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Effects of Various Ion Transport Inhibitors on the Water Response in the Superior Laryngeal Nerve in Rats

Takamitsu Hanamori

Department of Physiology, Miyazaki Medical College, Kiyotake, Miyazaki 889-1692, Japan

Correspondence to be sent to: Takamitsu Hanamori, Department of Physiology, Miyazaki Medical College, 5200 Kihara, Kiyotake-cho, Miyazaki 889-1692, Japan. e-mail: thanamo@post1.miyazaki-med.ac.jp

Abstract

The effects of inhibitors [acetazolamide, an inhibitor of carbonic anhydrase; amiloride, an inhibitor of the Na channel; furosemide, an inhibitor of the Na/K/2Cl transporter; 4,4′-diisothiocyanostilbene-2,2′-disulfonic acid (DIDS), an inhibitor of the Cl channel] on the water response in the superior laryngeal nerve (SLN) were investigated using whole nerve recordings from the SLN of anesthetized and paralyzed rats. Changes in spontaneous activity in the SLN after i.v. injection of a hypo- or hypertonic solution were also investigated. The water response to higher concentration amiloride solutions (0.1, 1, 5 and 10 mM) were significantly smaller in comparison with the control, i.e. the water response to deionized water (88–59% of the control, Fisher's PLSD, $P < 0.05$). DIDS suppressed the water response significantly at concentrations of 0.5 and 2 mM by 18 and 33%, respectively ($P < 0.05$). Likewise, acetazolamide (2 mM) and furosemide (5 mM) significantly suppressed the water response by 9 and 40%, respectively ($P < 0.05$). An i.v. bolus injection of a hypertonic solution (1 ml of 1.5 M NaCl or 1.0 M mannitol) depressed spontaneous activity of the SLN. In contrast, an i.v. injection of a hypotonic solution (0.015 M NaCl) increased spontaneous activity. These results suggest that several ion transporters and ion channels, as well as carbonic anhydrase, that may exist in the dorsal surface in the epiglottis may regulate the water response in the SLN and that osmotic changes in the dorsal surface of the epiglottis and in the interstitial space can affect nerve activity in the SLN.

Introduction

Many studies have shown, in various mammalian species, that afferent fibers in the superior laryngeal nerve (SLN) respond to chemical stimulation of the larynx (Shingai, 1977, 1980; Stedman *et al.*, 1980; Bradley *et al.*, 1983; Shingai and Beidler, 1985; Smith and Hanamori, 1991). The characteristics of responsiveness in the SLN are quite different from those of other nerves that convey gustatory information from the tongue and the anterior oral cavity (Frank, 1973; Hanamori *et al.*, 1988; Harada *et al.*, 1997) and relatively similar to those of the pharyngeal branch of the glossopharyngeal nerve (Hanamori and Ishiko, 1993). Namely, afferent fibers in the SLN are more sensitive to water or hypotonic solutions than to other kinds of taste stimuli. Previous studies have shown that there may be two different types of water fibers in the SLN. One type is responsive to low chloride ions and the other is responsive to low osmolarity (Sant'Ambrogio *et al.*, 1993; Ghosh *et al.*, 1995, 1996). Concerning the transduction mechanism of the water response, no direct evidence has been presented. Some authors have suggested that Cl ions may have an important role in the water response (Shingai, 1977; Ghosh *et al.*, 1996). However, Shingai has shown that the water response does not depend on Cl ions in rats (Shingai, 1980). At present, the mechanism of the water response in fibers of the SLN is poorly understood. Histological studies have revealed numerous taste buds and free nerve endings in the rat larynx (Travers and Nicklas, 1990; Yamamoto *et al.*, 1998). Even the question of how these sensory receptor organs in the larynx contribute to the taste response, including the water response in the SLN, has not been answered.

In humans it has been reported that inhaled diuretic agents (for example furosemide and amiloride) can inhibit the cough response induced by inhalation of water or a low chloride solution (Ventresca *et al.*, 1990; Stone *et al.*, 1991; Foresi *et al.*, 1996). It is supposed that the cough response induced by inhalation of water or a low chloride solution may be caused by activation of the afferent fibers in the SLN (the water response) and that diuretic agents which act as ion transport inhibitors or ion channel inhibitors may depress the water response in the SLN. This possibility has been ascertained in electrophysiologically cats and dogs. Inhibitors could depress the water response in the SLN (Sant'Ambrogio *et al.*, 1993; Ghosh *et al.*, 1995), indicating that some ion channels or ion transporters have the ability to modify the water response in the SLN. However, no experiments have been conducted concerning the effects of inhibitors on the water response in the SLN in rats. Only one report about the water response in the SLN in rats has been

published (Shingai, 1980). More studies are needed to understand the water response in the SLN. In the present study we have examined electrophysiologically the effect of ion transport inhibitors, ion channel inhibitors and a carbonic anhydrase inhibitor on the water response in the SLN using whole nerve recordings. Moreover, we investigated concentration–response functions in the SLN for mannitol, KCl and NaCl and changes in spontaneous activity in the SLN when changing the osmolarity in the interstitial fluid by i.v. injection of a hypo- or hypertonic solution.

Materials and methods

Animals and surgery

Recordings were obtained from the whole SLN of male Sprague–Dawley rats weighing 230–400 g (mean ± SE = 282.1 ± 7.1 g, n = 33). Rats were initially anesthetized with an i.p. injection of a mixture of urethane (1 g/kg) and α-chloralose (100 mg/kg). The trachea was cut and its lower portion was cannulated for artificial ventilation. The upper side of the dissected trachea was cut through longitudinally near the epiglottis and opened wide to view the inside of the trachea. The ventral side of the epiglottis was pushed with a cotton swab from the oral cavity so that the dorsal epiglottis could be seen in the uppermost portion of the opened trachea. The left femoral artery and vein were cannulated for measurement of blood pressure (BP) and administration of drugs, respectively. End-expired CO_2 was constantly monitored and maintained at 3.5–4.5%. Rectal temperature was maintained 37–38°C by a thermostatically regulated heating pad. During the recordings the rat was paralyzed with tubocurarine (2 mg/kg i.v.) and artificially ventilated by a Harvard respiratory pump (model 683). Whenever paralysis seemed to wear off (usually 30–60 min) the level of anesthesia was assessed by pinching the tail. In addition, the level of anesthesia was continuously checked during the experiment by continuous monitoring of BP, heart rate (HR) and expiratory CO_2, which are useful parameters for this purpose. If required, supplemental doses of α-chloralose or urethane were administered. The left SLN was dissected from surrounding connective tissue and transected for whole nerve recording. In all experiments the right SLN, bilateral glossopharyngeal nerves and bilateral hypoglossal nerves were transected to prevent inadvertent tongue and trachea movements. The esophagus was tied up to prevent the solutions (delivered over the epiglottis) from flowing into the stomach.

Stimulation

A small amount of taste solution (0.1–0.2 ml) was manually delivered around the epiglottal surface for ~30–60 s using a 1 ml syringe. Solution flowing in the inside of the trachea was constantly removed by aspiration using a glass capillary tube whose tip was placed near the surface of the epiglottis.

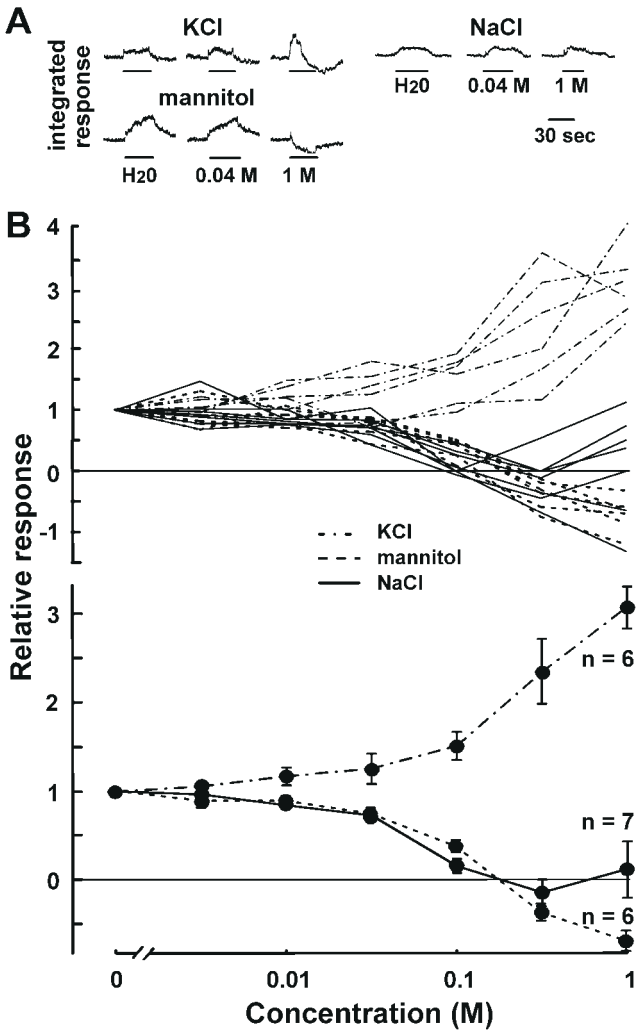

Figure 1 SLN responses to stimulation of the larynx by deionized water (H_2O), KCl, NaCl and mannitol. **(A)** Sample recordings of integrated responses of the SLN to deionized water (H_2O) and hypotonic (0.04 M) and hypertonic (1 M) solutions of KCl, NaCl and mannitol. The SLN showed an excitatory response to H_2O: 0.04 M for KCl, NaCl and mannitol (water response). On hypertonic stimulation (1 M) the SLN showed a strong excitatory response to KCl followed by an inhibitory phase and a relatively small excitatory response to NaCl. **(B)** Upper graphs show the concentration–response function for KCl, NaCl and mannitol in each rat. The lower graphs show the mean concentration–response function. For NaCl the concentration–response function was similar to that of mannitol. However, in some rats (4/7) the SLN showed an excitatory response to 1 M NaCl, as in (A). The magnitude of the response is relative, obtained from the height of the integrated response in the SLN (response to H_2O = 1.0).

Usually the epiglottis remained covered with 0.15 M NaCl to keep it from drying. Taste stimuli used were concentration series of NaCl (0.003–1 M), KCl (0.003–1 M), mannitol (0.003–1 M), amiloride (0.001–10 mM, an inhibitor of the sodium channel) and 4,4′-diisothiocyanostilbene-2,2′-disulfonic acid (DIDS) (0.02–2 mM, an inhibitor of the chloride channel), deionized water, 2 mM furosemide (an inhibitor of the Na/K/2Cl transporter) and 2 mM acetazoramide (an inhibitor of carbonic anhydrase).

All stimuli were dissolved in deionized water except furosemide. The solution of 2 mM furosemide contained 22 mM NaCl. After taste stimulation the epiglottis was rinsed with 1 ml of 0.15 M NaCl since it has been determined that the SLN shows the least response to 0.15 M NaCl (Shingai, 1980). The stimulus interval was ~5 min. The taste stimuli and rinse were delivered at room temperature (22–24°C).

To change the osmolarity in the interstitial space in the larynx we i.v. injected 1 ml of hypotonic (0.015 M NaCl) or hypertonic solution (1.5 M NaCl or 1 M mannitol). This stimulation may evoke complex changes in the autonomic system. It is supposed that the ion environment of the interstitial space in the larynx will change immediately after i.v. injection of the hypo- or hypertonic solution. To investigate the effects of changes in BP and HR on nerve activity in the SLN two drugs, methoxamine hydrochloride (20 μg/0.1 ml, increasing BP by a vasoconstrictor effect) and sodium nitroprusside (10 μg/0.1 ml, decreasing BP by a vasodilator effect), were i.v. injected.

Recording and data analysis

BP and HR (obtained from blood pressure pulsation) were amplified using a conventional amplifier and recorded on a pen recorder. For whole nerve recording of the SLN the peripheral portion of the SLN was placed on a pair of platinum wire electrodes. Neural activity was amplified and fed into a pulse counter (spikes/s) and integrator (time constant 0.3 s).

In the present study we called a response to H_2O or hypotonic solution (NaCl, KCl, amiloride, etc.) a water response (a water response to low chloride solution was not observed in the present work). Although it is difficult to definitely describe the response to hypotonic slutions such as NaCl or KCl as a water response, we describe the response to 0.04 M as a water response in consideration of the consentration–response function (Figure 1B).

The magnitude of SLN responses to chemical stimuli was obtained by measuring the maximum height of the integrated response. For statistical analysis the magnitude of the response was expressed as a relative value: the response to deionized water was defined as unity (1.0). The changes in spontaneous discharge rate (spikes/s) after i.v. injection of a hypo- or hypertonic solution were also expressed as a relative value. The average spontaneous discharge rate before stimulation was defined as unity (1.0). The values in the text are means ± SE. A statistical analysis was made using ANOVA.

Results

Water response: concentration–response functions for NaCl, KCl and mannitol

In the whole nerve recordings the rate of spontaneous activity in the SLN was usually high (of the order of several hundred Hertz). For all three stimuli (NaCl, KCl and mannitol) spontaneous activity in the SLN increased during epiglottal stimulation with a hypotonic solution (the water response; Figure 1A, H_2O and 0.04 M). The water response was gradually depressed as the concentration of NaCl or mannitol increased and almost disappeared at around the level of saline solution (0.15 M NaCl, Figure 1B). At higher concentrations (>0.15 M) an inhibitory response was recorded; spontaneous activity in the SLN was depressed during stimulation. As the concentration increased further the inhibitory response became larger for mannitol, while for NaCl an excitatory response was noted in some rats (4/7 rats, Figure 1B, 1 M NaCl). On the other hand, KCl showed a unique concentration–response function. The SLN showed an excitatory response at all concentrations in the KCl series (Figure 1B). The time course of the response for KCl at high concentration (KCl response, Figure 1A, 1 M) was different from that at lower concentrations (the water response, Figure 1A, H_2O or 0.04 M). Namely, the response to 1 M KCl increased rapidly, then decreased after reaching a maximum to a level even below that of spontaneous SLN activity before stimulation (Figure 1A).

Effects of various inhibitors on the water response

The effects of four drugs (DIDS, acetazolamide, amiloride and furosemide) on the water response were investigated. Compared with deionized water, the responses to 2 mM DIDS and 5 mM amiloride was depressed (Figure 2A). A depressed water response was also observed for both 2 mM acetazolamide and 5 mM furosemide. The results are summarized in Figure 2B. All the stimuli used were dissolved in deionized water except for 5 mM furocemide, the solution of which contained 22 mM NaCl. As the solutions used are expected to have weak osmolarity, we adopted the water response to 25 mM NaCl as a control. Figure 2B shows that all the inhibitors used in the present study have a depressant effect on the water response [$F(4,43) = 34.049$, $P < 0.0001$]. The magnitudes of the response to 25 mM NaCl, 2 mM acetazolamide, 2 mM DIDS, 5 mM furosemide and 5 mM amiloride were 1.01 ± 0.01 ($n = 8$), 0.91 ± 0.03 ($n = 9$), 0.68 ± 0.04 ($n = 10$), 0.61 ± 0.03 ($n = 6$) and 0.63 ± 0.02 ($n = 15$), respectively (the magnitude for deionized water = 1). The water response for all the inhibitors used in the present study was significantly depressed compared with 25 mM NaCl (Fisher's PLSD, $P < 0.05$).

Concentration–response function for DIDS and amiloride

The water response for DIDS was significantly decreased with increasing concentration of DIDS [$F(4,25) = 5.285$, $P < 0.0032$; Figure 3]. The magnitudes of the water response to 0.02, 0.2, 0.5 and 2 mM DIDS were 0.95 ± 0.04 ($n = 6$), 0.93 ± 0.09 ($n = 6$), 0.82 ± 0.07 ($n = 6$) and 0.67 ± 0.04 ($n = 6$), respectively. The water response for both 0.5 and 2 mM was significantly depressed compared with the water response to deionized water (Fisher's PLSD, $P < 0.05$). The

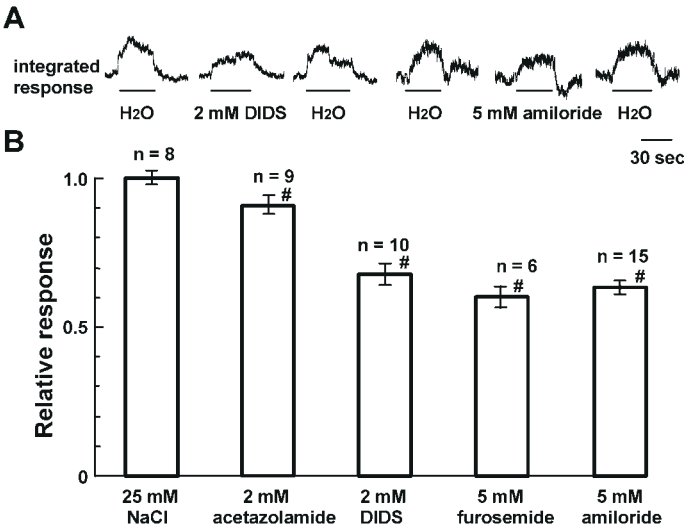

Figure 2 Effects of the inhibitors on the water response in the SLN. **(A)** Sample recordings of integrated responses of the SLN to H_2O, 2 mM DIDS and 5 mM amiloride. Both inhibitors dissolved in deionized water depressed the water response. H_2O stimulation was conducted before and after test stimulation as a control for the water response. **(B)** Summary of the effect of the inhibitors (2 mM acetazolamide, 2 mM DIDS, 5 mM furosemide and 5 mM amiloride) on the water response. The magnitude of the response is shown as a relative value (response to H_2O = 1.0). The solution of 25 mM NaCl was used as a control for statistical analysis, since the 5 mM furosemide solution contained 22 mM NaCl (all other inhibitors were dissolved in deionized water). All inhibitors used in the present study significantly depressed the water response. $^{\#}P < 0.05$.

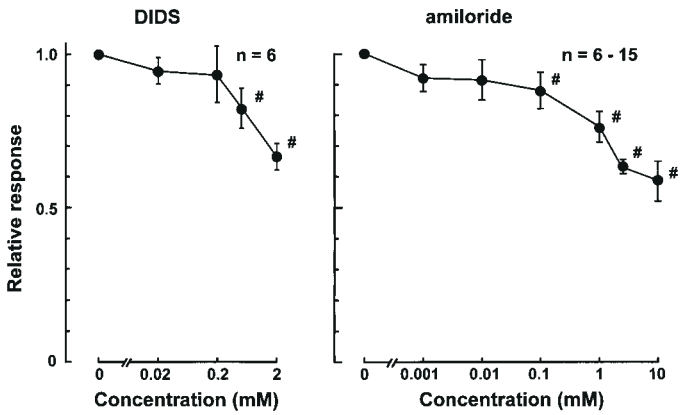

Figure 3 Concentration–response function for DIDS and amiloride in the SLN. The water response was depressed with increasing concentration of DIDS or amiloride. The depressant effect of the inhibitors on the water response was statistically examined by comparison with the water response to deionized water. $^{\#}P < 0.05$.

concentration–response function for amiloride was also significantly decreased with increasing amiloride concentration [$F(6,55) = 20.188$, $P < 0.0001$; Figure 3]. The magnitudes of the water response to 0.001, 0.01, 0.1, 1, 5 and 10 mM amiloride were 0.92 ± 0.04 ($n = 6$), 0.92 ± 0.07 ($n = 6$), 0.88 ± 0.06 ($n = 6$), 0.76 ± 0.05 ($n = 7$), 0.63 ± 0.03 ($n = 15$) and 0.59 ± 0.06 ($n = 7$), respectively. The water response to 0.1, 1, 5 and 10 mM amiloride was significantly depressed in comparison with the water response to deionized water (Fisher's PLSD, $P < 0.05$).

Effects of an i.v. injection of hypo- or hypertonic solution on spontaneous activity in the SLN

The spontaneous discharge rate in the SLN was depressed by i.v. application of hypertonic solutions, either 1.5 M NaCl or 1 M mannitol (1 ml, Figure 4A). Changes in BP and HR were also elicited by i.v. injection of a hypertonic solution. However, no depression in spontaneous activity was seen when BP and HR were changed by i.v. injection of methoxamine hydrochloride (20 μg/0.1 ml) or sodium nitroprusside (10 μg/0.1 ml) (Figure 4B). On the other hand, spontaneous activity in the SLN was slightly increased by i.v. injection of a hypotonic solution (0.015 M NaCl, 1 ml) (Figure 4A). Figure 5 shows the time course of relative spontaneous discharge rate after i.v. application of 1.5 M NaCl, 0.015 M NaCl and 1 M mannitol (spontaneous rate before i.v. application = 1). Each time course showed significant changes in spontaneous activity in the SLN on i.v. application of a hypo- or hypertonic solution (1 M NaCl, $P < 0.0001$; 1 M mannitol, $P < 0.003$; 0.015 M NaCl, $P < 0.047$). For 1.5 M NaCl significant depression was seen at 0.25 and 0.5 min (Fisher's PLSD, $P < 0.05$). Mannitol showed a similar time course to 1.5 M NaCl but slightly longer depression; significant depression was shown until 1 min after i.v. injection (Fisher's PLSD, $P < 0.05$). The changes in spontaneous activity in the SLN induced by hypotonic injection were not as large as those induced by injection of hypertonic solutions, but showed a significant increase. The changes in spontaneous activity induced by 0.015 M NaCl seemed to continue longer than those induced by 1.5 M NaCl. Spontaneous activity in the SLN did not change on i.v. injection of saline (0.15 M NaCl, 1 ml; data not shown).

Discussion

The present study shows that the inhibitors DIDS, furosemide, amiloride and acetazolamide depress the water response in the SLN. This suggests that various ion channels and ion transporters, as well as carbonic anhydrase, may exist on the epiglottal surface and can regulate the water response in the SLN. In addition, changes in the ion environment or osmolarity in the interstitial space also affect spontaneous activity in the SLN.

Water response in the SLN

In dogs (Anderson *et al*., 1990; Sant'Ambrogio *et al*., 1993) and cats (Ghosh *et al*., 1996) it has been reported that the water fibers in the SLN are of two types. One type is responsive to low chloride solution, while the other is responsive to hypotonic solutions. On the other hand, in the mouse only the latter type (fibers responsive to hypotonic

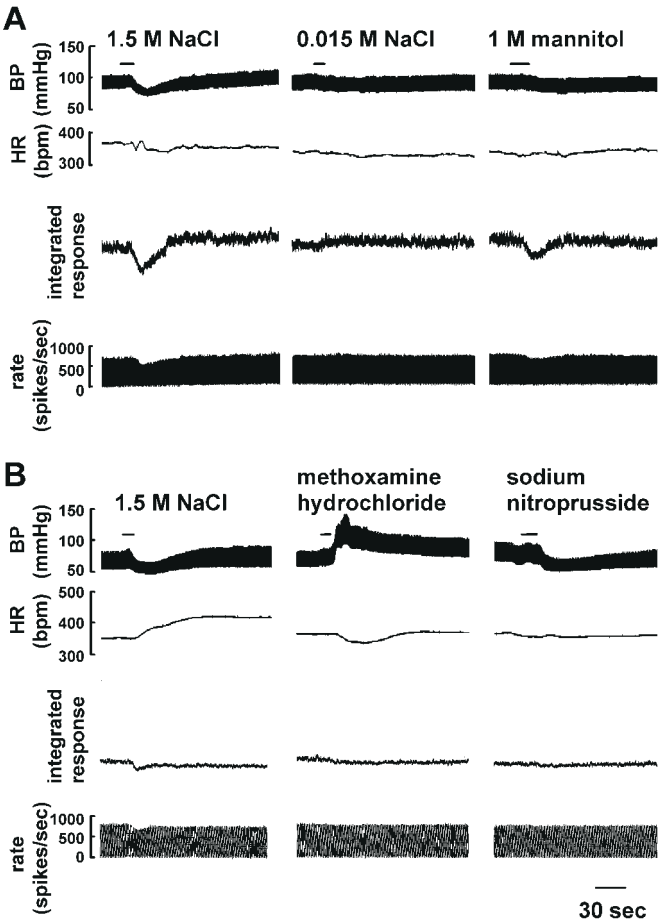

Figure 4 Effect of an i.v. injection of hypo- or hypertonic solution on spontaneous activity in the SLN. **(A)** Spontaneous activity was decreased by i.v. injection (1 ml) of a hypertonic solution (1.5 M NaCl and 1 M mannitol). Spontaneous activity was slightly increased by i.v. injection of a hypotonic solution (0.015 M NaCl). Simultaneous recordings of HR, arterial BP and nerve activity in the SLN (integrated response and rate) are shown. **(B)** Similar recordings as in (A) but from a different rat. The basal activity of the SLN was depressed by i.v. injection of 1.5 M NaCl. To ascertain the effects of the changes in HR and BP on spontaneous activity in the SLN two drugs, methoxamine hydrochloride (20 μg/0.1 ml, 0.1 ml) and sodium nitroprusside (10 μg/0.1 ml, 0.1 ml), were injected i.v.. There was no effect of changes in BP and HR on spontaneous activity in the SLN.

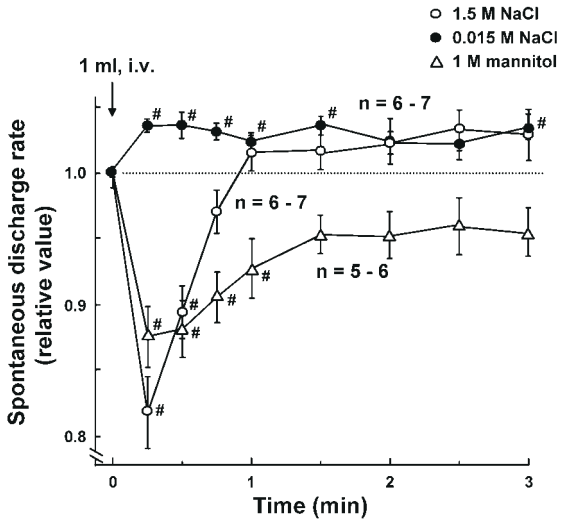

Figure 5 Time course of the changes in spontaneous activity in the SLN on i.v. injection of a hypo- or hypertonic solution. The magnitude of spontaneous discharge rate is indicated as a relative value (spontaneous discharge rate before i.v. injection = 1.0). Spontaneous discharge rate changed significantly compared with that before stimulation. $^{\#}P < 0.05$.

solutions) seem to be contained in the SLN, since isotonic glucose solution (which does not contain Cl) was an ineffective stimulus for the water response (Shingai and Beidler, 1985). In the present study, as in studies on mice, we could not demonstrate a low chloride-sensitive water response in the rat SLN, since mannitol solution (which does not contain Cl) was not an effective stimulus at around the isotonic concentration level (Figure 1B). The reason for the lack of water fibers sensitive to a low chloride solution in the rat SLN may be a species difference.

There was no inhibitory response for KCl at 0.1 M or higher concentrations (Figure 1B), which has a high enough osmolarity to depress the water response. On the contrary, the SLN showed an excitatory response to KCl stimulation. It may be reasonable to assume that the excitatory response is induced by K ions. It is supposed that the concentration–response function for KCl shown in Figure 1B is composed of two response curves (water and K ions).

The concentration–response function for NaCl is similar to that for mannitol and seems to be dependent on an osmolarity change (Figure 1B). However, the degree of depression by mannitol was larger than that by NaCl considering the osmolarity. In addition, the responsiveness to NaCl is clearly different from mannnitol at higher concentrations (Figure 1B). This difference may be due to Na ions having an excitatory effect on SLN activity, as do K ions (although it seems that the excitatory effect of Na ions is much weaker than that of K ions). On the other hand, mannitol is thought to be an ineffective chemical stimulus. Therefore, it is considered that the concentration–response function for mannitol indicates solely the SLN response to the osmolarity change in the epiglottal surface.

There is a species difference in the water response in the SLN. In all species the SLN seems to respond to hypotonic solutions. However, a water response due to low chloride could not be demonstrated in rats. The functional role of the water fibers in the SLN and the meaning of the exsistence of two types of water fibers remain to be resolved. Other effective chemical stimuli for the SLN seem to differ depending on the animal: KCl in rats and sheep (Bradley *et al*., 1983); HCl in hamsters (Smith and Hanamori, 1991). In addition, the hamster SLN is sensitive to NaCl at high concentrations (Smith and Hanamori, 1991).

Ion transport inhibitors

It has been reported that pretreatment with furosemide,

amiloride or acetazoramide depresses the cough response evoked by low chloride aerosol stimulation in humans (Ventresca *et al*., 1990; Stone *et al*., 1991; Foresi *et al*., 1996). The possibility that receptors that are sensitive to low chloride and are innervated by the SLN may be modified by inhibitors has been ascertained in dogs and cats; furosemide and amiloride reduced the water response in the SLN (Sant'Ambrogio *et al*., 1993; Ghosh *et al*., 1995, 1996). In the present study similar results were obtained in rats. The water response in the SLN to 5 mM furosemide and 5 mM amiloride was ~60% of that of the control (the water response to deionized water) (Figure 2). In addition, the present study shows that DIDS and acetazolamide also have an inhibitory effect on the water response in the SLN. In contrast to our results for DIDS, there was no significant reduction in the water response for DIDS in the cat (Ghosh *et al*., 1996). This discrepancy may be due to a species difference.

It is well known that amiloride inhibits the NaCl response in taste receptor cells by blocking the Na channel (amiloride-sensitive Na channel) (cf. DeSimone and Ferrell, 1985). However, it has been shown that amiloride is not a specific inhibitor of the Na channel. At higher concentrations (>0.1 mM) amiloride also has an inhibitory effect on other ion transporters, such as the Na–H exchanger and the Na–Ca transporter (Lundy *et al*., 1997). Several reports have shown relatively non-specific inhibitory effects on the taste response of amiloride (cf. Nakamura and Kurihara, 1990). The present study shows that amiloride is effective on the water response at relatively high concentrations (>0.1 mM). Furthermore, the rat SLN was not responsive to NaCl except at high concentrations. Therefore, we consider that there is no amiloride-sensitive Na channel on the epiglottal surface or, if there is one, its role in the NaCl response is insignificant. Amiloride might act on the system regulating ionic balance in the epiglottis by blocking ion transporters and/or ion exchangers (mentioned above) and then may indirectly inhibit the water response in the SLN.

It is known that furosemide and acetazolamide inhibit the Na/K/2Cl transporter and the Na-H exchanger, respectively. Although it has not been shown that the Na/K/2Cl transporter exists on the epiglottal surface in rats, it has been reported that carbonic anhydrase was found on the surface of the respiratory tract (Okamura *et al*., 1996) and gustatory epithelium in the taste buds in the posterior part of the tongue (Daikoku *et al*., 1999). As in the case of amiloride, these inhibitors may indirectly depress the water response in the SLN by acting on the ionic balance regulation system in the epiglottis.

Previous studies that have shown a low chloride water response suggest that passive exclusion of Cl from the epithelial cells is important for the water response (the low chloride water response) (Sant'Ambrogio *et al*., 1993; Ghosh *et al*., 1996). However, Ghosh *et al.* have shown that DIDS did not significantly depress the water response (Ghosh *et al.*, 1996). Therefore, it seems that in cats the Cl channel does not have a role in the water response or that a Cl channel insensitive to DIDS is involved in the water response. The present study indicates that the rat SLN has only one type of water response, namely the low osmolarity water response, and that it does not have a low chloride water response. Since the present study showed inhibitory effects of DIDS on the water response, it is supposed that a DIDS-sensitive Cl channel has an important role in the low osmolarity water response.

It is known that osmotic changes induce Cl movement through the membrane. Therefore, there is a possibility that the DIDS-sensitive Cl channel at work in osmotic change is involved in water transduction. In the frog the glossopharyngeal nerve shows a water response. Okada *et al.* have suggested that exclusion of Cl from the taste cells contributes to the water response in the frog glossopharyngeal nerve (Okada *et al.*, 1993).

Ion environment in the interstitial fluid

It is known that i.v. injection of hypo- or hypertonic solutions affects various autonomic systems. Shingai *et al*. have shown that i.v. injection of 1.75% NaCl or 10% mannitol depressed spontaneous activity in the SLN in rabbits over a 15 min period (Shingai *et al*., 1991). They suggested that hypothalamic osmoresponsive neurons activated by i.v. injection of hypertonic solutions depresses spontaneous SLN activity via the sympathetic nervous system. Similary, the present study shows that spontanous activity in the rat SLN is depressed following i.v. injection of a hypertonic solution (NaCl or mannitol). However, the mechanism for the depression in the SLN observed in the present study seems to be different from that in the rabbit. In the present study depression of spontaneous activity may be induced by a hypertonic change in the interstitial space in the larynx (high osmolarity in the interstitial space may induce depression of spontaneous activity in the SLN). This assumption may be supported by the following data. First, changes in BP and HR during i.v. injection of a hypertonic solution did not correlate with the changes in spontaneous activity in the SLN (Figure 4). In addition, spontaneous activity in the SLN did not change when BP and HR were changed by i.v. injection of methoxamine hydrochloride (increase in BP by a vasoconstrictor effect) or sodium nitroprusside (decrease in BP by a vasodilator effect). The changes in BP and HR are expected to induce changes in parasympathetic and sympathetic nerve activity. Second, the duration of changes in spontaneous activity in the SLN were relatively short (~1 min, Figure 5) in comparison with those in rabbits [>15 min (Shingai *et al*., 1991)]. On the other hand, spontaneous activity of the SLN was increased following i.v. injection of a hypotonic solution.

The effect of i.v. injection of hypo- or hypertonic solutions on spontaneous activity in the SLN was similar to that shown for SLN responses to an osmolarity change in

the epiglottal surface. The change in osmolarity in the interstitial space may affect various ion transporters and/or ion channels that may exist on the terminals of the afferent nerve or basolateral side of receptor cells. The results suggest that spontaneous activity in the SLN will decrease in the case of high osmolarity in the interstitial space, as in a dehydrated state.

Conclusions

The present study has shown that the water response is elicited by application of a low osmolarity solution to the epiglottal surface and that a DIDS-sensitive Cl channel may be involved in the primary process of the water transduction mechanism. However, the osmotic response may be modified by various cations (Na, K, H, etc.), anions and non-ionic solutes. Therefore, the concentration–response function may differ according to the stimulus used. It may be reasonable to conclude that amiloride, acetazolamide and furosemide modify the water response. All these inhibitors could affect an environmental ionic balance change around the receptor cells or afferent nerve terminals. To understand the mechanism of the water response in the SLN several issues still need to be resolved. The most complicated one is that it is still uncertain whether the receptor organ for the water response is free nerve endings or receptor cells in the taste buds or epithelial cells.

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