Low environmental temperature modulates gustatory nerve activity and behavioral responses to NaCl in rats

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Shimizu, Yasutake, and Keichi Tonosaki. Low environmental temperature modulates gustatory nerve activity and behavioral responses to NaCl in rats. Am. J. Physiol. 277 (Regulatory Integrative Comp. Physiol. 46): R368–R373, 1999.—We studied the effects of cold ambient temperature on chorda tympani nerve responses to taste stimuli such as sucrose, NaCl, quinine HCl (QHCl), and HCl in rats. The electrophysiological recordings of the whole chorda tympani nerves from control (22°C) and cold-exposed (4°C) rats revealed that the responses to sucrose, HCl, and QHCl were unaffected by cold exposure. In contrast, the nerve responses to NaCl were enhanced time dependently, reaching a maximum 7–14 days after cold exposure. Responses to sodium acetate were likewise elevated as they were to NaCl, whereas those to KCl were unchanged after cold exposure. In addition, the residual NaCl responses after lingual application of the sodium-channel blocker amiloride in cold-exposed rats were similar to those in control animals. It is thus most likely that cold exposure potentiates the chorda tympani nerve responses to Na⁺, but not to Cl⁻. Behavioral studies with the two-bottle preference test showed that the cold-exposed rats refused to drink NaCl solutions at 0.05 and 0.3 M, the concentrations being preferred by control animals. These results suggest that the ambient temperature influences taste cell function, and that the enhanced NaCl response of the chorda tympani nerve is related to the avoidance of NaCl intake under cold environment.

METHODS

Subjects. We used 76 male Wistar rats, weighing 180–230 g (6–7 wk old) at the time of delivery from Japan SLC (Shizuoka, Japan). The rats were housed individually in plastic cages at 22 ± 1°C with a 12:12-h light-dark cycle (lights on 0700–1900) and given free access to laboratory chow (LABO MR Stock, Nihon-Nosan, Yokohama, Japan) and water, unless otherwise stated. The chow contains 0.18% NaCl. The rats were maintained in the laboratory for ≥2 wk after arrival to permit them to acclimate to their surrounding before experiments began (8–9 wk old, 250–300 g at the start of experiments). When the rats were exposed to a cold environment, they were transferred to a cold room along with the cages in which they were housed and kept for 1–14 days. The cold room into which rats were transferred was kept at 4 ± 1°C with the same lighting schedule (lights on 0700–1900). During cold exposure, the rats had free access to the

TASTE CAN COMMONLY BE classified into four distinct groups: 1) sweet, 2) salty, 3) bitter, and 4) sour (12, 15, 21). Each taste group has some biological meanings, such as supply for energy sources (sweet), warning of spoiled and toxic food (sour and bitter), and regulation of osmotic and ionic condition (salty and sour). Recent evidence has demonstrated that taste receptor function can be modulated by environmental factors, indicating that the plasticity of peripheral gustatory system may contribute to a favorable feeding behavior under certain environmental conditions in which animals are placed (21). The most representative example of environmental factors that affect taste receptor function is the restriction of dietary sodium. In the sodium-restricted rats, the chorda tympani nerve response to NaCl was reduced, and the reduced responsiveness would permit the animals to intake high-sodium foods and solutions (5, 21).

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same chow and water as the animals housed at 22°C. The temperature of the cold room, i.e., 4°C, was easily tolerated by rats and they remained in good health throughout the cold-exposure period.

Neural recording procedure. Each rat was deeply anesthetized by an intraperitoneal injection of pentobarbital sodium (50 mg/kg body wt), and the trachea was cannulated. The animal was fixed with a headholder, and its body temperature was regulated between 36 and 38°C by a water-circulating heating pad. The chorda tympani nerve branch was then exposed through a mandibular approach. The nerve was severed at the point where it enters the bulla, desheathed, and placed on a pair of silver wire electrodes. The nerve was covered and bathed with mineral oil to prevent drying. The electrical activity of the whole chorda tympani nerves was fed to an AC amplifier and then displayed on an oscilloscope screen. Neural responses resulting from chemical stimulation on the tongue were integrated (time constant 1.0 s) and recorded on a chart recorder. An audiomonitor was also used. The tongue was gently extended with a hook, and test solutions were applied for 20 s at a constant rate of 10 ml/20 s by a gravity-flow system. Bottles containing distilled water and taste solutions were incubated in the water bath at 30°C and set on the gravity-flow system immediately before each application. The height of steady-state responses of the chorda tympani nerve activity, which were measured at 10 s after the onset of stimuli, was normalized to means of responses with 0.1 M NH₄Cl being taken as unity (1.0). The stability of the nerve responses was monitored by periodic application of 0.1 M NH₄Cl. Responses were also recorded after lingual treatment of the epithelial sodium-transport blocker amiloride. The solvent for NaCl, KCl, and NH₄Cl solutions and the rinse consisted of 500 µM amiloride. Neural responses in cold-exposed rats were recorded at room temperature. They were anesthetized immediately after removal from the cold room. All procedures for neural recording were equivalent to those in control rats as previously described.

Solutions. Distilled water was used to prepare test solutions of sucrose, NaCl, sodium acetate (NaAc), KCl, HCl, and NH₄Cl, amiloride solution, and for rinsing the tongue. All of the test solutions were made fresh at least each week and refrigerated when not in use. Amiloride solution (500 µM) was prepared immediately before use.

Preference test. The two-bottle choice methods were used for the preference test, in which one bottle contained distilled water and the other a test solution made with NaCl dissolved in distilled water. The positions of the bottles were altered daily to control for position preference. Volumes of intake were measured each afternoon (between 1 and 2 PM), and the bottles were returned to the cages after replacement with new solutions. Solutions were offered for 48 h each in ascending order of concentration at the values shown in the abscissa of Fig. 1. The food intake was greater in cold-exposed rats (17.6 ± 0.7 g/day for control vs. 25.6 ± 0.8 g/day for cold-exposed rats), whereas water intake was equal (33.9 ± 1.1 ml/day for control vs. 34.6 ± 0.4 ml/day for cold-exposed rats). The rate of weight gain was 5.7 ± 0.3 g/day in control and 1.7 ± 0.6 g/day in cold-exposed rats.

Results. The nerve response to NaCl was substantially increased in rats exposed to cold environment for 7 days. In contrast, responses to sucrose, QHCl, and HCl were unchanged after cold exposure.

Concentration-response relationships for NaCl in both groups are shown in Fig. 2. There was no significant difference in the responses to NaCl less than 0.005 M between the two groups. However, at NaCl concentrations more than 0.01 M, the relative responses of cold-exposed rats were significantly greater than those of the control animals. Similar effects were observed on the responses to NaAc (data not shown).

Time course of increase in NaCl response under cold environment. We then examined the time course of increase in NaCl response (Fig. 3). Cold exposure for 1 day did not change the chorda tympani nerve responses to NaCl (1.56 ± 0.09 for control vs. 1.58 ± 0.13 for cold-exposed rats). Significant increase in the response was observed on day 3 of cold exposure (1.96 ± 0.10, P < 0.05). The response increased further in a time-dependent manner for up to day 7. The increased response to NaCl on day 14 was comparable to that on day 7 (2.52 ± 0.12 on day 7 vs. 2.68 ± 0.15 on day 14), indicating that an adaptive change in the chorda tympani nerve response is accomplished almost completely within a week.

To see if the enhanced responsiveness of the chorda tympani nerve to NaCl is reversible, rats kept at 4°C for...
7 days were returned to a room with temperature at 22°C, and the nerve responses were recorded on days 1, 3, and 7 after rewarming. As shown in Fig. 4, the response to NaCl on day 1 was similar to that in cold-exposed rats. However, the increased response gradually declined with time of rewarming and approached the control level (1.56 ± 0.09 for control vs. 1.80 ± 0.14 at day 7 of rewarming). The time dependency of recovery was similar to that of the response enhancement.

Effects of amiloride on sodium response of chorda tympani nerve. It has been known that NaCl response of the chorda tympani nerve was effectively, but not totally, suppressed by the epithelial sodium-channel blocker amiloride. This implies that the NaCl response is composed of both amiloride-sensitive and -insensitive pathways (7, 13). However, the residual NaCl response after amiloride is not due to an amiloride-insensitive sodium response but related to chloride response (9). To estimate a physiological significance of the cold-induced hypersensitivity to NaCl, it is advisable to see whether it is derived from the increased response to sodium or to chloride.

Figure 5 shows the effects of amiloride on the chorda tympani nerve responses to NaCl, NaAc, and KCl. Lingual application of 500 µM amiloride suppressed NaCl response 64 ± 4% in control rats. In cold-exposed rats, in which NaCl response was increased, the residual response after amiloride was comparable to that in control rats (0.58 ± 0.10 for control vs. 0.60 ± 0.07 for cold exposure). As expected from increased NaCl responses with identical remains after amiloride, percent suppression was greater in cold-exposed rats (77 ± 2%). The response to NaAc was almost completely suppressed by amiloride in both control and cold-exposed rats, although its level before amiloride treatment was much higher in cold-exposed animals. In contrast, the response to KCl was not affected by amiloride treatment.

Behavioral response to NaCl during cold exposure. We also examined behavioral response to NaCl solutions in the cold environment by the two-bottle choice methods. Total fluid intake by cold-exposed rats did not significantly differ from that by control animals when they were kept in the cold for more than 4 days, whereas it was reduced slightly for the first 2 days of exposure (data not shown). As shown in Fig. 6, total fluid intake (water plus NaCl solution) by control and cold-exposed rats during the preference testing was not significantly different. Calculated preference ratio shows that rats kept at 22°C preferred 0.05–0.1 M NaCl solutions. In contrast, rats under the cold environment did not prefer NaCl solutions at any concentration to water and avoided 0.05–0.1 M solutions preferred by control animals. Both groups of rats did not discriminate 0.01 M solution with distilled water and showed strong aversion to 0.5 M NaCl solution.
DISCUSSION

We have investigated the effects of cold exposure on peripheral gustatory function of rats by recording the chorda tympani nerve responses to various taste stimuli. Major findings of the present study are 1) the response of the chorda tympani nerve to NaCl is enhanced by cold exposure with time dependency, whereas responses to sucrose, QHCl, and HCl are unaffected, 2) the enhancement of NaCl response is attributable to increased responsiveness of taste receptor cells to Na\(^+\) but not to Cl\(^-\), and 3) the enhanced responsiveness to NaCl could be associated with avoidance of salt intake under cold environment. Although the peripheral taste cells have often been considered passive transducers of taste stimuli, our results suggest that the taste cell function can be modulated by an environmental temperature, which may contribute to an appropriate ingestive behavior.

It has been known that taste cells are continuously renewed over an average of 10 days (1, 8, 15). Time course for the enhancement of the nerve responses to sodium salts after cold exposure was found to be approximately identical with the turnover time of the taste cells. Furthermore, a similar time course was observed for the recovery of nerve response to NaCl, when the rats were reintroduced to a warm environment (Fig. 4). In contrast to acute changes in taste responses (e.g., differential responses to the same stimuli with different temperature), relatively long-term changes are thought to be taking place during differentiation of the taste cells from basal stem cells. For instance, Matsuo et al. (16) pointed out that the reduction of chorda tympani nerve responses after desalivation depends on the renewal of taste cells; the time course for the reduction of taste responses after desalivation was almost identical with that for the change in sodium responses after cold exposure, as observed in the present study. It is thus probable that sensitivity of taste receptor cells to sodium salts is substantially refined during the process of cell replacement under the cold environment. It should be noted, however, that this does not necessarily rule out the possibility that responsiveness of fully differentiated taste cells can also be improved under cold environment.

An important early event in sodium taste transduction is the entry of Na\(^+\) into the receptor cells via apical sodium channels (12, 15, 21). In accordance with this, the epithelial sodium-channel blocker amiloride is effectively, but not totally, capable of suppressing the chorda tympani nerve response to NaCl (7, 13). It has been demonstrated that the residual NaCl response after amiloride application is related to halogen (e.g., Cl\(^-\)) response, rather than to an amiloride-insensitive Na\(^+\) transduction pathway (9). This is based largely on the evidence that responses to nonhalogenated sodium...
salts such as NaAc are completely abolished by amiloride (Ref. 9 and Fig. 5). In the present study, the residual responses of NaCl after amiloride application were found to be equivalent before and after cold exposure. Furthermore, the nerve responses to KCl were unchanged by cold exposure. If Cl⁻ responses were elevated, both the KCl and amiloride-insensitive NaCl responses may have been increased. It is thus most likely that Cl⁻ responses were not modified. In addition, responses to NaAc were likewise elevated as observed with NaCl and blocked completely by amiloride even after cold exposure. Considering that NaAc is theoretically composed by Na⁺ only as a taste cell stimulant (9), it is conceivable that Na⁺ responses of the chorda tympani nerves are exclusively potentiated by cold exposure. Collectively, these results suggest that the sodium taste transduction pathway, which relates to the amiloride-sensitive sodium channels, is positively modulated under cold environment.

Typically, the rats ingest preferentially hypotonic and isotonic NaCl solutions more than plain water, whereas they strongly avoid ingestion of the hypertonic solutions (17, 19). To address the physiological significance of the increase in NaCl responses after cold exposure, it is important to clarify whether it is linked with preferential or aversive behavior. In two-bottle preference tests, cold-exposed rats avoided 0.05 and 0.1 M NaCl solutions, the concentrations of which were preferred by control animals. If these behavioral changes would be a consequence of a shift in the concentration-response curve to the left, it is reasonable to assume that the enhancement of the nerve responses plays a role for the detection of a very small amount of NaCl in cold-exposed rats. However, this is probably not the case, because the threshold of the chorda tympani nerve responses to NaCl were not changed by cold exposure. Accordingly, it is most likely that the enhanced NaCl responses are fundamental for the avoidance of excess NaCl intake. Considering that the blood pressure is generally increased under cold environment (22, 23), the enhancement of the nerve responses would be rational as an adaptive change of the peripheral taste function.

It has been known that depletion of sodium in rats by dietary sodium restriction, adrenalectomy, or injection of natriuretic increases intake of concentrated NaCl solutions that are normally avoided (4, 15, 18, 20, 21). Interestingly, the depletion-induced sodium appetite is accompanied by a decrease in the chorda tympani nerve responses to NaCl, which is based on the reduction of amiloride-sensitive component (3, 5). These behavioral and neural alterations are quite opposite to those observed after cold exposure. It is thus noteworthy that the magnitude of amiloride-sensitive responses directly affects ingestive behavior for NaCl. In accordance with this, Fischer 344 rats, which did not prefer NaCl solutions at any concentration and avoided NaCl solutions preferred by other strains, are known to be more sensitive to amiloride (2, 3). Taken together, it can be inferred that plasticity of the peripheral taste receptor cells may contribute to the changes in feeding behavior favorable for the maintenance of sodium homeostasis.

Dejima et al. (6) reported that acute cold exposure induces sodium appetite in rats. However, this is not incompatible with our present observation showing sodium avoidance after cold exposure. In their experiments the rats were exposed to cold for 6 h, and drinking behavior was examined after transferring the animals to a room at warm temperature. Because the cold exposure reduces body fluid by the reduction of surface blood flow and diuresis, rewarming of the cold-exposed rats would result in dehydration (11, 23). Dehydration has been known as a powerful inducer of NaCl appetite in animals (24). Thus their observation of NaCl appetite is possibly caused by dehydration. Importantly, the sensitivity of the chorda tympani nerves to NaCl was unchanged by acute (at least within 1 day) cold exposure. This indicates, as is well known, that the avoidance of or preference for NaCl does not depend solely on taste information from the chorda tympani nerves, although it is certainly one of the important factors affecting NaCl preference.

Perspectives

Recent investigations suggest that function of the peripheral gustatory system can be modulated by environmental factors. Particularly, the sodium-sensing system seems to have high plasticity and is variable even in the matured animals. As reported here, enhancement of the chorda tympani nerve responsiveness to Na⁺ under the cold environment may be a suitable example for peripheral gustatory plasticity in rats. The physiological advantages of the increase in Na⁺ responses in adapting and living in the cold may be an important subject for future study. In addition, cellular mechanism for hypersensitivity to Na⁺ is another interest. For this, recent advances in the molecular biology of the amiloride-sensitive Na⁺ channel may serve as useful tools. Taking into consideration the similarity between Na⁺ channel in taste cells and that in other Na⁺-reabsorbing epithelia, it may become possible to identify specific circulating hormones, growth factors, and cytokines that account for hypersensitivity to Na⁺ in the taste-sensing system.

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