USA) on the recurrent laryngeal nerve, through which the majority of fibers enter the trachea [8]. The recording electrode was placed within the nodose ganglion and manipulated until single unit activity was detected. When electrically evoked action potentials were detected, the stimulator (Grass Model S48, AstroMed, W. Warwick, RI, USA) was switched off and the trachea and bronchi were gently brushed with a von Frey filament (Stoelting, Wood Dale, IL, USA). Mechanically-sensitive receptive fields were revealed when a burst of action potentials was elicited in response to von Frey filament stimulation. Conduction velocities were determined prior to and after 20 min after the addition of the indicated concentration of mexiletine to the buffer solution superfusing the tracheal preparation. Conduction velocity was determined by electrically stimulating the receptive field (at approximately twice threshold voltage; see below) and monitoring the time elapsed between the shock artifact and the recorded action potential. This delay was divided by the distance between the receptive field and the recording electrode to yield a conduction velocity. In some cases, a second stimulating electrode was placed at a point of the nerve trunk that carried the axon of the fiber. Conduction velocity from this point was determined by electrically stimulating the nerve trunk and monitoring the time elapsed between the shock artifact and the recorded action potential. Only mechanically sensitive neurons were studied. These nerve fibers had little or no activity at rest, if spontaneous activity exceeded 0.5 action potential s^{-1}, the fiber was not studied further.

2.4. Electrical threshold

Electrical threshold of identified receptive field was determined as previously described [9] prior to and 20 min after the addition of the indicated concentration of mexiletine to the buffer solution superfusing the tracheal preparation. Briefly, electrical stimulation of tracheal afferent nerve endings was achieved by placing the concentric stimulating electrode in juxtaposition to identified mechanically sensitive receptive fields. Stimuli delivered to the electrode were a 0.1 ms square pulses, delivered at various voltages, at 1 Hz. Stimuli voltage was initially supramaximal, evoking a shock artifact, each successfully followed by an action potential that was easily observed on the oscilloscope screen. Threshold stimulation voltages were determined by decreasing the voltage to the electrode until at least three consecutive shock artifacts (delivered at 1 Hz) were not followed by action potentials. Care was taken to avoid movement of the stimulating electrode throughout the experiment.

2.5. Citric acid challenge

Citric acid challenge was performed essentially as previous described [10] prior to and 20 min after the addition of the indicated concentration of mexiletine to the buffer solution superfusing the tracheal preparation. Briefly, the plastic tip of a pipette, filled with 200 μl of a 1 mM citric acid solution made up in 0.9% saline, was positioned just beneath the surface of the superfusion solution at the site of the receptive field. The contents of the pipette tip was expelled over a time of approximately 2 s. Particular caution

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