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# Evidence for Nicotinic Acetylcholine Receptors on Nasal Trigeminal Nerve Endings of the Rat

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## Abstract

The peripheral chemoreceptors of the trigeminal system in the nasal cavity are presumed to be free nerve endings arising from Aδ and C fibers. These fibers appear to be scattered throughout the nasal epithelium, and arise from the nasopalatine and ethmoid branches of the trigeminal nerve. In the present study, the effects of nicotinic acetylcholine receptor (nAChR) blockers on ethmoid nerve responses to nicotine and cyclohexanone were examined. Multiunit neural recordings were obtained from the ethmoid nerve of Sprague–Dawley rats. Vapor-phase nicotine (12.5 p.p.m.) and cyclohexanone (450 p.p.m.) were delivered to the rats' nares via an air-dilution olfactometer. The magnitude of the response to nicotine decreased after the administration of the nAChR blockers dihydro-β-erythroidine hydrobromide (DHBE) and mecamylamine hydrochloride. DHBE is a competitive nicotinic receptor antagonist specific for the α4β2 receptor subtype and mecamylamine is known to bind α3β4 and α4β2 receptors. The nAChR blockers had no effect on ethmoid nerve responses to cyclohexanone. These results suggest that the mechanism by which at least one irritant stimulates nasal trigeminal nerve endings involves the binding of irritant with a specific receptor.

## Introduction

In most mammals the nasal cavity houses chemoreceptors for the olfactory, vomeronasal and trigeminal systems. Olfactory and vomeronasal receptors are found in discrete locations and zones within the nasal cavity and play significant roles in a variety of functions, ranging from detection of food to reproductive behavior. In contrast, trigeminal nerve endings are distributed throughout the nasal cavity and appear to function, in part, as a detection system for irritants and potentially noxious chemicals. Trigeminal nerve fibers respond to a variety of substances and are part of what has been traditionally called the common chemical sense. More recently, the term *chemesthesis* has been used to denote that trigeminal chemoreceptors are actually temperature and pain fibers, and therefore part of the somatosensory system (Green *et al.*, 1990).

Nasal trigeminal chemoreceptors are believed to be intraepithelial free nerve endings arising from Aδ and C fibers of the nasopalatine and ethmoid branches of the trigeminal nerve (Silver, 1992). The anterior nasal mucosa is innervated by the ethmoid nerve, which upon entering the nasal cavity bifurcates repeatedly, sending unmyelinated and thinly myelinated fibers with diameters of 0.2–1.5 μm throughout the respiratory epithelium. Stimulation of these nerve endings can potentially produce chemogenic pain (tingling, burning and stinging) and trigger protective reflex movements of rejection or withdrawal (Silver, 1992). These reflex movements, along with the sensory perceptions of irritation and pain, serve to minimize exposure to noxious substances.

Although we know that various chemical stimuli elicit responses from trigeminal nerve fibers, the stimulatory mechanisms and the processes mediating the response are not fully understood. Several lines of evidence suggest that the chemosensitivity of trigeminal nerve endings in the nasal cavity may be receptor mediated. For example, sensory irritation effects can be described by Michaelis–Menten or equivalent equations, which would be consistent with a reversible bimolecular reaction between a receptor and its ligand. Also, sensory irritation responses can fade, indicating that a desensitization process, a well-known phenomenon related to receptors, is occurring (Nielsen, 1991).

One of the most potent stimuli of nasal trigeminal chemoreceptors is nicotine. Electrophysiological recordings from rat ethmoid nerves show high trigeminal nerve sensitivity to vapor-phase nicotine, with the threshold response at 5 p.p.m. (Silver, 1992). Evidence for the involvement of specific receptors in nasal trigeminal chemosensitivity to nicotine comes from experiments utilizing electrophysiological recordings from the rat ethmoid nerve, which showed differing trigeminal response to the stereoisomers of nicotine (Walker *et al.*, 1996). Differential responses to *S*(–)-

and *R*(+)-nicotine suggest that specific receptor proteins are involved in trigeminal chemosensitivity to nicotine.

In the protonated form, nicotine bears a strong structural similarity to acetylcholine, and one of the principal biological targets of nicotine action is the nicotinic acetylcholine receptor (nAChR). Nicotinic acetylcholine receptors are diverse members of the ligand-gated ion channel superfamily of neurotransmitter receptors, and are found throughout the central nervous system, in the autonomic ganglia and at the vertebrate–neuromuscular junction. These receptors are fairly large transmembrane glycoproteins, with two functions: (i) to recognize and bind ligand; and (ii) to open a channel in the cell membrane through which cations can flow. The receptor–channel complex consists of a pentameric array of homologous subunits, with a combined molecular weight of ~275 kDa. Nicotinic acetylcholine receptor subunits are encoded by at least 16 different genes (α1–α9128, β1–β4, γ, δ, ε), 10 of which (α2–α7, α9 and β2–β4) are known to be expressed by trigeminal ganglion neurons (Lukas *et al.*, 1996; Liu *et al.*, 1998).

Functional neuronal nAChRs can be formed from combinations of α and β subunits, and in certain instances, a single type of α subunit. It is the α subunit that is believed to be primarily involved in receptor–ligand interactions, although recent experiments have shown that both neuronal subunits may be involved in agonist and antagonist binding (Stafford *et al.*, 1998). Neuronal nAChRs are found throughout the brain and have also been found in the peripheral nervous system, where they may be involved in sensory irritation responses. Experiments performed on human subjects show that perceived irritation in the lungs and lower airways in response to high nicotine cigarette smoke is drastically reduced by pretreatment with hexamethonium (Lee *et al.*, 1993). Hexamethonium, which is a nicotinic receptor antagonist, exerts its effects by binding nAChRs, blocking nicotine binding without opening the ion channel.

Evidence for nAChR expression in trigeminal sensory neurons has come from experiments utilizing electrophysiological and biochemical techniques. Using whole-cell patch-clamp recordings, nAChRs were identified in a group of cultured rat trigeminal ganglia neurons (Liu *et al.*, 1993), and sensory neurons of the rat trigeminal ganglion have been shown to express several nAChR subunits at the mRNA and protein levels (Flores *et al.*, 1996; Liu *et al.*, 1998). Although these studies provide evidence that sensory neurons in the rat trigeminal ganglion express nAChR subunits, they neither demonstrate the expression of receptors on trigeminal free nerve endings nor provide any direct information on what is occurring at the periphery, where the stimulus and nerve cell interact.

In the present study, we attempted to determine whether nAChRs might be present on trigeminal free nerve endings at the periphery. The nicotinic receptor antagonists dihydro-β-erythroidine hydrobromide (DHBE) and mecamylamine hydrochloride were employed to assess whether nAChRs may be playing a role in eliciting trigeminal nerve responses to nicotine and cyclohexanone. DHBE is a competitive nAChR antagonist specific for the α4β2 nicotinic receptor subtype and mecamylamine is a ganglionic blocking agent, known to bind α3β4 and α4β2 receptors. If these compounds block ethmoid nerve responses to nicotine, it would provide evidence that specific receptors at the periphery may be involved in the trigeminal nerve response to at least one irritant.

## Materials and methods

### Recording procedure

A total of 30 adult male Sprague–Dawley rats (350–750 g) were used in this study. Prior to surgery, each rat was anesthetized with an i.p. injection of urethane (ethyl carbamate: 1.0 g/kg). The procedure for recording from the ethmoid nerve has been described previously (Silver *et al.*, 1990).

### Stimulus delivery

Stimuli were delivered to the nares of the rats using a computer-controlled air-dilution olfactometer. The layout and operation of this olfactometer have been thoroughly described elsewhere (Silver *et al.*, 1990). In brief, the olfactometer operates by mixing a clean airstream with an airstream saturated with a volatile chemical. By mixing the two airstreams in specific proportions, airflows of different percent vapor saturation can be presented. Stimuli were delivered to the rats by positioning the end of the stimulus delivery tube directly in front of the nares. The stimuli used in this study, (–)-nicotine (Eastman Kodak Co., Rochester, NY) and cyclohexanone (Sigma, St Louis, MO), were delivered at 12.5 and 450 p.p.m. respectively. In preliminary experiments, these concentrations were shown to elicit equivalent responses from the rat trigeminal nerve, generating quantitatively similar levels of integrated neural activity. The olfactometer delivered a 10 s stimulus presentation to the nares of the rat at a flow rate of 2 l/min. Using a nasopharyngeal cannula attached to a vacuum, this airstream was pulled through the rat's nasal cavity at a controlled rate of 255 ml/min. Rats were stimulated once every 300 s, and each rat received a total of seven stimulus presentations over a 30 min period.

### Receptor blocker administration

The nicotinic acetylcholine receptor antagonists dihydro-β-erythroidine hydrobromide (DHBE) and mecamylamine hydrochloride (Research Biochemicals Inc., Natick, MA) were administered through an i.p. injection. Rats were injected with either DHBE or mecamylamine, at a concentration of $2.5 \times 10^{-5}$ mol/kg. Blockers were administered only once, immediately following the first stimulus presentation. One group of rats stimulated with nicotine was injected with

a physiological saline solution (1 ml/kg i.p.) in place of a receptor blocker. Rats in control groups did not receive any injections.

### Data analysis

The magnitudes of integrated responses were used to generate all data. Response magnitudes were defined as the amplitude difference from baseline activity 10 s after onset of the stimulus, and were measured using AcqKnowledge 3.2 software (Biopac Systems Inc., Goleta, CA) running on an IBM 365XD ThinkPad computer. In order to compare different rats, response magnitude data were normalized through a percent change calculation. Each response magnitude was calculated as a percentage of the first response, equating each rat's first response to 100%. Percent-response curves were generated by taking the means for each of the seven experimental groups and plotting the values against time. The seven percent-response curves were analyzed using multiple analyses of variance at each stimulus presentation. Significance was examined using Tukey's HSD ($P < 0.05$).

## Results

### Qualitative analysis

In rats stimulated with nicotine, the ethmoid nerve displayed a sharp and sustained decrease in response upon administration of either DHBE or mecamylamine (Figure 1a). This trend was in contrast to control and saline-injected rats, whose ethmoid nerves exhibited a steady response to nicotine over the entire length of the experiment. A considerable amount of inter-animal variability was observed within the control and saline-injected rats, with several of the animals exhibiting oscillations in response magnitude upon subsequent stimulus presentations. This pattern was not as pronounced in the mecamylamine and DHBE-injected rats, due to the antagonistic effects of the nAChR blockers. Variability in response magnitude in these rats was masked by the blocking action of the injections.

All rats responding to cyclohexanone showed a steady level of response over the whole 30 min period (Figure 1b). The same pattern of response was observed in control rats and rats injected with either DHBE or mecamylamine, indicating the ineffectiveness of nAChR blockers on the trigeminal nerve response to cyclohexanone. Based on these observations, cyclohexanone trials utilizing saline injections were not conducted.

### Analysis of variance

For rats stimulated with nicotine, no significant differences were found between non-injected rats (control) and rats injected with physiological saline (Figure 2a). This was true for the entire length of the experiment, at every stimulus presentation. Similar results were observed when comparing DHBE-injected rats and rats injected with mecamylamine.

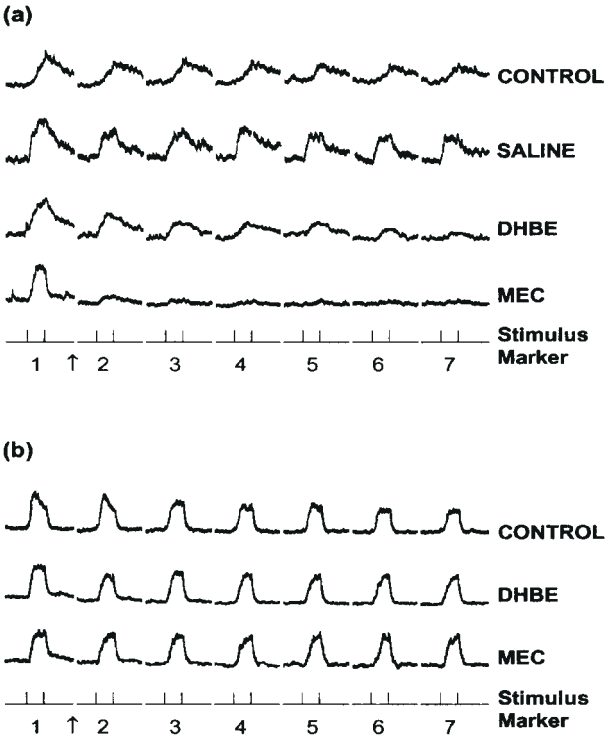


**Figure 1** Representative integrated ethmoid nerve responses to **(a)** nicotine and **(b)** cyclohexanone for non-injected rats (CONTROL), rats injected with 0.1 ml/kg physiological saline (SALINE), $2.5 \times 10^{-5}$ mol/kg dihydro-β-erythroidine hydrobromide (DHBE) and $2.5 \times 10^{-5}$ mol/kg mecamylamine hydrochloride (MEC). Each trace was obtained from a different rat. The two marks above each stimulus number correspond to the onset and offset of stimulus presentation. The time between the two marks is 10 s, with 300 s between each stimulus presentation. All injections were made i.p. (represented by the ↑). Note that the first responses on the graph were recorded before any injections were made.

Throughout the whole duration of the experiment, no significant differences in response magnitude were found between rats injected with either of the nAChR blockers. Significant differences were found when the control and saline groups were compared with the DHBE and mecamylamine groups ($P < 0.05$). Analysis of variance showed significant differences between the control and saline group rats and rats injected with nAChR blockers at every stimulus presentation (excluding the first response, which by definition is the same for all percent-response curves). For rats stimulated with cyclohexanone, analysis of variance showed no significant differences between control rats, rats injected with DHBE and rats injected with mecamylamine (Figure 2b).

## Discussion

The results of this study provide strong evidence for the expression of nAChRs in the nasal cavity, presumably on intraepithelial trigeminal nerve endings. Upon administration of either of the nAChR blockers DHBE and mecamylamine, the ethmoid nerve lost the ability to respond

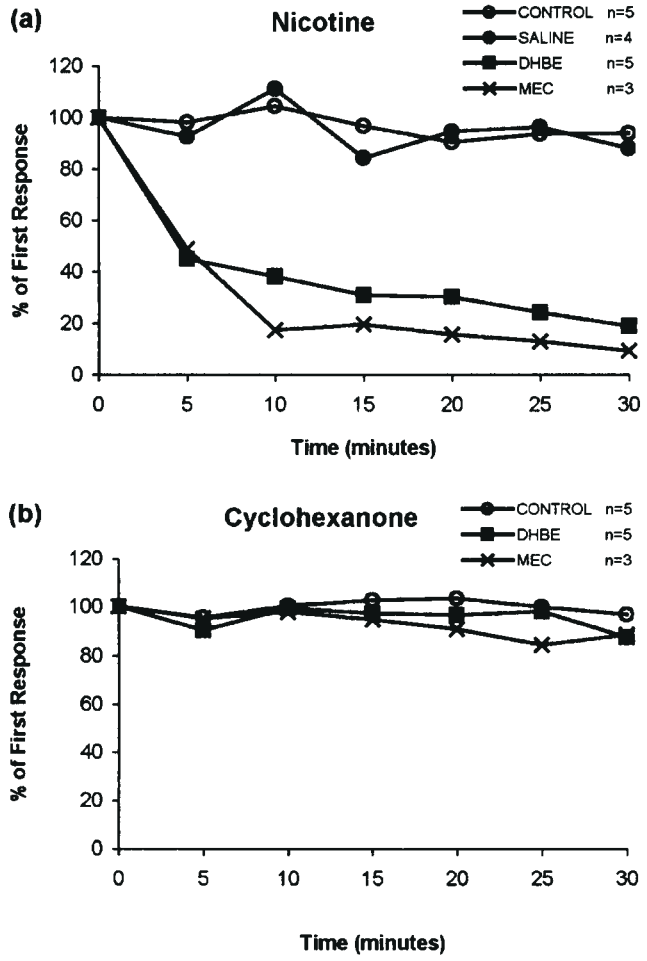


**Figure 2** Percent response curves showing changes in the magnitude of the ethmoid nerve response to **(a)** nicotine and **(b)** cyclohexanone over a 30 min time period (seven stimulus presentations). Administration of either dihydro-β-erythroidine hydrobromide (DHBE) or mecamylamine hydrochloride (MEC) resulted in a significant decrease in the ethmoid nerve response to nicotine ($P < 0.05$), whereas response to cyclohexanone remained unchanged. See text for details.

to nicotine, whereas responses to cyclohexanone remained unchanged. This research represents the first time that nAChR antagonists have been used to investigate trigeminal chemoreception of airborne stimuli entering the nasal cavity. Although DHBE, mecamylamine and other antagonists have previously been used to investigate the diversity and function of nAChRs in the central and peripheral nervous systems (Alkondon and Albuquerque, 1993), they have not been utilized to study the mechanisms underlying nasal trigeminal chemoreception. Most of the available information on the pharmacology of DHBE comes from receptor binding studies using brain nAChRs. It appears that in the brain, DHBE competitively binds the alpha subunits of high affinity nicotinic receptors (Yang *et al.*, 1994). The antagonistic effect of DHBE on different nAChR subtypes is diverse, with the α4β2 subtype being the most sensitive (Luetje *et al.*, 1990).

Compared with DHBE, there is more information available on mecamylamine and its pharmacological actions on nicotinic receptors. Mecamylamine is known to have diverse interactions with different nicotinic receptor subtypes, including α3β4 and α4β2 nAChRs, and acts as an antagonist to both the peripheral and central actions of nicotine (Lerner-Marmarosh *et al.*, 1995). For example, in the nicotinic synapse of frog and mammalian skeletal muscle, mecamylamine interacts with the open conformation of the ion channel, thereby acting as a noncompetitive antagonist. In contrast, neuronal nAChRs (those composed of only α and β subunits) are competitively blocked by mecamylamine. In these receptors, mecamylamine appears to bind to the agonist recognition sites of the alpha subunits (Varanda *et al.*, 1985).

The effectiveness of mecamylamine as a blocker of central neuronal nAChRs has been demonstrated in several experiments. In oral self-administration experiments performed on rats, administration of mecamylamine (5 mg/kg i.p.) has been shown to completely abolish nicotine preference, presumably through a blockade of the central rewarding property of nicotine (Glick *et al.*, 1996). Similarly in humans, mecamylamine has been shown to block the rewarding effects of cigarette smoking and to help reduce craving (Rose *et al.*, 1996).

In addition to the central neuronal nAChR data, a small number of experiments have been performed to test the effects of mecamylamine on sensory irritation by nicotine. Mecamylamine has been shown to reduce oral irritation by blocking the burning sensation elicited by nicotine, suggesting a role for nAChRs in the observed oral trigeminal chemosensitivity to nicotine (Jarvik and Assil, 1988; Dessirier *et al.*, 1998). Although these experiments do not address the question of nasal trigeminal chemoreception, they do show that nAChRs may have a function as peripheral oral receptors for at least one sensory irritant.

In the present study, both DHBE and mecamylamine were shown to be effective at selectively reducing nasal trigeminal sensitivity. As can be seen in Figures 1a and 2a, nasal chemosensitivity to nicotine was significantly reduced within 5 min of nAChR blocker administration. The magnitude of the response to nicotine sharply decreased after the administration of either nAChR blocker and stayed between zero and 20% of the first response (Figure 2a). In contrast, DHBE and mecamylamine had no effects on the trigeminal nerve response to cyclohexanone (Figures 1b and 2b).

Based on these results and what is known about the specificity of DHBE and mecamylamine for nAChRs, it appears that nicotinic receptors, specifically α3β4 and α4β2 subtypes, are expressed in the nasal cavity, presumably on trigeminal free nerve endings. Although up to 10 different nAChR subunits have been isolated in rat trigeminal ganglion neurons (Flores *et al.*, 1996; Liu *et al.*, 1998), it cannot be concluded that all are necessarily present in the periphery, contributing to functional chemoreceptors. What

can be concluded from these experiments is that receptors blocked by DHBE and mecamylamine are responsible for the trigeminal nerve response to nicotine, and that trigeminal sensitivity to cyclohexanone and nicotine is more than likely mediated through different receptive pathways.

The results of the present study are compatible with the results of recent studies examining neuronal nAChR expression in sensory neurons of the rat trigeminal ganglion (Flores *et al.*, 1996). These experiments suggest the expression of at least two different nAChR subtypes in the trigeminal ganglion with different densities and markedly differing affinities for [$^3$H]epibatidine. The results of these experiments offer compelling evidence for the possibility that in trigeminal ganglion cells, α3b4 nAChRs constitute a low affinity, high density binding site and that α4β2 nAChRs constitute a high affinity, low density binding site. The number of low affinity binding sites (α3β4) was measured to be ~2.5 times higher than the number of high affinity sites (α4b2) (Flores *et al.*, 1996). These experiments were limited to cell bodies in the trigeminal ganglion, and may not provide information as to what is actually being expressed on intraepithelial nerve endings within the nasal cavity. Nevertheless, if trigeminal ganglion neurons expressing nAChR mRNA also express nAChRs along their axons, then a greater proportion of the trigeminal nerve response to nicotine may be due to α3β4 receptors. This assumption would explain the observed qualitative differences between DHBE and mecamylamine-injected rats responding to nicotine (Figures 1a and 2a). Qualitatively, an injection of mecamylamine appears to have a larger impact than a DHBE injection. Although non-specific binding can occur with DHBE, one would expect a mecamylamine injection to have a slightly larger impact, since it is the less specific blocker and the one with the greater number of binding sites.

Interestingly, electrophysiological and pharmacological evidence shows that trigeminal ganglion cells with soma diameters ≤28 μm do not contain nAChRs (Liu *et al.*, 1993), and nicotinic receptor subunit mRNAs have been detected mostly in large- and medium-diameter neurons (Flores *et al.*, 1996). This suggests that the sensory perception of nicotine is not mediated by small C fiber neurons, but instead by large and medium neuronal populations which give rise to Aδ fibers. More extensive experimentation is required to verify these possibilities.

Within the nasal epithelium, peptidergic Aδ fibers immunoreactive to substance P and calcitonin gene-related peptide are found in the spaces between epithelial cells, rising to within a few micrometers of the surface (Finger *et al.*, 1990). When present in the nasal cavity, nicotine may diffuse down through epithelial layers, moving through transcellular and paracellular pathways to reach these chemosensitive fibers. All forms of nicotine have been shown to be similarly permeable through the nasal mucosa (Nair *et al.*, 1997). Whether charged or uncharged, nicotine can readily diffuse through epithelial layers to reach trigeminal nerve fibers. Nicotine may also reach these intraepithelial fibers through the line of tight junctions near the apical surfaces of respiratory epithelial cells. Tight junctions have previously been shown to be readily permeable to chemicals that activate trigeminal nerve fibers in the tongue (Simon *et al.*, 1991). If the same is true in the nasal cavity, paracellular pathways around nasal epithelial cells may be another route by which nicotine can reach free nerve endings. Once there, nicotine interacts with nAChRs on the nerve fiber, eliciting a trigeminal nerve response.

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## References

**Alkondon, M.** and **Albuquerque, E.X.** (1993) *Diversity of nicotinic acetylcholine receptors in rat hippocampal neurons. I. Pharmacological and functional evidence for distinct structural subtypes*. J. Pharmacol. Exp. Ther., 265, 1455–1473.

**Dessirier, J.-M., O'Mahony, M., Sieffermann, J.-M.** and **Carstens, E.** (1998) *Mecamylamine inhibits nicotine but not capsaicin irritation on the tongue: psychophysical evidence that nicotine and capsaicin activate separate molecular receptors*. Neurosci. Lett., 240, 65–68.

**Finger, T.E., St Jeor, V.L., Kinnamon, J.C.** and **Silver, W.L.** (1990) *Ultrastructure of substance P- and CGRP-immunoreactive nerve fibers in the nasal epithelium of rodents*. J. Comp. Neurol., 294, 293–305.

**Flores, C.M., DeCamp, R.M., Kilo, S., Rodgers, S.W.** and **Hargreaves, K.M.** (1996) *Neuronal nicotinic receptor expression in sensory neurons of the rat trigeminal ganglion: demonstration of the α3β4, a novel subtype in the mammalian nervous system*. J. Neurosci., 16, 7892–7901.

**Glick, S.D., Visker, K.E.** and **Maisonneuve, I.M.** (1996) *An oral self-administration model of nicotine preference in rats: effects of mecamylamine*. Psychopharmacology, 128, 426–431.

**Green, B.G., Mason, J.R.** and **Kare, M.R.** (eds) (1990) Chemical Senses, Vol. 2. Irritation. Marcel Dekker, New York.

**Jarvik, M.E.** and **Assil, K.M.** (1988) *Mecamylamine blocks the burning sensation of nicotine on the tongue.* Chem. Senses, 13, 213–217.

**Lee, L.Y., Gerhardstein, D.C., Wang. A.L.** and **Burki, N.K.** (1993) *Nicotine is responsible for airway irritation evoked by cigarette smoke inhalation in men*. J. Appl. Physiol., 75, 1955–1961.

**Lerner-Marmarosh, N., Kende, A.S., Wang, D.X.** and **Abood, L.G.** (1995) *Probing ion channels and recognition sites of neuronal nicotinic cholinergic receptors with novel nicotine affinity and other ligands.* Ann. NY Acad. Sci., 757, 120–132.

**Liu, L., Chang, G.-Q., Jiao, Y.Q.** and **Simon, S.A.** (1998) *Neuronal nicotinic acetylcholine receptors in rat trigeminal ganglia*. Brain Res., 809, 238–245.

**Liu, L., Pugh, W., Ma. H.** and **Simon, S.A.** (1993) *Identification of acetylcholine receptors in adult rat trigeminal ganglion neurons*. Brain Res., 617, 37–42.

**Luetje, C.W., Patrick, J.** and **Seguela, P.** (1990) *Nicotine receptors in the mammalian brain.* FASEB J., 4, 2753–2760.

**Lukas, R.J., Ke, L., Bencherif, M.** and **Eisenhour, C.M.** (1996) *Regulation by nicotine of its own receptors.* Drug Dev. Res., 38, 136–148.

**Nair, M.K., Chetty, D.J., Ho, H.** and **Chien, Y.W.** (1997) *Biomembrane permeation of nicotine: mechanistic studies with porcine mucosae and skin*. J. Pharm. Sci., 86, 257–262.

**Nielsen, G.D.** (1991) *Mechanisms of activation of the sensory irritant receptor by air-borne chemicals.* CRC Crit. Rev. Toxicol., 21, 183–208.

**Rose, J.E., Westman, E.C.** and **Behm, F.M.** (1996) *Nicotine/mecamylamine combination treatment for smoking cessation*. Drug Dev. Res., 38, 243–256.

**Silver, W.L.** (1992) *Neural and pharmacological basis for nasal irritation*. Ann. New York Acad. Sci., 641, 152–163.

**Silver, W.L., Walker, D.B., Ogden, M.W.** and **Walker, J.C.** (1990) *Nasal trigeminal responses to toluene presented by an automated delivery system*. Chem. Senses, 15, 701–712.

**Simon, S.A., Holland, V.F.** and **Zampighi, G.A.** (1991) *Tight junctions in taste buds: possible role in perception of intravascular gustatory stimuli*. Chem. Senses, 16, 69–80.

**Stafford, G.A., Oswlad, R.E., Figl, A., Cohen, B.N.** and **Weiland, G.A.** (1998) *Two domains of the beta subunit of the neuronal nicotinic acetylcholine receptors contribute to the affinity of substance P*. J. Pharmacol. Exp. Ther., 286, 619–626.

**Varanda, W.A., Aracava, Y., Sherby, S.M., VanMeter, W.G., Eldefrawi, M.E.** and **Albuquerque, E.X.** (1985) *The acetylcholine receptor of the neuromuscular junction recognizes mecamylamine as a noncompetitive antagonist*. Mol. Pharmacol., 28, 128–137.

**Walker, J.C., Kendal-Reed, M., Keiger, C.J., Bencherif, M.** and **Silver, W.L.** (1996) *Olfactory and trigeminal responses to nicotine*. Drug Dev. Res., 38, 160–168.

**Yang, X., Buccafusco, J.J.** and **Pauly, J.R.** (1994) *Pharmacological evaluation of methylcarbamylcholine induced drinking behavior in rats*. Pharmacol. Biochem. Behav., 49, 1–6.

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