Selectivity of Lingual Nerve Fibers to Chemical Stimuli

Y. WANG, R. P. ERICKSON, and S. A. SIMON

From the Departments of Neurobiology, Anesthesiology, and Psychology, Duke University, Durham, North Carolina 27710

ABSTRACT The cell bodies of the lingual branch of the trigeminal nerve were localized in the trigeminal ganglion using extracellular recordings together with horseradish peroxidase labeling from the tongue. Individual lingual nerve fibers were characterized with regard to their conduction velocities, receptive fields, and response to thermal, mechanical, and chemical stimuli. Fibers were classified as C, Aδ, Aβ, cold, and warm. The chemical stimuli included NaCl, KCl, NH_4Cl, $CaCl_2$, menthol, nicotine, hexanol, and capsaicin. With increasing salt concentration the latency of the response decreased and the activity increased. The responses elicited by salts (to 2.5 M), but not nonpolar stimuli such as menthol, were reversibly inhibited by 3.5 mM of the tight junction blocker, $LaCl_3$. These data suggest that salts diffuse into stratified squamous epithelia through tight junctions in the stratum corneum and stratum granulosum, whereupon they enter the extracellular space. 11 C fibers were identified and 5 were characterized as polymodal nociceptors. All of the C fibers were activated by one or more of the salts NaCl, KCl, or NH_4Cl. Three C fibers were activated by nicotine (1 mM), but none were affected by $CaCl_2$ (1 M), menthol (1 mM), or hexanol (50 mM). However, not all C fibers or even the subpopulation of polymodals were activated by the same salts or by nicotine. Thus, it appears that C fibers display differential responsiveness to chemical stimuli. Aδ fibers also showed differential sensitivity to chemicals. Of the 35 characterized Aδ mechanoreceptors, 8 responded to NaCl, 9 to KCl, 9 to NH_4Cl, 0 to $CaCl_2$, menthol, or hexanol, and 2 to nicotine. 8 of 9 of the cold fibers (characterized as Aδ's) responded to menthol, none responded to nicotine, 8 of 16 were inhibited by hexanol, 9 of 19 responded to 2.5 M NH_4Cl, 5 of 19 responded to 2.5 M KCl, and 1 of 19 responded to 2.5 M NaCl. In summary, lingual nerve fibers exhibit responsiveness to chemicals introduced onto the tongue. The differential responses of these fibers are potentially capable of transmitting information regarding the quality and quantity of chemical stimuli from the tongue to the central nervous system.

INTRODUCTION

Chemical stimuli introduced on the anterior two-thirds of mammalian tongues elicit responses from both special sensory (chorda tympani) and general sensory (lingual

Address correspondence to Dr. Sidney A. Simon, Department of Neurobiology, Duke University, Durham, NC 27710.

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nerve) fibers (Beidler, 1969). Chorda tympani fibers form synapses with taste cells (Murray, 1971; Kinnamon, 1988) in fungiform papillae and thus relay information regarding the quality and concentration of chemical stimuli to the taste centers in central nervous system (CNS). Lingual nerve fibers terminate in the lingual epithelium itself or in the papillary layer, and a few even terminate (without forming synapses) in taste buds (Dastur, 1961; Whitehead, Beeman, and Kinsella, 1985; Yamasaki, Kubota, and Tohyama, 1985; Kinnman and Aldskogius, 1988). Lingual fibers are responsive to chemical, thermal, and mechanical stimuli and therefore also contribute to the sensations produced by food placed on the tongue (Zotterman, 1936; Hensel and Zotterman, 1951*b*; Benzing, Hensel, and Wurster, 1969).

Activation of chorda tympani fibers by chemical stimuli occurs indirectly. That is, chemical stimuli initially interact with receptors on microvilli on taste cells that project from the taste cells into the oral cavity. The taste cells then depolarize and release transmitters and/or peptides onto receptors on chorda tympani fibers (Roper, 1989). The mechanisms by which chemical stimuli elicit responses from lingual fibers are not as well understood. Chemical stimuli placed on lingual epithelium diffuse into the epithelium where they initially contact epithelial cells. Whether or not epithelial cells play an important role in the transduction processes involving lingual nerve fibers is not well understood. The reason for considering that lingual epithelium may play an important role in the chemical transduction process of lingual fibers is that many chemical stimuli alter ion transport across lingual epithelium at concentrations similar to those that activate or inhibit lingual nerve fibers (Simon and Sostman, 1991). Consequently, we have undertaken to investigate the entry pathway(s) of chemical stimuli into lingual epithelium and to determine which types of lingual nerve fibers are activated by particular stimuli.

Mammalian lingual epithelium is classified as stratified squamous and as such has a protective layer of corneocytes overlying the living epithelial strata. Morphological, radiotracer, and ion transport studies suggest that monovalent salts, for the most part, enter lingual epithelium by first diffusing across zonula-type tight junctions in the stratum corneum and at the interface between the stratum corneum and stratum granulosum (Holland, Zampighi, and Simon, 1989, 1991). Moreover, it has been shown that $LaCl_3$ blocks ion transport across lingual epithelial tight junctions (Holland et al., 1989, 1991) and also inhibits whole lingual nerve responses to monovalent salts (Sostman and Simon, 1991). Whole lingual nerve recordings do not provide information regarding fiber specificity to chemical stimuli and therefore in this study we investigated the fiber types that are activated by stimuli diffusing across tight junctions. In contrast to small electrolytes, nonelectrolytes penetrate into lingual epithelium by partitioning into the nonpolar lamellar bodies in the stratum corneum and then into the plasma membranes of epithelial cells and nerve fibers (Williams and Elias, 1987). Consequently, the permeability of nonelectrolytes across lingual epithelium is proportional to the compound's membrane/water partition coefficient (Mistretta, 1971; Squier and Johnson, 1975; Siegel, 1984; Simon and Sostman, 1991). Therefore, for fibers activated by nonelectrolytes it would be expected that inhibitors of ion transport across tight junctions would not inhibit their response.

There is a paucity of information regarding chemical responsiveness of lingual nerves. Ethanol activates a class of lingual fibers that are insensitive to mechanical

stimuli (Hellekant, 1965). Menthol, at low concentrations, activates cold fibers (Hensel and Zotterman, 1951*a*), whereas capsaicin and other pungent tasting compounds activate a specific class of fibers (Okuni, 1978). However, until now there has not been a systematic study to determine which fiber types are activated by specific stimuli. Several chemical stimuli were chosen to test their ability to activate specific types of lingual fibers including NaCl, KCl, NH_4Cl, $CaCl_2$, nicotine, capsaicin, hexanol, phenyl ethanol, and menthol. They were chosen for the following reasons: NaCl at high concentrations (<0.5 M) is an irritant (Green and Gelhard, 1989) and activates pain fibers (Cadden, Linsey, and Matthews, 1983); KCl elicits responses from the lingual and other branches of the trigeminal nerve (Anderson and Matthews, 1967; Sostman and Simon, 1991); NH_4Cl produces robust and reproducible whole lingual nerve responses (Sostman and Simon, 1991); $CaCl_2$ at high concentrations inhibits spontaneous activity of cold fibers (Hensel and Schäfer, 1974); menthol increases responses from cold fibers (Hensel and Zotterman, 1951*a*); nicotine causes a burning sensation when placed on the tongue at high concentrations (Jarvik and Assil, 1988); acetylcholine activates many primary afferent sensory fibers (Paintal, 1964); capsaicin activates a specific class of nociceptive C and Aδ fibers (Bevan and Szolcsanyi, 1990); phenyl ethanol is a classic trigeminal stimulant (Alarie, 1990); and hexanol elicits responses from ethmoid and lingual nerves (Silver, Farley, and Finger, 1986; Sostman and Simon, 1991) and is also a general anesthetic at high concentrations (Seeman, 1972).

MATERIALS AND METHODS

The salts used were reagent grade made up in distilled water. All organic compounds (L-nicotine, capsaicin, menthol amyl acetate, phenyl ethanol, and hexanol) were obtained from Sigma Chemical Co. (St. Louis, MO). The pH's of these solutions were between 5 and 7.5 except for solutions containing nicotine, which had pH's between 8.5 and 9.

Adult female Sprague-Dawley rats (250–450 g) were used in all experiments. Animals were anesthetized by interperitoneal injection of sodium pentobarbital (50 mg/kg i.p., supplemented as necessary), and after tracheal cannulation were placed in a nontraumatic headholder. The tongue was secured on a platform, thus exposing it for the presentation of stimuli. Body temperature was maintained at 36–38°C by placing the rat on a heated block.

A craniotomy was performed on one side, and the overlying hemisphere was excised, exposing the trigeminal ganglion (TG). The recording sites representing the location of cell bodies of the sensory component of the lingual nerve are presented in Figs. 1 and 2.

The lingual map was obtained electrophysiologically by placing microelectrodes in different areas of the TG and searching for mechanically sensitive regions on the anterior of the tongue which activated these fibers. This electrophysiological map corresponded to regions that were labeled with horseradish peroxidase (HRP) from the tongue in this study, as described below.

The locations of the cell bodies representing the lingual nerve in the TG were obtained as follows. Rats were anesthetized with Na pentobarbital (50 mg/ml), whereupon 1-μl injections of HRP (type 1V; Sigma Chemical Co.) were injected subepithelially on the ipsilateral side. After 24–36 h the rats were reanesthetized and then perfused transcardially with 0.9% NaCl followed by perfusion with a solution of 10% paraformaldehyde and 1% gluteraldehyde in phosphate-buffered saline (PBS) at pH 7.4. The TGs were incubated in PBS containing 20% sucrose for 48 h, whereupon frozen 75-μm-thick sections in the transverse direction were reacted with 3,3′ diaminobenzidine, 1% $CoCl_2$, 1% $Ni(NH_4)_2(SO_4)_2$, and 0.003% H_2O_2 in PBS. The sections were

rinsed several times with PBS, mounted, counterstained with thionin, dehydrated with ethanol, and placed on a coverslip.

Extracellular recordings were made with glass microelectrodes filled with 1–2 M NaCl and having resistances of ~1–4 MΩ, which were placed into the TG where the cell bodies of the lingual nerve are located. A silver wire, serving as a reference ground electrode, was placed near the recording area. The signal from the microelectrode was amplified using a Grass P15 preamplifier (Grass Instrument Co., Quincy, MA), displayed with an oscilloscope, and simultaneously stored on video tape for further analysis. The tapes were either digitized and analyzed using pClamp programs (Axon Instruments, Inc., Foster City, CA) or played into an integrator (Frederick Haer & Co., Brunswick, ME) and visualized on a chart recorder.

The method used to identify lingual nerve fibers was to stimulate the tongue mechanically. Thus all fibers except for those that exhibited spontaneous activity (19 cold fibers and 1 warm fiber) were sensitive to mechanical stimuli. The receptive field of the mechanically sensitive fibers was determined by mechanically stimulating the tongue, measuring the evoked activity, and recording the coordinates relative to the midline and tongue tip. This was accomplished by placing the tongue on a trough having a ruler along one edge and also by measuring the distance from the midline to the edge. For three cold fibers the receptive fields were obtained by injecting small streams of cold water (15°C) on different regions of the tongue.

The conduction velocities were obtained by dividing the distance between the receptive field and the cell bodies by the time it took an action potential to traverse that distance. The distance between the lingual nerve cell bodies to the tip of the tongue was 47 ± 0.8 mm (mean ± SE; $n = 5$). The distance from the receptive field to the cell bodies of the lingual nerve was obtained by subtracting its distance from the tongue tip from 47 mm. The time for an action potential to propagate between these two locations was measured by electrically stimulating the tongue using bipolar silver pin electrodes connected to a model SD 9 stimulator (Grass Instrument Co.) and measuring the time between the electrical impulse and the elicited action potential (see Fig. 3 *A*). The tongue's temperature was measured with a small thermistor placed on its surface whose output was amplified, digitized, and then fed into the computer to coordinate with the changes in neural activity.

Liquid stimuli were applied to the surface of the tongue using a computer-controlled flow system that permitted up to seven stimuli to be applied in any order over a wide temperature range (8–51°C). Solutions were flowed over the tongue at 15 ml/min and 33 ± 1°C unless the temperature was changed in order to identify the fiber type. The stimuli were applied in different sequences and the ability of a fiber to respond to a particular stimulus (electrolyte) did not depend on the order of application to the tongue. Each stimulus was flowed for 45 s, followed by water rinses of 5–25 min, the time depending on the reversibility of the stimuli. The time the stimuli contacted the tongue was recorded by manually pressing a button generating a pulse. This time could be readily identified because a small (visible) air bubble always preceded the stimuli. The chemical stimuli included salts (NaCl, KCl, NH_4Cl, $CaCl_2$, and $LaCl_3$) and hydrophobic compounds (nicotine, hexanol, capsaicin, and phenyl ethyl alcohol), all dissolved in water or with water containing small concentrations of ethanol when necessary. Gentle mechanical stimuli were applied to the tongue by brushing a cotton swab over the dorsal surface. Stronger (nociceptive) mechanical stimuli were elicited by pinching with a tweezers or using a pin.

For determining whether or not a change in activity occurred upon presentation of a stimulus, the following criteria were used. For fibers that did not exhibit spontaneous activity (C, Aδ mechanoreceptors, and Aβ fibers) the activity is simply the amount of recorded activity. The smallest number of spikes thus counted as a response was four. Moreover, these fiber types did not exhibit activity for at least 45 s before the application of the stimuli (which lasted 45 s). For fibers exhibiting a spontaneous discharge (e.g., cold and warm fibers), a response was

termed excitatory or inhibitory if the rate of discharge increased or decreased by ≥30% with respect to the rate of the spontaneous activity.

RESULTS

Location of Cell Bodies of Lingual Nerve

Fig. 1 shows a dorsal view of the rat skull with the location of the intracranial portion of the trigeminal nerve, including the ganglion. The location of the cell bodies of the lingual nerve, obtained using electrophysiological recordings and HRP labeling, is

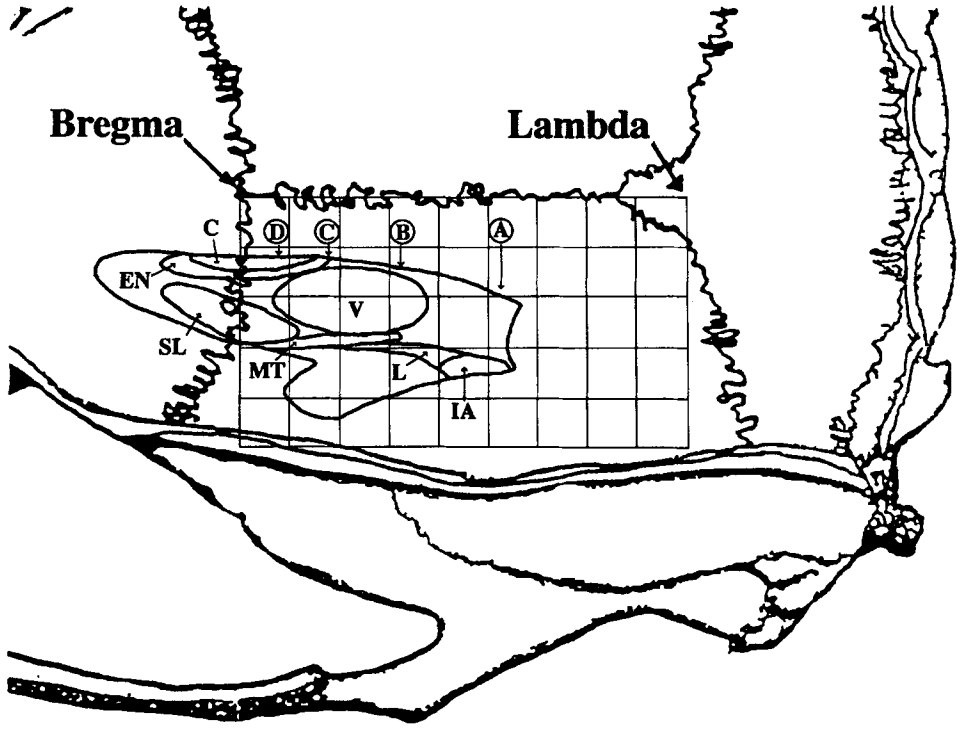

FIGURE 1. Dorsal view of rat skull adapted from Paxinos and Watson (1982). This view shows the portion of TG and trigeminal nerve assessable within the cranium. In its course from the lateral pons, the nerve enters posteriorly from beneath the bony tentorium, and then the mandibular portion exits laterally through the foramen ovale and the maxillary and ophthalmic divisions exit anteriorly through the anterior lacerated foramen; the area shown includes all of the trigeminal ganglion. The area labeled *L* reflects the location of the cell bodies of the lingual nerve found in this study by electrophysiological and to a lesser extent by HRP labeling. The other areas, shown for completeness, were approximated from other sources. *C,* cornea (Arvidson, 1977; Marfurt and Del Toro, 1987); *IA,* inferior alveolar (Mazza and Dixon, 1972); *EN,* external nasal (Mazza and Dixon, 1972); *MT,* maxillary teeth (Arvidson and Arvidsson, 1990); *SL,* superior labial (Mazza and Dixon, 1972); *V,* vibrissae (Zucker and Welker, 1969). The size and shape of the illustrated area remain fairly constant between animals, but the medial–lateral stereotactic coordinates vary by up to 0.2 mm, and anterior–posterior coordinates by up to 0.4 mm. The squares are 1 mm on a side.

labeled L. Fig. 2 shows coronal sections through the trigeminal nerve and TG. In this figure the blackened regions represent the cell bodies. The location of the lingual nerve cell bodies is in general agreement with previous HRP labeling experiments (Jacquin, Semba, Egger, and Rhoades, 1983). Other regions of the face and oral cavity whose cell bodies are in the TG are also shown.

FIGURE 2. (*opposite*) Typical coronal sections through trigeminal nerve and TG are shown together with diagrams of surrounding regions as given in Paxinos and Watson (1982). Areas containing cell bodies are blackened. The anatomical structure between animals is fairly constant posteriorly but is quite variable anteriorly. (*A*) The motor tract is bordered by the diagonal line in the ventral part of nerve; cell bodies from mandibular branch laterally (blackened area represents the tongue). (*B*) Maxillary and ophthalmic portion of nerve medially, cell bodies from lingual and maxillary divisions centrally, motor root ventrally, and mandibular branch laterally. (*C*) Ophthalmic and maxillary roots and cell bodies medially, cell body–free mandibular branch laterally. (*D*) Ophthalmic and maxillary roots and cell bodies. Abbreviations used:

AF	amygdaloid fissure
AHY	anterior hypothalamic area
alv	alveus of the hippocampus
AP	area postrema
Aq	cerebral aqueduct (sylvius)
Arc	arcuate hypothalamic nucleus
cg	cingulum
CG	central grey
cp	cerebral peduncle, basal part
csc	commissure of the superior colliculus
dhc	dorsal hippocampal commissure
Dk	nucleus of Darkschewitsch
DM	dorsomedial hypothalamic nucleus
DMC	dorsomedial hypothalamic nucleus, compact part
f	fornix
FC	fasciola cinereum
fr	fasciculus retroflexus
HiF	hippocampal fissure
ICPC	intracommissural nucleus of the posterior commissure
IF	interfascicular nucleus
IMCPC	interstitial magnocellular nucleus of the posterior commissure
InC	interstitial nucleus of Cajal
IPF	interpeduncular fossa
LH	lateral hypothalamic area
lo	lateral olfactory tract
LOT	nucleus of the lateral olfactory tract
LPO	lateral preoptic area
ME	medial eminence
mfb	medial forebrain bundle
ml	medial lemniscus
MP	medial mammillary nucleus, posterior part
MPO	medial preoptic area
MT	medial terminal nucleus of the accessory optic tract
mtg	mammillotegmental tract
opt	optic tract
ox	optic chiasm
PBP	parabrachial pigmented nucleus
pc	posterior commissure
PeF	perifornical hypothalamic nucleus
PPT	posterior pretectal nucleus
RF	rhinal fissure
RCh	retrochiasmatic area
RLi	rostral linear nucleus of the raphe
RPC	red nucleus, parvocellular part
S	subiculum
SC	superior colliculus
scc	splenium of the corpus callosum
scp	superior cerebellar peduncle
SO	supraoptic hypothalamic nucleus
sox	supraoptic decussation
st	stria terminalis
VMHC	ventromedial hypothalamic nucleus, central part
VMHDM	ventromedial hypothalamic nucleus, dorsomedial part
VMHVL	ventromedial hypothalamic nucleus, ventrolateral part
VTA	ventral tegmental area

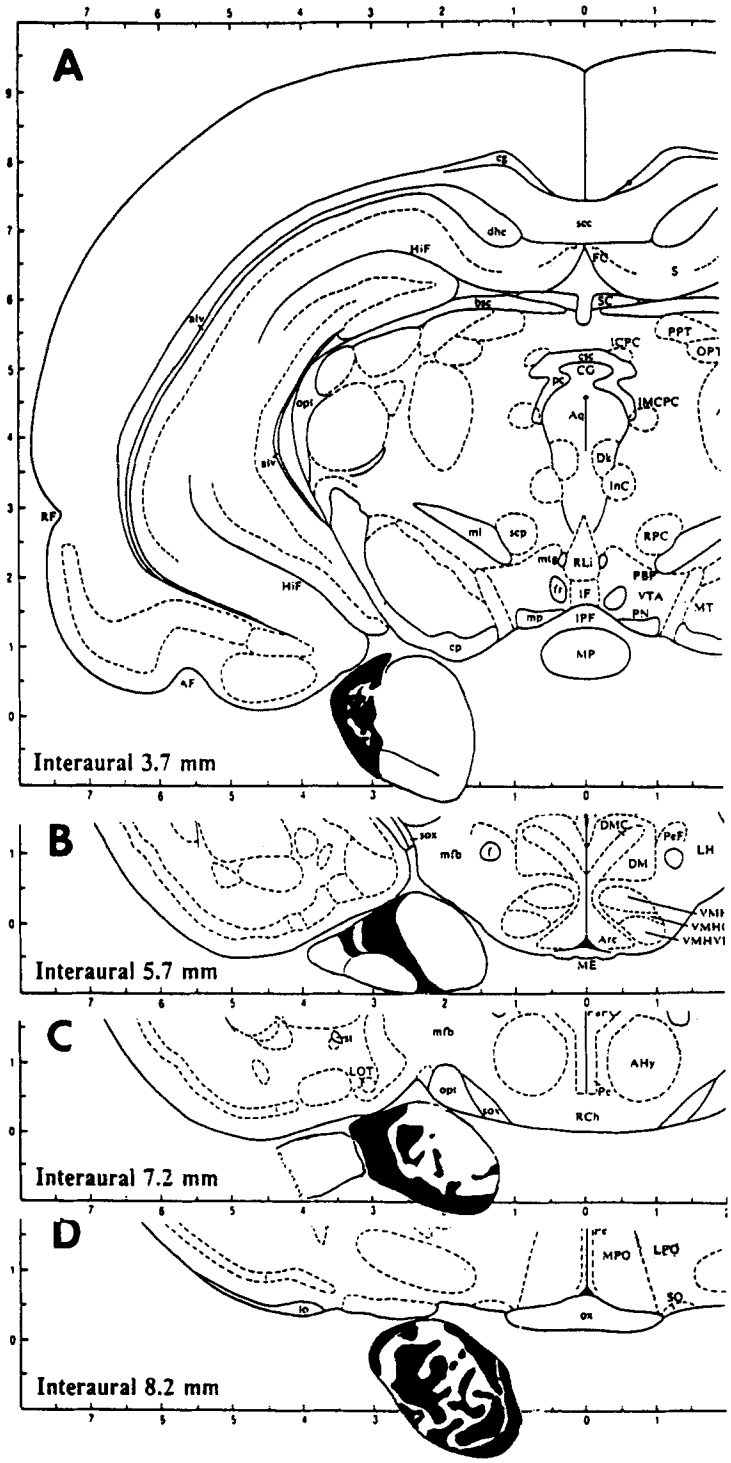
A
Interaural 3.7 mm
B
Interaural 5.7 mm
C
Interaural 7.2 mm
D
Interaural 8.2 mm

FIGURE 2.

Identification of Fiber Types in Lingual Nerve

A total of 83 fibers were characterized with regard to chemical stimuli. However, the conduction velocity was measured for only 67 of them. Of the 25 remaining fibers, 13 were spontaneously active (Aδ) cold fibers, 2 were Aδ mechanoreceptors, 9 were either Aδ mechanoreceptors or Aβ fibers, and 1 was a warm fiber.

The lingual nerve contains C, Aδ, and Aβ fibers as characterized by measurements of conduction velocity (Fig. 3 *A*). A histogram of the conduction velocities of 67 fibers of the C, Aδ, and Aβ types is shown in Fig. 3 *B*. Most of the fibers investigated were of the Aδ type.

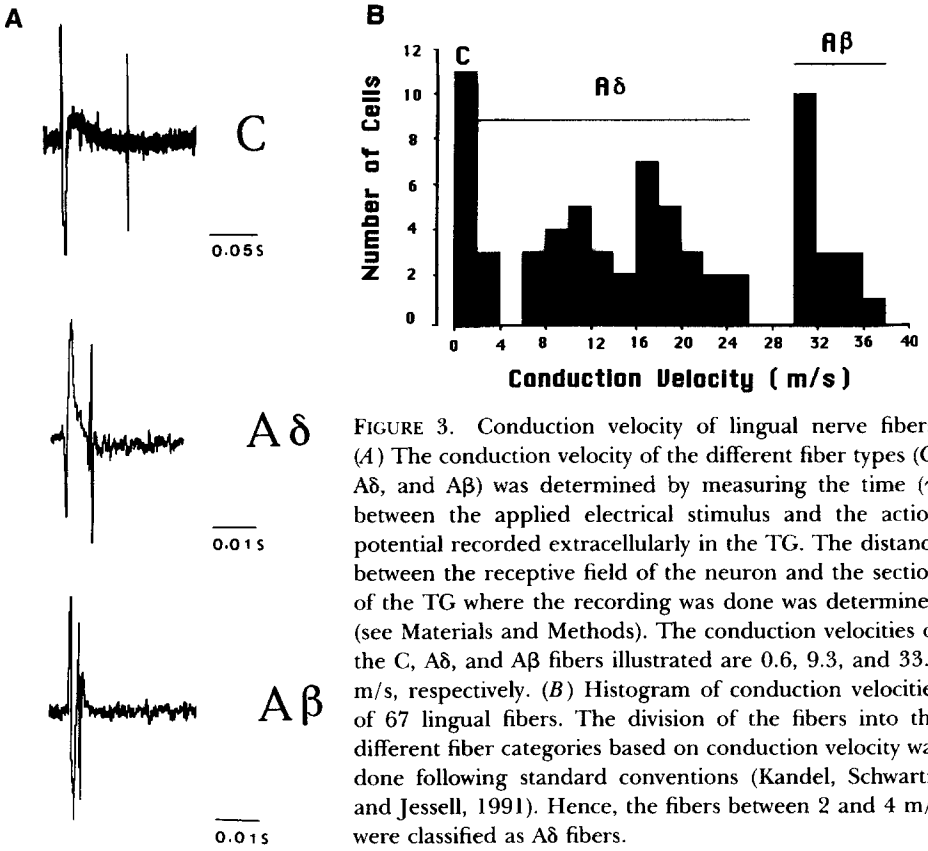

FIGURE 3. Conduction velocity of lingual nerve fibers. (*A*) The conduction velocity of the different fiber types (C, Aδ, and Aβ) was determined by measuring the time (τ) between the applied electrical stimulus and the action potential recorded extracellularly in the TG. The distance between the receptive field of the neuron and the section of the TG where the recording was done was determined (see Materials and Methods). The conduction velocities of the C, Aδ, and Aβ fibers illustrated are 0.6, 9.3, and 33.3 m/s, respectively. (*B*) Histogram of conduction velocities of 67 lingual fibers. The division of the fibers into the different fiber categories based on conduction velocity was done following standard conventions (Kandel, Schwartz, and Jessell, 1991). Hence, the fibers between 2 and 4 m/s were classified as Aδ fibers.

The chemical sensitivities of a total of 83 fibers were investigated. The stimuli most frequently tested were NaCl, KCl, NH_4Cl, $CaCl_2$, nicotine, and menthol (Table I). Other stimuli were also tested, but not as often or on as many fibers. The difference between the 83 fibers tested in their response to chemical stimuli and the 67 fibers whose conduction velocity was measured is accounted for by 13 cold fibers, 2 Aδ mechanoreceptors, and 1 warm fiber (Table I). Cold fibers were identified by their spontaneous activity as well as their responses to menthol and temperature changes

TABLE I

Responses of Lingual Nerve Fibers to Chemical Stimuli

Afferent fiber type	Conduction velocity (mean ± SD)	Cells*	Mechanal stimuli	Thermal stimuli‡ (8–51°C)	Chemical stimuli							
					NaCl (2.5 m)	KCl (2.5 M)	NH_4Cl (2.5 m)	$CaCl_2$ (1.0 M)	Nicotine (0.1–10 mM)	Menthol (1.0 mM)	Hexanol (50 mM)	Capsaicin (1 μM)
	m/s											
C fibers	1.2 ± 0.5	11/11	11/11	5/11	7/11	9/11	10/11	0/11	3/11	0/3	0/5	0/3
1			Yes	No	Yes	Yes	Yes	No	No	—	No	—
2			Yes	No	No	Yes	No	No	No	—	—	—
3			Yes	No	Yes	Yes	Yes	No	No	—	No	No
4			Yes	No	Yes	Yes	Yes	No	No	—	—	No
5			Yes	No	No	Yes	Yes	No	Yes	No	No	—
6			Yes	No	Yes	Yes	Yes	No	No	No	No	—
P1			Yes	Yes	Yes	Yes	Yes	No	No	—	—	No
P2			Yes	Yes	Yes	No	Yes	No	Yes	—	—	—
P3			Yes	Yes	No	Yes	Yes	No	Yes	No	No	—
P4			Yes	Yes	Yes	Yes	Yes	No	No	—	—	—
P5			Yes	Yes	No	No	Yes	No	No	—	—	—
Aδ mechanoreceptors	14.7 ± 6.5	33/35	35/35	0/35	8/35	9/35	9/35	0/35	2/35	0/35	0/17	
Cold	13.3 ± 4.2	6/19	0/19	19/19	2/19	5/19	9/19	0/19	0/19	8/9	8/16	
Warm		0/1	0/1	1/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	
Aβ fibers	32.1 ± 2.0	17/17	17/17	0/17	0/17	0/17	0/17	0/17	0/17	0/15	0/9	
Total		67/83										

*Number of cells with known conduction velocity/total number of cells.

‡C fibers were activated in the temperature range from 38 to 51°C.

(Braun, Bade, and Hensel, 1980; for example, see our Figs. 8 and 9). The single warm fiber was identified by its spontaneous activity and by the fact that its activity increased when the temperature was increased to ~43°C (Dubner and Bennett, 1983). The reasons for classifying the 13 cold fibers as Aδ fibers are: (*a*) they did not respond to mechanical stimuli (including pin prick) as do C fibers, (*b*) they responded to some salts (unlike Aβ fibers), and (*c*) the conduction velocities of the six cold fibers measured placed them in the Aδ category. The single warm fiber was placed in the Aδ category because others have found warm fibers to be Aδ fibers (Dubner and Bennett, 1983). The reason for placing two fibers in the Aδ mechanoreceptor category is because (*a*) they were unresponsive to temperatures as high as 51°C, as are C fibers, and (*b*) they responded to 2.5 M NH_4Cl, unlike Aβ fibers.

Receptive Fields

The receptive fields of the C, Aδ, and Aβ fibers differ in area and shape. The receptive fields of the C fibers were the largest and most asymmetric, having a length (2.6 ± 0.5 mm; mean ± SE) about twice their width (1.2 ± 0.2 mm; $n = 11$). The receptive fields of Aδ fibers were both smaller and more symmetric than for C fibers, having lengths and widths of 2.1 ± 0.1 and 1.6 ± 0.1 mm ($n = 39$), respectively. Aβ fibers have the smallest and most symmetric receptive field of the three fiber types, being 1.6 ± 0.2 mm long and 1.2 ± 0.1 mm ($n = 17$) wide.

Chemical Selectivity of Lingual Nerve Fibers

The thermal, mechanical, and chemical responses of all fibers are presented in Table I. All the fibers except the 19 cold fibers and the warm fiber responded to mild mechanical stimulation. Their responses to mechanical stimuli would classify them as slowly adapting mechanoreceptors (see Figs. 5, 7, and 10). For clarity, the Aδ fibers were divided in three categories: mechanoreceptors, cold fibers, and one warm fiber.

C fibers. All 11 of the C fibers responded to mechanical stimuli, but only 5 responded to temperatures between 8 and 51°C (Table I). All five C fibers that responded to both mechanical and thermal (>38°C) stimuli also responded to chemical stimuli and thus, from this characterization, can be classified as polymodal nociceptors (labeled P1–P5 in Table I). All of the C fibers responded to one or more 2.5-M solutions of NaCl, KCl, or NH_4Cl. None of the fibers responded to 1 M $CaCl_2$ and none of the fibers tested responded either to hexanol, menthol, or capsaicin (Table I). Three fibers responded to nicotine. There is a large variability in the responses of the C fibers to the univalent salts. For example, fiber P1 responded to all three salts, whereas fiber P2 responded to NaCl and NH_4Cl but not to KCl (Fig. 5, Table I). This selectivity among the C fibers for univalent salts was an intrinsic property of them since some fibers responded to all three univalent salts and others to fewer stimuli (Table I). The ability of the polymodal C fibers to respond differentially among chemicals was not limited to the electrolytes, since two of the five polymodal nociceptors tested were activated by nicotine (Fig. 5) and none of those tested were activated by hexanol, menthol, and capsaicin (Table I).

The temporal responses of C fibers to solutions containing 2.5 M NaCl ($n = 7$), KCl ($n = 9$), and NH_4Cl ($n = 10$) are shown in Fig. 4, *A–C*, respectively. As with most sensory afferent fibers, lingual nerve C fibers have both phasic and tonic components.

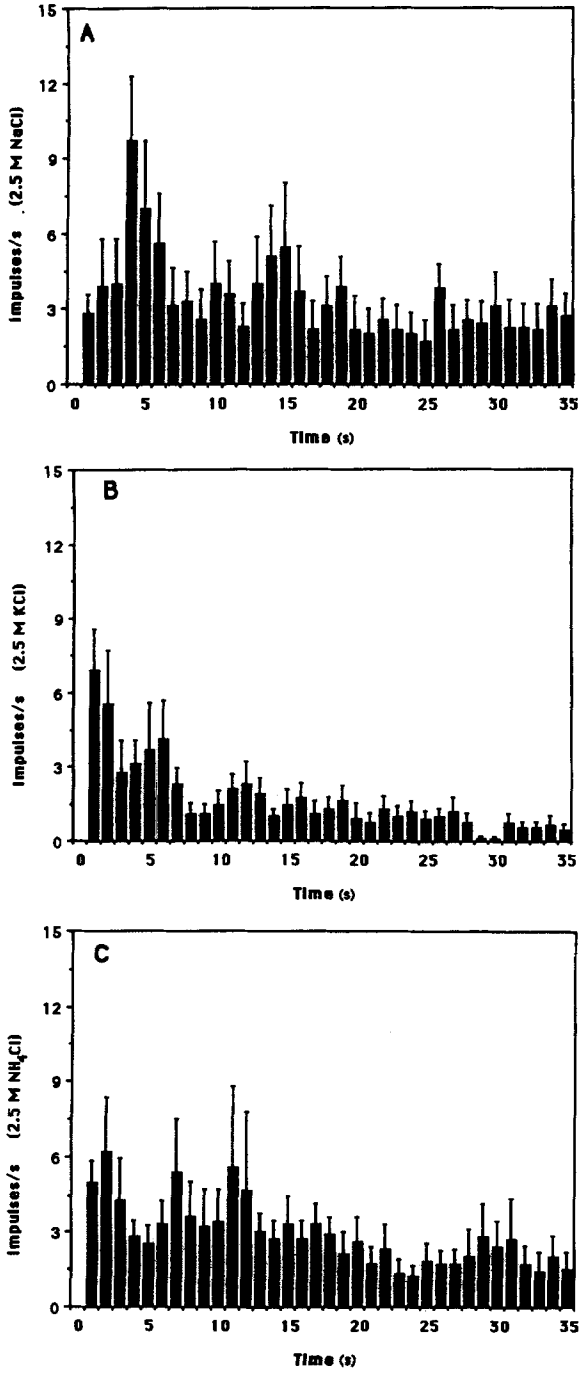

FIGURE 4. Responses of C fibers to 2.5-M solutions of NaCl (*A*), KCl (*B*), and NH_4Cl (*C*). The responses represent the mean ± SE of 7, 9, and 10 fibers that responded to NaCl, KCl, and NH_4Cl, respectively.

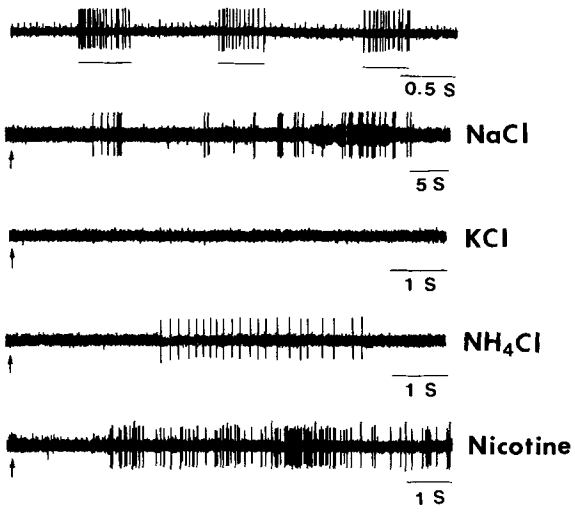

FIGURE 5. Chemical selectivity of C fiber (P2 in Table I). Response to mechanical stimulation (*upper trace*) and 2.5-M solutions of NaCl, NH_4Cl, and KCl and 10 mM nicotine. After each stimulus was applied, the tongue was rinsed with water.

After coming in contact with the tongue, the maximum activity for KCl, NH_4Cl, and NaCl occurred at 1, 2, and 4 s, respectively (see Figs. 5 and 6 for examples). The maximal (peak) activity is 9.7 ± 2.5, 6.9 ± 1.7, and 6.2 ± 2.2 impulses/s (mean ± SE) for NaCl, KCl, and NH_4Cl, respectively. The steady-state activity (after 35 s) is 2.71 ± 0.89, 0.44 ± 0.24, and 1.5 ± 0.72 impulses/s for NaCl, KCl, and NH_4Cl, respectively. In this regard, the activity decrease of C fibers to their tonic activity is faster in the presence of KCl than in the presence of NaCl or NH_4Cl. A particularly interesting point regarding these response profiles is that all three profiles exhibit periods of increased activity (bursts) before reaching their final tonic activity (see Figs. 5–7 for examples).

Aδ fibers. Approximately 26% of the 35 Aδ mechanoreceptors tested responded to 2.5-M solutions of NaCl, KCl, or NH_4Cl (Fig. 6, Table I). In contrast, none of the 35 identified Aδ fibers responded to 1 M $CaCl_2$. In other words, 74% of Aδ mechanoreceptors did not respond to any of the four salts tested. Nevertheless, even

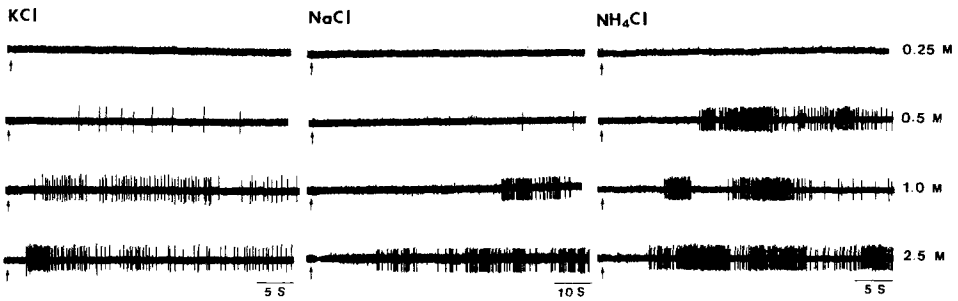

FIGURE 6. Responses of lingual fibers to monovalent salts. (*Left*) Response of an Aδ fiber to KCl. (*Middle*) Response of a C fiber to NaCl. (*Right*) Response of another C fiber to NH_4Cl. The arrow points to the time the stimuli contacted the tongue. The stimuli were present throughout the entire trace. Note that the latency decreases with increasing salt concentration.

among the eight or nine Aδ fibers that responded to the three salts, not all responded to all three univalent salts. Cold fibers responded most frequently to NH_4Cl (9 of 19) and least frequently to NaCl (2 of 19) among the three univalent salts tested (Table I). Cold fibers were the only type of Aδ fibers to be activated by menthol (Table I). A small proportion (2 of 35) of the Aδ mechanoreceptors was activated by nicotine. Nicotine did not alter the activity of cold fibers nor of the single warm fiber investigated (Table I). The anesthetic, hexanol, did not activate the Aδ mechanoreceptors but inhibited the activity of 8 of 16 cold fibers tested (Fig. 9; Table I). Finally, 0.1 M phenyl ethanol did not elicit responses from three Aδ mechanoreceptors, but in three cold fibers it completely inhibited their spontaneous activity (not shown).

The single identified warm fiber did not respond to any of the chemical stimuli listed in Table I.

Aβ fibers. None of the 17 Aβ fibers investigated responded to any to the chemical stimuli tested, nor did they respond to thermal stimuli from 8 to 51°C (Table I).

Chemical Stimuli

Threshold concentrations. The frequency of action potentials elicited from C and Aδ fibers to NaCl, KCl, and NH_4Cl increased with increasing salt concentration (Fig. 6). The lowest salt concentration from which responses were obtained in either C or Aδ fibers was 0.25 M (not shown, but see Fig. 8 for examples of large responses to 0.5 M KCl and NH_4Cl). However, among the concentrations used, the usual threshold concentration was 0.5 M, as illustrated in Fig. 6.

Latency and temporal patterns. Latency is defined as the time between the stimulus reaching the tongue and the onset of electrical activity. The latency of the responses of trigeminal fibers always decreased with increasing salt concentration (Fig. 6). It is not particularly useful to assign a numerical value for the latency for a given stimulus type and concentration because the latency varies among fibers of the same class and sometimes is influenced by stimuli previously placed on the tongue. For a given fiber, the latency for KCl was usually less than the latency for NaCl or NH_4Cl (Figs. 6 and 7). The patterns of the action potentials elicited by salts also depended on the salt type and concentration (Figs. 4 and 6). The temporal patterns were variable even among the same fiber types. Finally, the responses to all salts exhibit bursts of activity (Figs. 6 and 7).

Lanthanum inhibition of salt responses. The ability of 3.5 mM $LaCl_3$ to inhibit responses to 2.5-M solutions of NaCl, KCl, and NH_4Cl was tested in a total of 10 C or Aδ fibers. Lanthanum inhibited the responses to NaCl and KCl in nine fibers and to NH_4Cl in eight fibers. The inhibition of the responses of three C fibers and one cold fiber to 2.5-M solutions of NaCl, KCl, and NH_4Cl by 3.5 mM $LaCl_3$ is presented in Figs. 7 and 8, respectively. $LaCl_3$, even at concentrations as high as 1.0 M (not shown), did not elicit responses from any fiber. The inhibition of salt responses by $LaCl_3$ is partially reversible, but only after ~20 min of flowing water over the tongue (bottom trace of Fig. 7). Finally, $LaCl_3$ does not inhibit the responses of fibers to mechanical stimuli (Fig. 7).

In contrast to inhibiting the response to monovalent salts, $LaCl_3$ does not inhibit the response to hydrophobic stimuli such as menthol. Fig. 8 shows that menthol

increases the activity of a cold fiber and that the spontaneous activity is undiminished in the presence of 2.5 mM $LaCl_3$.

Hexanol and capsaicin. Hexanol decreases the spontaneous activity of cold fibers and also inhibits their ability to respond to menthol (Fig. 9). The responses of C fibers to 2.5 M NaCl (and other monovalent salts) can be reversibly (or partially reversibly) inhibited by nonpolar compounds such as capsaicin and hexanol (Fig. 10). Although both capsaicin and hexanol inhibited the response to NaCl, they differed in their ability to inhibit responses to mechanical stimuli. That is, responses to mechanical stimuli can be elicited soon after treatment with capsaicin but not after treatment with 50 mM hexanol.

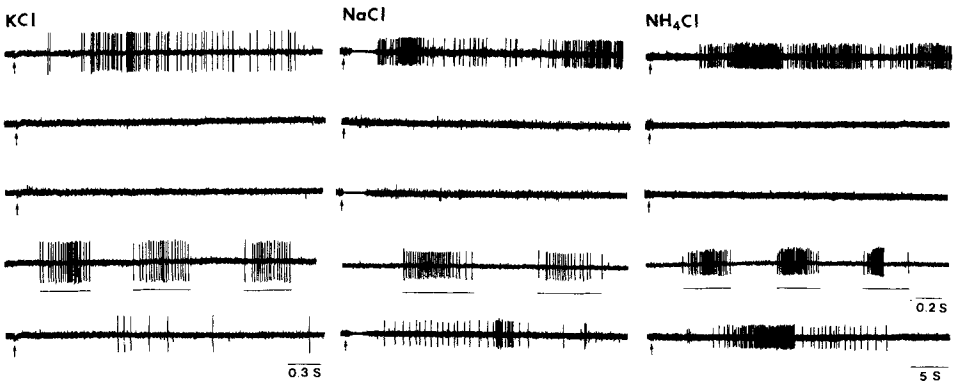

FIGURE 7. Lanthanum blockage of salt responses in C fibers. The upper trace in each panel shows the responses to 2.5 M KCl (*left*), 2.5 M NaCl (*middle*), and 2.5 M NH_4Cl (*right*). The tongue was then rinsed with water for 5 min (not shown). The second trace in each panel shows that 3.5 mM $LaCl_3$ by itself does not elicit a response. Immediately after this stimulus, a solution containing 2.5 M salt plus 3.5 mM $LaCl_3$ was flowed over the tongue (*third trace*). $LaCl_3$ treatment did not alter the fiber's ability to respond to mechanical stimuli (*fourth trace*). After rinsing with water for 15–20 min (not shown), a solution containing 2.5 M salt was flowed over the tongue (*bottom trace*). The arrows point to the times the stimuli first contacted the tongue. The time line on the bottom of the KCl panel corresponds to all the KCl traces. The time lines on the bottom trace of the NH_4Cl response apply to all the NH_4Cl and NaCl responses except those in the records showing the response to mechanical stimuli above the bottom traces. For these two traces the time line corresponds to the one of 0.2 s.

DISCUSSION

Location of Cell Bodies of Lingual Nerve

The cell bodies of general sensory afferent fibers from the anterior two-thirds of the tongue (lingual nerve) have been identified. They are in close proximity to cell bodies of afferent fibers from other tissues in or around the oral cavity (the lower incisors, chin, upper cheek, and vibrissae), suggesting that the TG is spatially organized (Figs. 1 and 2). The front of the TG contains the cell bodies from afferent fibers projecting from the front and upper part of the face (ophthalmic and maxillary divisions; nose,

regions near and in the eye, vibrissae area), whereas the rear contains fibers from the oral cavity and lower regions of the face (mandibular division).

Fiber Types

Anatomical studies of the fiber diameters in cat lingual nerve showed that ~5% have diameters ≤2 μm (C fibers), ~40% have diameters between 2 and 5 μm (Aδ fibers), and the remainder (55%) had diameters >5 μm and thus were Aβ fibers (Bieden-

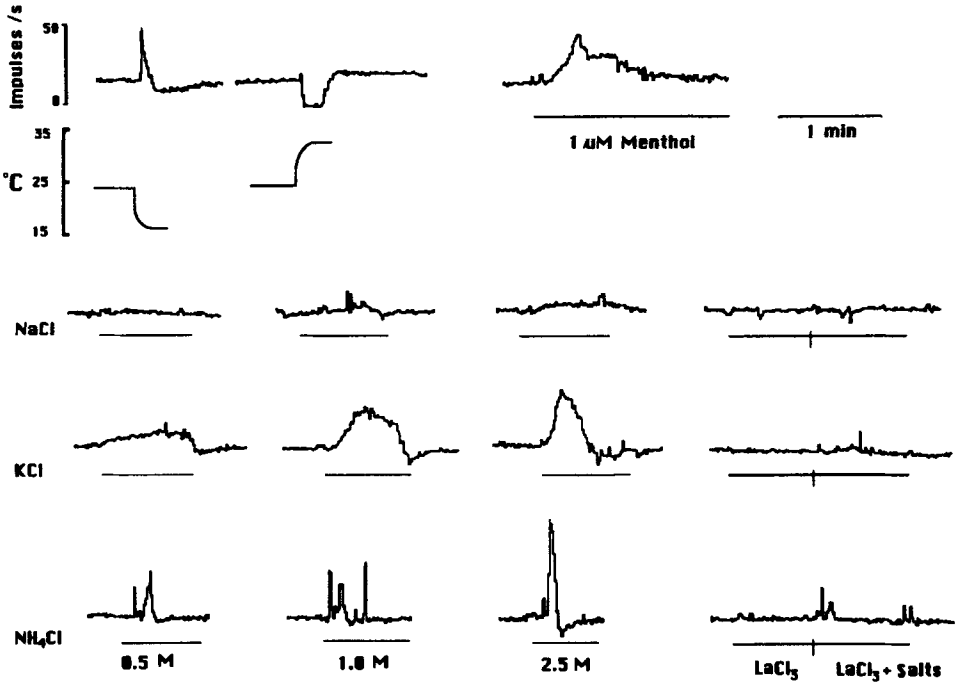

FIGURE 8. Lanthanum inhibition of salt responses in a cold fiber. Change in activity (impulses per second) of a cold fiber, initially at 24°C, due to decreasing (to 16°C) and increasing (to 32°C) the tongue temperature. The fiber activity was increased by 1 μM menthol (*top right*). The lower panels show the responses of this same fiber to 0.5–2.5-M solutions of NaCl, KCl, and NH_4Cl. The traces on the right side show that 3.5 mM $LaCl_3$ by itself does not elicit a response but that 3.5 mM $LaCl_3$ inhibited the responses to 2.5-M solutions of NaCl, KCl, and NH_4Cl. The calibration for the activity of all traces is in the top left corner.

bach, Beuerman, and Brown, 1975). This distribution reflecting fiber types mostly reflects what we found electrophysiologically in rat lingual nerve. That is, C fibers were very difficult to find, although this was partly due to sampling bias toward larger cells. To this point, among the 31 cells bodies recorded from the ethmoid nerve, no C fibers were identified (Lucier and Egizii, 1989). In contrast, Aδ and Aβ fibers were readily found. The distribution of Aβ fibers shown in Fig. 2 *B* does not accurately

reflect the number identified, since once it was determined that these fibers do not respond to chemical stimuli (Table I) they were not investigated further.

Chemical Sensitivity of Lingual Nerve Fibers

C FIBERS

Salts. About half the fibers identified as C fibers were classified as polymodal nociceptors and the other half are probably high threshold mechanical nociceptors. It is well established that many pain fibers are activated by KCl, NaCl, and NH_4Cl (Jyvasjarvi, Kniffki, and Mengel, 1988; Markowitz, Bilotto, and Kim, 1991), and in this respect lingual neurons are no exception. What was not known previously is that both polymodal and other types of C fibers, by their differential sensitivities, carry the information that distinguishes between monovalent salts and other more hydrophobic stimuli such as hexanol, menthol, and nicotine (Table I).

Nicotine. A small proportion of the C (3 of 11) and Aδ (2 of 35) fibers were activated by nicotine. Whether nicotine activates these fibers via nicotinic acetylcho-

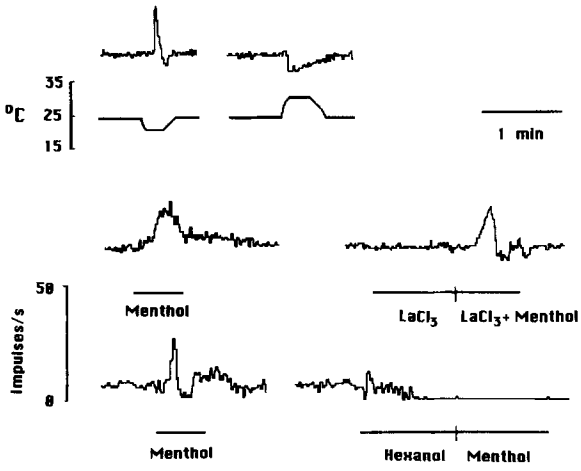

FIGURE 9. Integrated responses of cold fiber to heating and cooling (*top traces*). 1.0 mM menthol activated this fiber and the activation by menthol was not inhibited by 3.5 mM $LaCl_3$ (*middle traces*). Hexanol (50 mM) inhibited the activation of menthol in the same cold fiber (*bottom traces*). The bars indicate the time the stimuli were on the tongue. The activity scale (in impulses per second) for all traces is given on the left.

line receptors, as it does with other primary afferent fibers (Paintal, 1964), cannot be determined from these experiments. What can be concluded here is that nicotine's action is not simply a consequence of its solubility in the axolemmas of C or Aδ fibers. The reason is that neither hexanol, which has approximately the same octanol/water partition coefficient as nicotine (Leo, Hansch, and Elkins, 1971), nor menthol, which has ~10 times nicotine's octanol/water partition coefficient, activated C fibers or Aδ mechanoreceptors (Table I). In this regard, nicotine is likely to be interacting specifically with a small population of C and Aδ fibers.

Capsaicin. Only three C fibers were tested for their ability to respond to capsaicin, so conclusions deduced from results must be considered tentative. Although none of these three fibers responded to capsaicin, capsaicin clearly interacted with some of these fibers since it reversibly inhibited the response to 2.5 M NaCl without altering the ability of the fiber to respond to mechanical stimuli (Fig. 10).

Thus, capsaicin did not inhibit stretch-activated channels (and other structures involved in mechanical transduction) but inhibited, either directly or indirectly, the transport pathways involved in the generation of the response to NaCl. We have not determined whether capsaicin can inhibit the responses of C fibers to compounds other than NaCl.

Hexanol. Hexanol behaved similarly to capsaicin in that it also inhibited the response to NaCl but, in addition, inhibited mechanically induced responses (Fig. 10). In this regard, hexanol behaved as a general anesthetic. Whether capsaicin and hexanol inhibited the response to NaCl by the same mechanisms is not known.

Aδ FIBERS

Aδ fibers also exhibited selectivity to chemical stimuli. Most of the Aδ mechanoreceptors did not respond to any of the three monovalent salts, and those that did

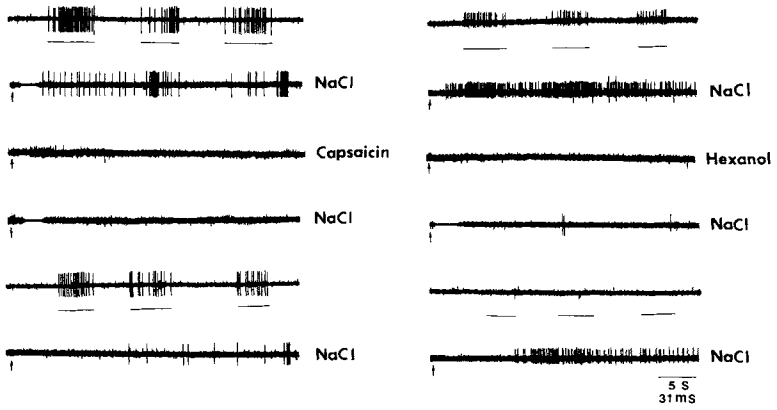

FIGURE 10. Blockage of C fiber responses to NaCl by capsaicin and hexanol. Response of two C fibers to mechanical (pinch) stimulation (*upper traces*) and 2.5 M NaCl (*second traces*). Subsequent to rinsing with water (not shown), 1 μM capsaicin or 50 mM hexanol was flowed over the tongue (*third traces*), whereupon 2.5 M NaCl was immediately flowed over the tongue (*fourth traces*). After capsaicin (or hexanol) treatment the fibers remained sensitive (capsaicin) or became insensitive (hexanol) to mechanical stimuli (*fourth traces*). After extensive washing with distilled water for 20 min the fibers responded to 2.5 M NaCl (*bottom traces*).

exhibited selectivity among them. Thus not all Aδ mechanoreceptors are identical in their sensitivity to chemical stimuli. Whether the selectivity among monovalent salts arises because the fibers differ in the number and/or type of ion transport proteins in their terminals or because they are in different epithelial environments cannot be determined from these experiments (see below).

All the Aδ fibers that responded to menthol were cold fibers. Thus, cold fibers that terminate in rat lingual epithelium, as in other epithelia (Hensel and Zotterman, 1951*a*; Andres and von During, 1973; Kosar and Schwartz, 1990), are the only ones that have receptors for menthol (Eccles, Griffiths, Newton, and Tolley, 1988). This statement can also be extended to the C fibers since none of the three tested for activation by menthol responded (Table I).

A small proportion of cold fibers responded to some or all of the monovalent salts tested (see Fig. 8 for the only one that responded to all three). As discussed above, it is not known whether the monovalent salt selectivity reflects intrinsic differences between fibers or microenvironmental differences. In general, if cold fibers respond to monovalent salts, the salt they are most likely to respond to is NH_4Cl (Table I). One possible reason is that the heat of solution is greater for NH_4Cl than for NaCl or KCl (Weast, 1979) and thus NH_4Cl might change the tongue temperature to a greater extent than the other salts. We considered it surprising that none of the 19 identified cold fibers responded to high concentrations of $CaCl_2$, since it was shown (in cats at least) that the spontaneous activity of fibers identified as cold fibers is diminished by the presence of high concentrations of $CaCl_2$ on the tongue (Schaffer and Braun, 1992). Moreover, our laboratory found that the integrated spontaneous activity recorded from whole rat lingual nerve was diminished by 1.0 M $CaCl_2$ (Sostman and Simon, 1991), a result consistent with $CaCl_2$ diminishing the activity of cold fibers. One possible explanation for the absence of a response to $CaCl_2$ is the location of cold fibers. If many cold fibers were at the base of the epithelium of filiform papillae (Andres and von During, 1973), which occupies >90% of the area of the anterior tongue (Holland et al., 1989), then the probability of increasing the Ca^{2+} concentration around the cold fiber terminal would be small since Ca^{2+} would have to diffuse across the very large cornified layer present in filiform papillae (Baratz and Farbman, 1975).

The single warm fiber investigated did not respond to any chemical stimuli. If this was found to be true in a large population of warm fibers, then one possible reason could be that warm fibers are located in the papillary layer (Holland, 1984); consequently, the concentration of stimuli reaching them may be below threshold.

Aβ FIBERS

Aβ fibers did not respond (in the sense of generating action potentials) to any of the tested chemical stimuli. Possible reasons why these fibers were unresponsive to these stimuli are: (*a*) they are located deep in the papillary layer and hence the concentration of stimuli reaching them will always be below threshold (see above), and (*b*) they are large myelinated fibers, many of which may have encapsulated endings that may "buffer" the responses to chemical stimuli.

Mechanisms of Chemical Stimulation of Lingual Fibers

PATHWAYS INTO THE EPITHELIUM

Previously it was shown using whole lingual nerve recordings that responses elicited by salts can be inhibited by incubating the tongue with the tight junction blocker $LaCl_3$ (Sostman and Simon, 1991), thus suggesting that salts enter lingual epithelium via tight junctions. This study has now been extended to the single fiber level where lanthanum inhibited responses to salts (Figs. 7 and 8) but not to the nonpolar compounds tested (Fig. 9). That is, while $LaCl_3$, even at concentrations as high as 1 M, did not elicit responses from C or Aδ fibers, 3.5 mM $LaCl_3$ inhibited responses to 2.5-M solutions of NaCl, KCl, and NH_4Cl (Figs. 7 and 8). These data suggest that the route by which these electrolytes enter lingual epithelium is across tight junctions in

the stratum corneum and stratum granulosum (Holland et al., 1989). The few cases where $LaCl_3$ did not inhibit the response to salts may reflect regions where the epithelium suffered some damage and the tight junctions were not functional, or that the tight junctions contained just a few junctional strands so that, in either case, lanthanum itself can penetrate into the epithelium (Schneeberger and Lynch, 1992) and thus would not act as an inhibitor. In contrast, hydrophobic compounds such as menthol, which enter epithelia by partitioning into their plasma membranes, were not inhibited by $LaCl_3$ (Fig. 9).

LATENCY

On first principles, the latency should increase as the distance from the surface, the thickness of the epithelium, and the fiber diameter increase (Kaaber, 1974; DeSimone and Heck, 1980; Siegel, 1984). Thus, there should not be, and indeed there is not, a standard number to assign to the latency for a particular salt and concentration. However, for a given fiber the latency decreases with increasing stimulus concentration (Fig. 6), which is consistent with direct interaction with the nerve terminal or an indirect interaction through epithelial or Schwann cells. From the latencies measured, it is difficult to distinguish between these alternatives without additional information regarding mechanisms of activation. The possibility that the latency (with salts) is determined by the development of a mechanically sensitive receptor caused by the buildup of osmotic pressure can be eliminated, since neither 1 M $CaCl_2$ nor 1 M $LaCl_3$ elicited responses. On average, for C fibers, the latency is greater for NaCl than KCl (Fig. 4). One possible reason is that the extracellular concentration of NaCl is much higher than that of KCl, so that for the same flux into the extracellular space, the percentage change of the NaCl concentration will be much smaller than that of KCl.

The latencies seen with nonpolar compounds such as menthol or nicotine probably reflect the time needed for these compounds to develop a threshold concentration at the nerve terminals in specific fiber types. Evidence for this assertion is that these compounds elicit responses from only a small subpopulation of fibers and therefore are not likely to interact nonspecifically through the epithelial or Schwann cells.

ACTIVATION OF FIBERS

Salts. Once the salts diffuse into the extracellular space, they may elicit responses from lingual C and Aδ fibers in several ways: (*a*) by diffusing into and hence changing the composition of the extracellular space, (*b*) by causing either nerve terminals and/or specialized epithelial cells (Merkel, mast, Langerhans) to release peptides or transmitters, or (*c*) by causing changes in epithelial or Schwann cells surrounding the fibers, thus changing the composition of the extracellular space. Since some fibers respond to all three univalent chloride salts, but some to only two or none, it is unlikely that Cl^- plays a major role in the activation of these fibers; hence, the selectivity must arise as a consequence of the different cations. The variability in the responses to the different salts reflects either a distinct subpopulation of intrinsically different fibers and/or fibers in different environments surrounding their terminals (i.e., depth from surface, responses of epithelial cells, volume of extracellular space) (Scriven, 1981; Lieberman and Hassan, 1988). Although there is no information

regarding these parameters, it is legitimate to inquire how these three salts may activate (depolarize) the same fiber. It is obvious that increasing the concentration of KCl in the extracellular space could result in increased activity since increasing KCl will decrease the resting potential (Anderson and Matthews, 1967; Markowitz et al., 1991; Sostman and Simon, 1991). However, simply increasing KCl in the extracellular space does not explain the temporal pattern of action potentials produced by KCl (or the other salts), or why some fibers that respond to NaCl and NH_4Cl are not activated by KCl (Fig. 5 and Table I).

It is not obvious how NaCl increases the activity of some C and Aδ fibers. Although many possibilities exist, without knowledge of the selectivity of leak channels responsible for determining the resting potential, the role of Na^+ exchangers, Na^+ pumps, and Na^+ cotransporters in influencing the membrane potential (Scriven, 1981), as well as the possible influence of the epithelium (Simon and Sostman, 1991) or Schwann cells (Lieberman and Hassan, 1988), at this time it is not productive to speculate.

The mechanisms by which NH_4Cl elicits responses from some C and Aδ fibers are also obscure. Possible considerations are: (*a*) NH_4^+ is permeable through K^+ and Na^+ channels (Hille, 1991) and thus can directly depolarize neurons by diffusing through them; (*b*) NH_4^+ can increase the intracellular pH (via $NH_4^+ = NH_3 + H^+$; pKa = 9.2, where NH_3 is membrane permeable), and (*c*) NH_4^+ can activate M channels in TG neurons (Pidoplichko, 1992). All of these mechanisms, whether direct or indirect, can, in principle, influence the activity of C and Aδ fibers. However, neither mechanism can explain why at most 33% (18 of 55) of these fibers were activated by a monovalent salt (Table I) unless the others were further from the surface.

Nicotine. Nicotine produces a burning sensation when placed on lingual epithelium. Moreover, the intensity of the burning sensation can be reduced by the nicotinic acetylcholine receptor (nAChR) antagonist, mecamylamine (Jarvik and Assil, 1988). These psychophysical experiments suggest that nicotine produces its burning sensation by interacting with nAChRs located on nociceptive fibers in lingual epithelia (Jarvik and Assil, 1988). Our data suggest that nicotine interacts with some C and Aδ fibers. Whether or not the activation occurred via nAChRs either directly or indirectly cannot be determined from these experiments. Some support for the hypothesis that nicotine interacts directly with nAChRs on lingual fibers is that nAChRs have been identified in TG neurons (Liu, Pugh, Ma, and Simon, 1993; Wada, Wada, Boulter, Deneris, Heinemann, Patrick, and Swanson, 1989).

Hexanol. The anesthetic effect of hexanol is evident in its ability to eliminate, in C fibers, responses elicited by both mechanical and chemical stimuli (Fig. 9), as well as spontaneous activity and responses to menthol in many cold fibers (Fig. 9).

Hexanol did not increase the activity of any of the C, Aδ, or Aβ fibers (Table I). This behavior was somewhat unexpected given that hexanol is an irritating trigeminal stimulant (Cometto-Muniz and Cain, 1991) and thus should elicit responses in nociceptive fibers. In fact, whole lingual and ethmoid nerve recordings show that the addition of hexanol increases the activity (from its basal activity) until it reaches a maximum and then decreases to or below basal activity as concentration continues to increase (Silver, Mason, Adams, and Smeraski, 1986; Simon and Sostman, 1991). The decrease below basal activity is clearly related to the anesthetic properties of hexanol.

The question then is which fiber types are activated by hexanol and give rise to the initial increase in activity. Silver et al. (1991) showed that the ethmoid nerve's response to irritating or nociceptive stimuli can be eliminated by elimination of capsaicin-sensitive fibers. This type of C fiber has not been identified in this study.

Capsaicin. The few studies performed with capsaicin revealed that it can selectively inhibit responses to chemical stimuli without affecting the generation of responses to mechanical stimuli. This behavior has also been found in the ethmoid nerve (Silver et al., 1991). The mechanism by which capsaicin inhibited the response to NaCl is unknown, but it is unlikely to occur via the cation-selective channel activated by capsaicin since in the particular fiber investigated capsaicin itself does not elicit a response (Fig. 10). As is well established, capsaicin can alter numerous other transport pathways besides the capsaicin-activated channel on classes of C and Aδ fibers by either direct interaction or via peptides released by capsaicin (Bevan and Szolcsanyi, 1990).

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