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Action of Calcitonin Gene-related Peptide (CGRP) and Substance P on Neurons in the Insular Cortex and the Modulation of Taste Responses in the Rat

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Abstract

Using multibarrel electrodes, recordings were made from single neurons in the insular cortex including the cortical taste area (CTA) of urethane-anesthetized rats. The effects of an iontophoretic application of calcitonin gene-related peptide (CGRP) and substance P (SP) on the spontaneous discharges and taste responses were tested. In a total sample of neurons (mostly non-taste), CGRP affected the spontaneous discharges in 35.6% of the 571 neurons studied and SP in 38.3% of the 775 neurons studied. The effects were mostly (~85–87%) facilitatory. Peptide-sensitive neurons were found at a similar frequency in all three insular areas—granular, dysgranular and agranular (areas GI, DI and AI). This is in contrast to previous reports that CGRP receptors were rich in area DI and CGRP-immunoreactive afferents numerous in area AI, but consistent with previous reports that the distribution of SP receptors and SP fibers was dense in the insular cortex. In ~40% of the 76 taste neurons recorded from areas GI and DI, the peptides affected the spontaneous discharges (mostly facilitated). When the taste responses were examined during application of the peptides, significant (mainly depressant) effects were seen in 61% of 18 neurons for CGRP and in 70.5% of 17 for SP. Such effects were not recognized on responses to specific taste stimuli and were not correlated with the effects on the spontaneous discharges. The findings indicate that both peptides modify taste coding in CTA neurons presynaptically and/or postsynaptically, independently of the existence of receptors on the neurons.

Introduction

Neuropeptides have been found in every region of the nervous system. However, because of the mismatch in location between the afferent terminals containing peptides and the receptors for them (Kuhar, 1985; Hokfelt and Terenius, 1987; Liu *et al.*, 1994), peptides are thought to be neuromodulators rather than neurotransmitters. The density of neurons containing a certain peptide or of the binding sites depends upon the area explored. Those for calcitonin gene-related peptide (CGRP) are sparsely distributed over the cerebral cortex, but they are dense in the insular cortex in rats, including the cortical taste area (CTA); this also obtains for substance P (SP) (Cuello and Kanazawa, 1978; Inagaki *et al.*, 1982; Mantyh *et al.*, 1989). CGRP-immunoreactive afferents and CGRP-binding sites have been found immunohistochemically along the gustatory pathway and in the insular cortex in rats (Mantyh and Hunt, 1984; Shimada *et al.*, 1985; Kruger *et al.*, 1988; Yasui *et al.*, 1989; Skofitsch and Jacobowitz, 1992).

The insular cortex is divided cytoarchitectonically into three areas—granular, dysgranular and agranular (areas GI, DI and AI) (Cechetto and Saper, 1987)—while the CTA consists of GI and DI areas (Ogawa *et al.*, 1991). The action of CGRP and SP in synaptic transmission and on the voltage-activated channels has been studied in the cortex (Zona *et al.*, 1991; Villa *et al.*, 1994). The effects of SP on taste coding have been reported in the brainstem (King *et al.*, 1993; Davis and Smith, 1997); however, they are largely unknown in the cortex, although one report suggested the involvement of CGRP in taste aversion learning (Yamamoto *et al.*, 1990).

In the present study, therefore, we first examined the responsiveness of single neurons in the insular cortices, including taste neurons, to CGRP and SP applied iontophoretically. We characterized the neurons sensitive to CGRP and/or SP by examining the sensitivity to glutamate simultaneously, since most insular neurons have been reported to respond to glutamate or its analogue (Otawa *et al.*, 1995). We then studied the effects of CGRP and SP on the taste responsiveness of single cortical taste neurons to clarify their involvement in taste coding in the CTA.

Materials and methods

Subjects and surgery

Female albino rats of the Sprague–Dawley strain, weighing 200–300 g, were anesthetized with urethane (1g /kg body wt; i.p.). After cannulation of the trachea and femoral vein, the animals were mounted with a pair of regular ear bars on a stereotaxic instrument following the Paxinos and Watson method (Paxinos and Watson, 1982). During the experiment, the rats were tilted by ~45° with the left side up, immobilized with D-tubocurarine (1.5 mg; i.v.) and artificially ventilated. The end-tidal CO_2 was maintained at 3.5–4.5%. Whenever the D-tubocurarine seemed to be wearing off, the level of anesthesia was checked at the corneal reflex and urethane (100 mg/kg) was supplemented if necessary. Body temperature was kept at 37°C with a water-heater. ECG was monitored throughout the experiment.

The bone covering the left middle cerebral artery was removed and a small opening was made in the dura to insert a multibarrel electrode. The left buccal wall was cut from the mouth corner to the anterior edge of the ramus of the mandible; the mouth was opened at ~30–40°, and the tongue was stretched out anteroventrally. Cut wounds were infiltrated with 1% Xylocain.

Stimulation and recording

The taste stimuli used were 0.1 M NaCl, 0.5 M sucrose, 0.01 N HCl, 0.02 M quinine–HCl and a 'search' stimulus containing all of the four basic chemicals at the above-mentioned concentrations. Taste stimulations were made through a 16-bit microcomputer (PC 9801RX, NEC, Tokyo, Japan): distilled water was delivered for 15 s, followed by a given taste stimulus for 10 s, then rinse water for 15 s. During the interstimulus period, the oral cavity was repeatedly rinsed with distilled water until it was confirmed with an audiomonitor that the rate of background discharges had returned to the level of the prestimulus period. Impulse discharges from the single neurons recorded were fed through a window discriminator to the same microcomputer as the controller of stimulus delivery to make peristimulus histograms to taste stimulation and to evaluate taste responses automatically. Taste responses were identified when, during the 10 s of taste stimulation, there was a change in the discharge rate at least 1.0 s long and 2 SD above or below the prestimulus average (Ogawa *et al.*, 1984). The magnitude of the response was calculated as the number of impulses in the first 5 s following the onset of stimulation minus the number of background impulses in a corresponding control (prestimulus) period.

Impulse discharges were also counted with a spike counter (DSE-325A, Dia-Medical, Tokyo, Japan) with a time bin of 0.1 s, and recorded on a pen recorder (RJG-4022, Nihon-Kohden, Tokyo, Japan).

Seven-barrel microelectrodes with a tip diameter of 5–10 μm were used for the iontophoretic application of drugs. These pipettes were attached to a recording pipette filled with 0.5 M sodium acetate and 2% pontamine sky blue. The recording pipette had a tip diameter of <1 μm which protruded 15–30 μm from the drug pipettes. The electrodes were inserted into the cortical taste area through a hole made at the dura around the middle cerebral artery. Single neurons with diphasic spikes or negative monophasic spikes were recorded because they indicate that the spikes were taken from the cell body or dendrites (Bishop *et al.*, 1962). In some experiments, the forms of the spikes were checked throughout with the aid of another computer (Forster and Handwerker, 1990) just in case neurons recorded changed over a long period of recording even if we recorded single neurons.

Drug application

The drugs employed were glutamate (0.5 M, pH 8.0; Kyouwa Hakko Kogyo, Tokyo, Japan), substance P (SP; 0.2 mM, pH 6.0; Peptide Institute, Osaka, Japan), calcitonin-gene-related peptide (CGRP, rat type; 0.02 mM, pH 6.0; Peptide Institute), and NaCl (165 mM) for balance. Retaining currents of 2–10 nA were routinely used to prevent the drugs from leaking, and ejection currents were <100 nA. The pH was adjusted with NaOH or HCl. The drugs were electrophoretically applied to recorded neurons for 10–30 s to examine the sensitivity. The standard ejection current used was 30 nA. Since the application of a retaining current to a drug electrode for several minutes decreases the concentration of the drug in the tip of the electrode, usually an ejection currrent of 30 nA was applied first. When it was effective, a smaller amount of current was used. In most cases, glutamate was first applied and followed by peptides; however, the order of application of the peptides was not predetermined. In the present experiments, the possibility that 'current effects' might have contributed to the effects we observed seemed unlikely in the range of the current intensities used (usually 20–100 nA), because these effects did not emerge immediately but with some delay, and also because the effects did not disappear immediately after stopping the current application.

Taste responses were recorded in the absence or presence of the drugs. The drugs of 2–10 nA were continuously applied by microiontophoresis for 30–40 min to obtain 3–5 series of taste responses to the four basic stimuli. After completion of the stimulus applications, drug ejection was discontinued and the neuron was allowed to recover. Reaching stable effects after the onset of ejection took 5–10 min, whereas dissipation of the effects after the offset of ejection took 20–30 min. Significant changes in the magnitude of taste responses in the two conditions were examined by the Mann–Whitney *U*-test at the level of 10%.

Histology

For histological identification of the cells recorded, extra-

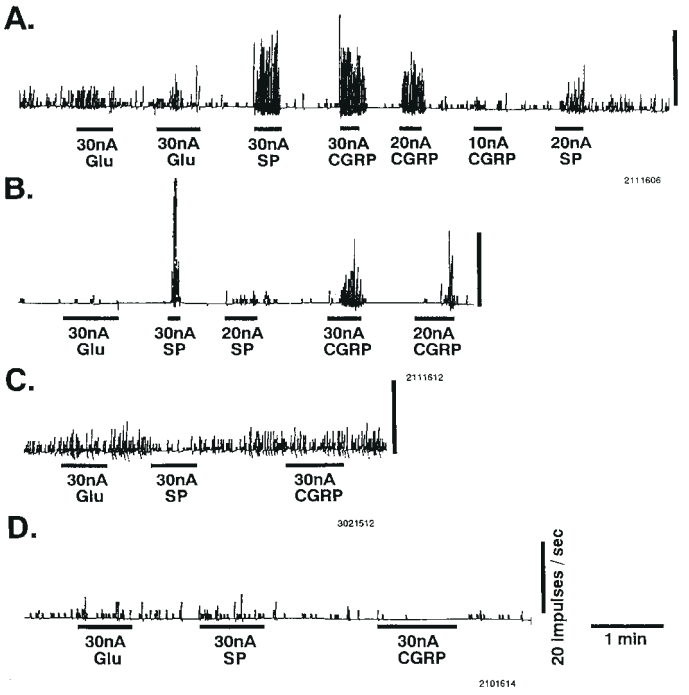

Figure 1 Responses of neurons in the insular cortex of rats to iontophoretic application of glutamate, SP and CGRP. **(A)** Neuron sensitive to all of the drugs. Spontaneous discharges were increased by all of the drugs. **(B)** Neuron sensitive to SP and CGRP. Spontaneous discharges were increased by the peptides but not by glutamate. **(C)** Neuron sensitive to SP only. Spontaneous discharges were decreased. **(D)** Neuron sensitive to all of the drugs. Spontaneous discharges were facilitated by glutamate and SP but decreased by CGRP.

cellular dye marks were produced by passing currents of 10 μA for 5 min through a recording electrode containing pontamine sky blue with its tip negative. In each penetration, the dye marks were made at two or three recording sites to reconstruct the electrode track. At the termination of the experiments, the animals were perfused through the heart with 10% formalin in a 0.1 M phosphate buffer. Blocks of tissue containing the recording sites were frozen, sectioned at 50 μm and stained with thionin. Reconstruction of the track of recording electrodes was done by referring to the two to three dye marks. Cytoarchitectonic identification of areas GI, DI and AI was based on the description by Cechetto and Saper (Cechetto and Saper, 1987).

Results

A total of 812 neurons were recorded from three subdivisions of the insular cortex, including the CTA in rats (386 in GI, 361 in DI, and 65 in AI). Among them, 76 taste neurons (39 in GI, 35 in DI and 2 in AI) were identified. Neuromodulators, such as CGRP and SP, were tested on the spontaneous discharges and taste responses of neurons in the insular cortex.

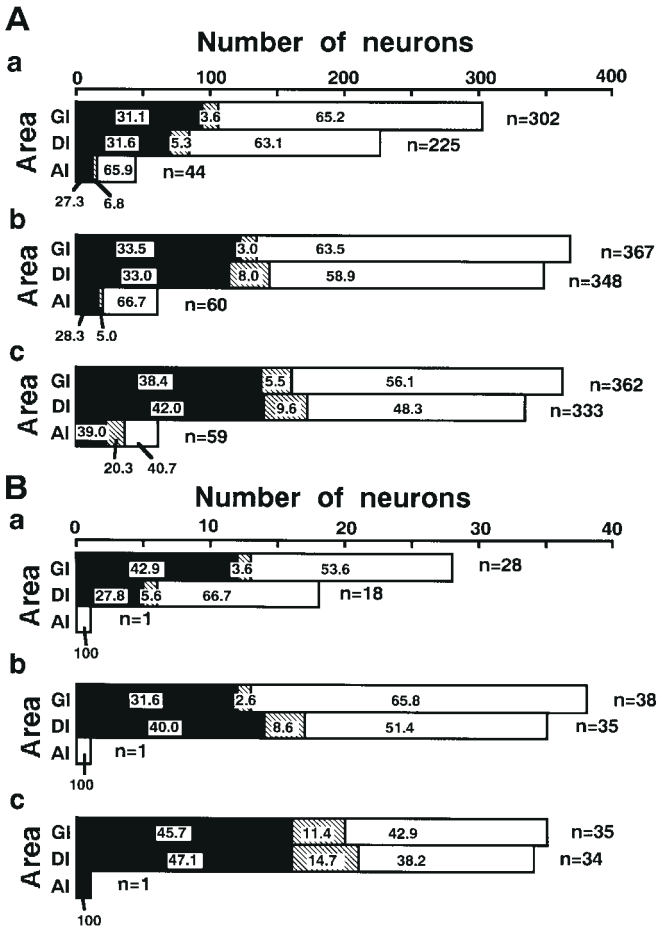

Figure 2 Fraction of neurons sensitive to each of the three drugs in a whole sample of neurons **(A)** or in a group of taste neurons **(B)**. Response to (a) CGRP, (b) SP and (c) glutamate. Solid, hatched and blank bars indicate the number of neurons showing facilitatory responses, inhibitory responses, and no responses. n: the number of neurons examined. Figures attached to the bars stand for the percentage of neurons showing the response types.

Effects of CGRP and SP on spontaneous discharges in a whole sample from the insular cortex

When the two peptides of the same dose (30 nA) were applied iontophoretically to neurons discharging spontaneously, they affected the discharges in different ways. SP produced responses in a short latency, but CGRP yielded sluggish responses in a long latency, outlasting the period of drug application in some neurons (Figure 1). Both peptides facilitated the spontaneous discharges in most neurons when they were effective.

To characterize the type of neurons sensitive to CGRP and/or SP, it was determined whether or not they were sensitive to glutamate. By setting ejection currents of three drugs, CGRP, SP and glutamate, at a certain intensity, e.g. 30 nA, the sensitivity of each neuron to these drugs was qualitatively surveyed (Figure 2A). Among the three drugs, glutamate was the most effective; it was effective in 48.5% of the 754 neurons studied in total—excitatory in 40.1% and

Table 1 Neurons (%) responsive to various combinations of CGRP (C), substance P (S) and glutamate (G) in the insular cortices in rats A

	Glutamate(+)					Glutamate(–)					Total
	C+S	C only	S only	None	Subtotal	C+S	C only	S only	None	Subtotal	
					(A) whole sample of neurons						
Area GI	33	18	27	59	137	27	22	15	93	157	294
Area DI	29	18	24	40	111	10	17	12	58	97	208
Area AI	6	5	5	13	29	2	0	0	11	13	42
Total	68	41	56	112	277	39	39	27	162	267	544
					(B) Taste neurons						
Area GI	1	7	2	6	16	2	1	0	7	10	26
Area DI	2	3	2	2	9	1	0	2	6	9	18
Area AI	0	0	0	1	1	0	0	0	0	0	1
Total	3	10	4	9	26	3	1	2	13	19	45

inhibitory in 8.5%. CGRP was effective in 35.6% of the 571 neurons studied in total; excitatory effects were seen in 31.0%, and inhibitory effects in 4.6%. On the other hand, SP was effective in 38.3% of the 775 neurons studied in total, being excitatory in 32.9% and inhibitory in 5.4%.

Frequency of CGRP- and SP-sensitive neurons in subareas of the insular cortex in rats

Recording sites of CGRP-, SP- or glutamate-sensitive neurons were constructed and displayed collectively in the histogram according to the subarea (Figure 2A). In any subarea of the insular cortex, the fraction of neurons sensitive to glutamate (Figure 2Ac) was larger than that to CGRP or SP (Figure 2Aa,b). Though the number of neurons examined in area AI was smaller than that in other areas, the fraction of neurons sensitive to CGRP (34.1%) or SP (33.3%) in area AI was comparable to that of CGRP (34.7%) and SP (36.5%) in both GI and DI (36.9 and 41.1% respectively). The frequency of neurons excited by CGRP or SP was similar in both the GI and DI areas, though SP inhibited neurons were more frequent in area DI than in any other area.

Relation between sensitivity to peptides and glutamate in insular neurons

Based on the sample of neurons to which the three drugs were successfully applied (n = 544), the sensitivity of neurons to both peptides was examined in relation to sensitivity to glutamate (Table 1A).

Peptide-sensitive neurons were significantly frequent among glutamate-sensitive insular neurons. Glutamate-sensitive neurons were more frequently sensitive to either peptide (165/277) than those insensitive to glutamate (105/267) ($P < 0.001$, χ^2-test, df = 1). The fraction of CGRP-sensitive neurons was higher in the glutamate-sensitive neuron group (109/277) than in the nonsensitive group (78/267) ($P < 0.05$, χ^2-test, df = 1). The same held true with the fraction of SP-sensitive neurons (124/277 versus 66/267) ($P < 0.001$, χ^2-test, df = 1).

A considerable fraction of peptide-sensitive neurons had receptors for three drugs (68/270) or for both SP and glutamate (56/270). Neurons sensitive to only one of the peptides comprised a very small fraction. The frequency of various effective combinations of drugs varied among the three areas. For example, there were twice as many neurons sensitive to both CGRP and SP in GI than in DI. Sensitivity to SP and glutamate in both the GI and DI areas or to CGRP and SP in all three areas occurred concomitantly with significant probability ($P < 0.05$, χ^2-test, df = 1).

Effects of CGRP and SP on taste neurons in the insular cortex

Spontaneous discharges

Taste neurons (n = 76) were recorded from the three areas of the insular cortex; however, almost all of them were from the GI and DI areas (Figure 2B, Table 1B). Both CGRP and SP were effective in ~40% of the taste neurons, which was less frequent than the fraction of neurons sensitive to glutamate (60%) (Figure 2B), as seen in the findings with a whole sample of neurons. Facilitatory effects (36.2%) were more frequent than inhibitory ones (4.3%) (see Figure 2B).

In the 45 taste neurons in which the three drugs were successfully applied, the fractions of taste neurons sensitive to peptides were examined in relation to those sensitive to glutamate. CGRP was found to be more effective among the glutamate-sensitive neurons than among the glutamate-nonsensitive (Table 1B): sensitivity to CGRP and glutamate occurred concomitantly with significant probability (P = 0.01, χ^2-test, df = 1). The fraction of SP-sensitivity was not different between the glutamate-sensitive (7/26) and -non-sensitive neuron groups (5/19): no significant combination of responses to SP and glutamate or CGRP and SP was

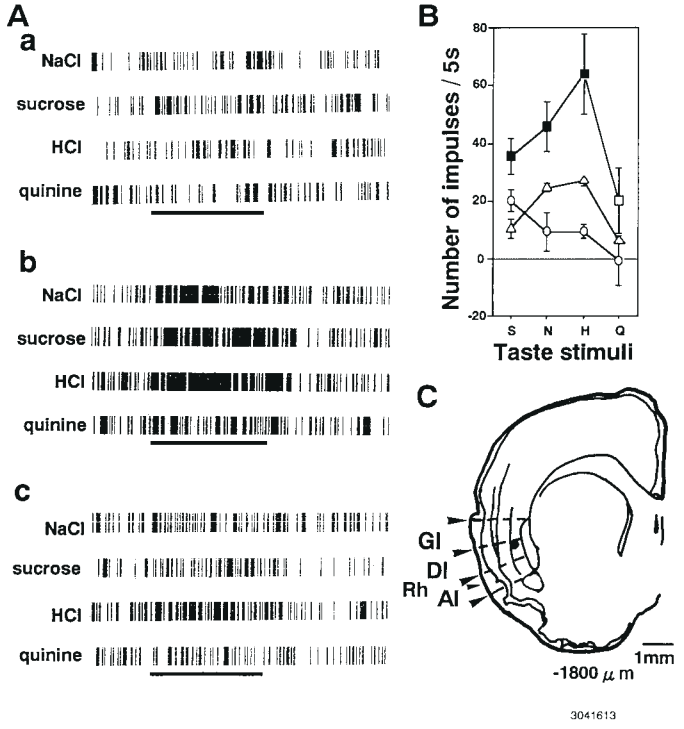

Figure 3 Effects of CGRP on taste responses in cortical taste neurons. **(A)** Reversible effects of CGRP on taste responses to the four basic stimuli. Impulse discharges in response to the stimulation are expressed in terms of impulse images. Horizontal bars under the responses indicate the period of taste stimulation. Aa, before CGRP; Ab, during CGRP application (5 nA); and Ac, 30 min after CGRP was discontinued. Impulses of a single neuron are expressed in terms of spike image. **(B)** Taste response profiles in the predrug period (circles), drug application period (squares) and recovery period (triangles). Average response magnitudes (± SD) of 3–5 trials are plotted against the four basic stimuli, which are arranged along the abscissa in the order of sucrose, NaCl, HCl and quinine according to the Frank method (Frank, 1973). Filled symbols indicate responses which significantly deviated from the control. **(C)** Recording site in the dysgranular insular area, unit 3041613 (area DI, layer VI).

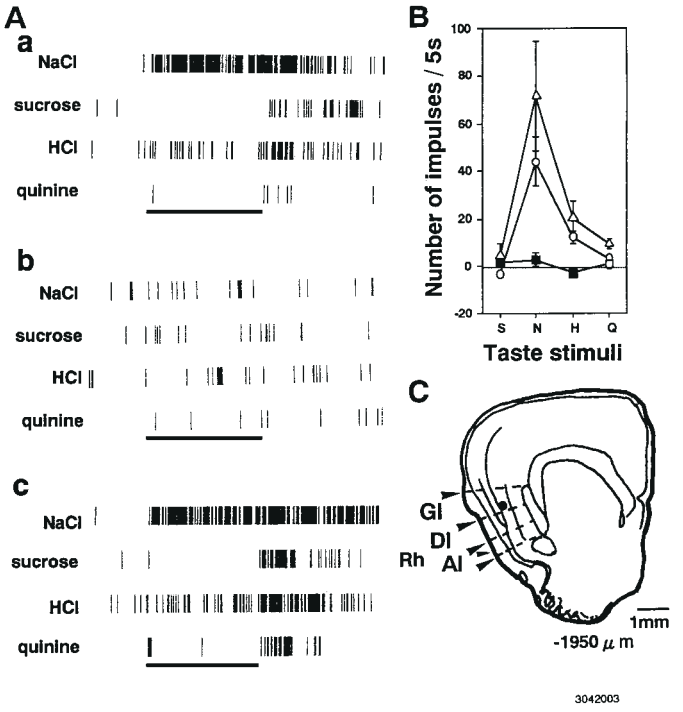

Figure 4 Effects of SP on taste responses in cortical taste neurons. **(A)** Reversible effects of SP on taste responses to the four basic stimuli. Aa, before SP; Ab, during SP application (3 nA); and Ac, 30 min after SP was discontinued. **(B)** Taste response profiles in the predrug period (circles), drug application period (squares) and recovery period (triangles). **(C)** Recording site in the granular insular area, unit 3042003 (area GI, layer V). For details, see the legend of Figure 3.

found, in contrast to the findings for the whole sample of insular neurons (Table 1B).

Taste responses

The action on taste responses was studied in a total of 40 neurons (26 in GI, 14 in DI). Taste responses recovered from the drug action in only 25 of them (14 in GI, 11 in DI). The findings were obtained from these neurons. Because the number of neurons examined was small, neurons in both areas were treated together to describe the characteristics.

CGRP

Effects of CGRP on taste responses were tested in 18 neurons (eight in GI and 10 in DI), whether or not the drug affected the spontaneous discharges (Figure 3). The figure shows facilitatory effects on responses to three of the four basic stimuli, i.e. sucrose, NaCl and HCl, when compared with the control responses before or after the iontophoretic application of the drug. Significant effects were seen in 11 neurons: facilitatory actions in three and depressant actions in eight. Effects were seen on responses to up to three of the four basic taste stimuli, mostly on responses to one stimulus (n = 6). Sucrose responses were most affected (n = 7), followed by HCl responses (n = 5), NaCl responses (n = 3) and quinine responses (n = 3). No particular relation was noted between kinds of action and taste stimuli. Depressant effects were more frequently seen in area DI (n = 7) than in area GI (n = 1) ($P < 0.05$, Fisher's exact probability test, df = 1), and taste responses were affected in a much larger number of neurons in area DI (9/10) than in area GI (2/8) ($P < 0.05$, Fisher's exact probability test, df = 1).

SP

Effects of SP on taste responses were tested in 17 neurons (11 in GI, six in DI), whether or not the effects were found on the spontaneous discharges (Figure 4). The figure shows the depressant effects on NaCl and HCl responses compared with the control responses before or after iontophoretic application of the drug. Significant effects were seen in 12 neurons, mostly in the GI area. Facilitatory actions were seen in four neurons and depressant actions in eight. Effects were seen on responses to one or two of the four basic taste stimuli, mostly on responses to one stimulus

(n = 9). No favorite responses were noted for SP action: responses to either stimuli were affected in 3–5 neurons. No particular relation was noted between kinds of action and taste stimuli. Depressant effects were more frequently seen in GI (n = 6) than in DI (n = 2), and taste responses were affected in a larger number of neurons in GI (9/11) than in DI (3/6), though both findings were statistically not significant ($P > 0.05$, Fisher's exact probability test, df = 1).

CGRP and SP action in single neurons

Actions of both peptides on taste responses were examined in ten neurons (five in GI, five in DI). In nine of them, the peptides were effective. In four neurons (one in GI, three in DI), both affected taste responses: both depressed the taste responses in two neurons but increased them in one (all in DI); however, the two drugs showed different effects in the other neuron (GI) (facilitatory by CGRP and depressant by SP). In the rest (n = 5), only SP depressed responses in three neurons and facilitated responses in one (all in GI), whereas CGRP depressed them in the other one (1 in DI).

Changes in response profiles

Taste responses were modified by the iontophoretic application of the peptides, but the modification was not the same for all four basic taste stimuli. Thus, the response profiles of neurons to taste stimuli were modified, and the best stimulus also changed in some neurons.

Some taste responses were greatly facilitated or depressed, as seen in Figures 3 and 4, but others were not affected at all. Significant effects of the peptides were most frequently seen in the responses to the best stimulus (in 7/11 neurons for CGRP and in 7/12 for SP). When changes in the best stimulus of the response profiles were evaluated by visual inspection, they were found in 8/11 cases modified by CGRP, including five neurons with best responses affected, and in 8/12 cases modified by SP, including five affected at the best stimulus.

Relation between actions of the peptides on spontaneous discharges and taste responses

Though the effect of peptides on taste response was examined in 25 CTA neurons, the drugs did not affect the spontaneous discharges in most of them.

CGRP facilitated the spontaneous discharges in eight of the 18 neurons examined. It increased the taste responses in three CGRP-nonsensitive neurons, but decreased them in three CGRP-sensitive and five CGRP-nonsensitive neurons. SP facilitated the spontaneous discharges in seven of the 17 neurons examined. It increased the taste responses in two SP-sensitive and two SP-nonsensitive neurons, but decreased them in three SP-sensitive and five SP-nonsensitive neurons. Neither peptide inhibited the spontaneous discharges in the taste neurons examined in this section. Neither peptide's action on the taste responses correlated significantly with the action on the spontaneous discharges ($P > 0.05$; Fisher exact probability test).

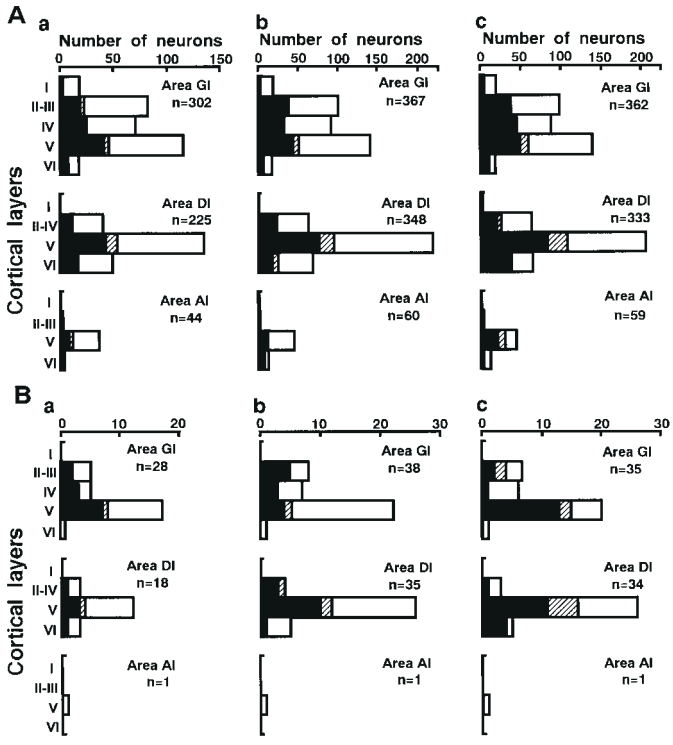

Figure 5 Laminar distribution of neurons sensitive to CGRP (a), SP (b) or glutamate (c) in the whole sample of neurons **(A)** or in the sample of taste neurons **(B)** in the insular cortices in rats. Solid, hatched and blank bars indicate neurons with spontaneous discharges facilitated, inhibited or unaffected, respectively.

Laminar distribution of neurons sensitive to CGRP and SP in the insular cortex of rats

The distribution of peptide-sensitive neurons across cortical layers, when examined by the action on the spontaneous discharges, is shown in Figure 5. In a total sample of neurons (Figure 5A), peptide-sensitive neurons were seen in layer V as frequently as in layers II–IV and VI in areas GI and DI. The same holds true in the taste neuron group (Figure 5B). It is reported that peptide-sensitive neurons are found in other cortical areas (Phillis and Limacher, 1974; Lamour *et al.*, 1983). In all layers, facilitatory effects dominated depressant ones.

The across-layer distribution of the neurons with taste responses affected by the peptides was investigated (Figure 6). Though the sample is small, neurons in layers II–V were frequently seen. Affected neurons were also numerous at these layers. Neurons whose taste responses were affected by CGRP were mostly found in layer V of area DI, whereas neurons with taste responses affected by SP were mostly distributed in layers II–V of area GI. The fraction of neurons in which taste responses were inhibited by CGRP was very large in layer V of area DI; however, the fraction facilitated by SP was high in layer V of area GI.

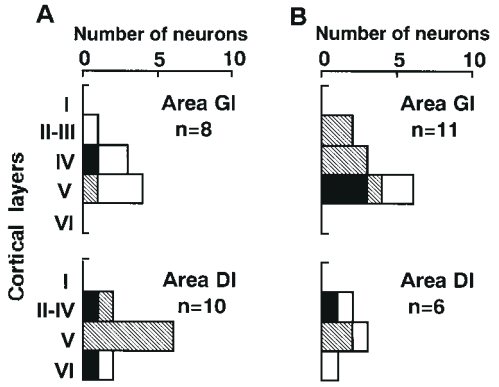

Figure 6 Laminar distribution of neurons whose taste responses were modified by CGRP **(A)** or SP **(B)** in the insular cortices in rats. Solid, hatched and blank bars indicate neurons with taste responses facilitated, inhibited or unaffected.

Discussion

Receptors for CGRP and SP in neurons in the insular cortex

The present study demonstrated that about the half of the neurons in the insular cortex, including the cortical taste area, are sensitive to the iontophoretically applied peptides CGRP and SP. Both CGRP- and SP-sensitive neurons were found in all three subareas of the insular cortex. SP and CGRP often increased spontaneous discharges, as seen in the somatosensory cortex (Lamour *et al.*, 1983). Thus, it is considered that neurons in the insular cortices have receptors for CGRP and/or SP. It is also shown that SP excites most neurons in the solitary tract nucleus of rats (King *et al.*, 1993; Davis and Smith, 1997). The present findings are partly consistent with immunohistochemical studies showing that CGRP receptors are concentrated in DI in rats (Skofitsch and Jacobowitz, 1992), but not with previous reports that CGRP fibers are numerous in area AI (Kruger *et al.*, 1988; Yasui *et al.*, 1989). However, the present findings on the distribution of SP-sensitive neurons are in agreement with the previous report that the distribution of SP binding sites is dense in the insular cortex (Mantyh *et al.*, 1989).

Peptide sensitivity was related to glutamate sensitivity. Numerous peptide-sensitive neurons were found among glutamate-sensitive neurons, though ~60% of the total sample or the taste neuron group were glutamate sensitive. The small fraction may be due to the lower effectiveness of glutamate as an agonist to the glutamate receptor compared with specific agonists (Watkins and Evans, 1981).

Non-taste neurons comprised >90% of the sample. The sensitivity of non-taste neurons in the insular cortex to SP and glutamate in both the GI and DI areas, or to CGRP and SP in GI, DI and AI, was found to occur concomitantly with significant probability; that is, non-taste neurons in the insular cortex tended to have receptors for SP and glutamate or for both CGRP and SP.

On the other hand, the sensitivity of taste neurons to CGRP and glutamate occurred concomitantly; that is, taste neurons tended to have receptors for both CGRP and glutamate, in contrast to non-taste neurons.

Peptides and taste processing in the insular cortex

Though the distribution of taste neurons sensitive to either of the peptides was even across the insular cortex when the effects on the spontaneous discharges were studied, it was rather localized to a given area when examined with regard to taste responses. CGRP mostly affected taste neurons in DI and SP mostly in GI, though they also affected them to some degree in other areas. CGRP-immunoreactive afferent terminals are dense in area AI (Yasui *et al.*, 1989) and area DI situates next to area AI, lying between the former and area GI. Thus, CGRP released from the afferents probably reaches taste neurons in area DI by diffusion in more densed concentration than taste neurons in area GI. On the other hand, there is no evidence that SP-binding neurons distribute differently between GI and DI, the two CTAs. The substantial difference of the two peptides between the two CTAs, in spite of the small sample size, suggests a difference in their participation in taste processing.

Peptide action on cortical cells

Responses to CGRP in some neurons were sluggish and outlasted the period of drug application. It is suggested that CGRP activates intracellular second messengers to depolarize cortical neurons, as reported for both peptides (Watson and Downes, 1983; van Valen *et al.*, 1990; Nakajima *et al.*, 1991; Zona *et al.*, 1991; Villa *et al.*, 1994). Though the peptides themselves cannot open or close ionic channels, they may modulate ionic currents elicited by glutamate or GABA through secondary intracellular messengers, such as cGMP or inositol-1,4,5-triphosphate. This is consistent with the present findings that CGRP or SP receptors are mostly found in neurons with glutamate receptors. Thus, CGRP and SP are accessible to glutamate receptors to modulate cortical responses evoked through the latter. It has been reported that these peptides may act at the postsynaptic cells to modulate, and in most cases potentiate, ionic channels through NMDA receptors (Rusin *et al.*, 1992), the receptors which present at the membrane of many cortical taste neurons (Otawa *et al.*, 1995). Though some cortical taste neurons have $GABA_A$ receptors (Ogawa *et al.*, 1998), it is not known whether or not the peptide-sensitive neurons are also sensitive to GABA.

On the other hand, the peptides decreased taste responses in most cases, in contrast to the effect on evoked responses in the taste neurons in the hindbrain of hamsters (Davis and Smith, 1997). Moreover, the drugs did not always affect both spontaneous discharges and taste responses in a single neuron, only one or the other in some neurons. Even when they modified both, taste responses and spontaneous discharges did not change in the same direction. Thus, the

findings suggest the possiblity that the peptides act at the presynaptic terminals in some neurons to affect taste responses. The peptides probably modulate the release of glutamate or GABA from the presynaptic terminals of the thalamic taste afferents or intracortical interneurons, as suggested in the peripheral nervous system (Otsuka and Yoshioka, 1993). However, it is not known immunohistologically whether or not the presynaptic terminals opposing the neurons with receptors for peptides have peptide receptors.

Previously it was reported that glutamate receptor blockers greatly affected taste responses in cortical taste neurons but not always spontaneous discharges (Otawa *et al.*, 1995). It is suggested then that, even if afferents mediating the two activities use glutamate as a transmitter, they could make contact with different parts of the dendrite (Otawa *et al.*, 1995). This may be partly true for the action of the peptides; however, in the present case, the peptides did not always modify the taste responses even when they affected the spontaneous discharges.

Physiological significance of CGRP and SP action on cortical taste neurons

Anatomical studies have shown that CGRP-immunoreactive neurons run along the taste pathway in the brain (Mantyh and Hunt, 1984; Kruger *et al.*, 1988). It has also been reported that CGRP- or SP-immunoreactive nerve fibers are present near the taste buds in the tongue (Welton *et al.*, 1992). Furthermore, it has been suggested that CGRP-immunoreactive neurons represent the visceral organs (Yasui *et al.*, 1989; Rusin *et al.*, 1992). These findings indicate that CGRP has something to do with taste processing, particularly the integration of taste and visceral information in the brain. On the other hand, CGRP levels in the insular cortex have been shown to increase with the formation of taste aversion (Yamamoto *et al.*, 1990). Thus, it is postulated that CGRP modulates taste input pre- and/or postsynaptically in cortical taste neurons during the formation of taste aversion. However, the places where CGRP is released in the insular cortex and where the peptide interacts with taste neurons have not been found. It remains to be clarified whether or not the peptide interacts with receptors to excitatory amino acids in the CTA in the formation of taste memory trace.

On the other hand, SP afferents originate from intrinsic neurons, such as the glutaminergic pyramidal neurons (Conti *et al.*, 1992) or the GABAergic interneurons (Penny *et al.*, 1986; Kaneko *et al.*, 1994). SP co-exists with fast excitatory or inhibitory substances, which are probably co-released. Thus, it may modulate activities of neurons nearby, whereas fast transmitters act on postsynaptic neurons directly; the targets of the collaterals of pyramidal neurons or the inhibitory interneurons and their surrounding neurons may be differently affected. Long-lasting effects of SP may participate in maintaining the effects of a single sensory event for some time.

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