Neuropeptides modulate rat chorda tympani responses

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There have been several studies regarding the modulation of gustatory responses by neuropeptides (8, 11, 20, 21, 26, 33, 43, 44). Because neuropeptides and their receptors are widely distributed in the central nervous system (CNS), their physiological effects on gustatory processes, including feeding (15, 17), could arise centrally or at the level of taste receptor cells (TRCs) and their associated primary neurons. Neuropeptides may rapidly reach the extracellular space surrounding the TRCs by being released from the TRCs themselves [cholecystokinin (CCK), vasointestinal peptide (VIP)], and/or their accompanying neurons via the activation of intra- and perigeminal peptidergic neurons, whose peripheral terminals may contain calcitonin gene-related peptide (CGRP) and/or substance P (SP) (13, 14, 27, 28, 34, 35, 41, 47, 48). Neuropeptides may also be secreted into the bloodstream from other locations (e.g., leptin from adipose tissue) that may also influence gustatory responses (17, 33). However, in these cases, any modulation of the gustatory responses should require a longer latency (33). To avoid effects on CT responses that take long times to develop, such as those arising from intraperitoneal injections or from protein synthesis, we investigated the effects of selected neuropeptides injected directly into the LA on rat chorda tympani (CT) nerve responses during the initial few minutes a tastant was placed on the tongue.

Although many experiments have pointed to a role for neuropeptides in influencing taste, in some cases important controls were not performed, and in others, raw data were not shown (21). In many of the early experiments, neuropeptides were used that could be degraded by endogenous peptidases leaving open the possibility that some biologically active metabolite could produce this effect. In addition, many vasoactive peptides (e.g., CGRP) can produce changes in the blood pressure (BP) or the tongue’s temperature and consequently may also alter CT responses through these mechanisms (23, 42, 47). For these cases it is not clear whether the effects of the peptides on CT responses are a consequence of interacting with TRCs, CT, and/or lingual nerve neurons, or whether they are a consequence of local changes in oxygen tension, BP, or the tongue’s temperature. Here we have performed experiments that control for many of these variables.

One extensively studied neuropeptide is CCK, which, like leptin, modulates (decreases) food intake (17, 18), including a decrease in the intake of sucrose (17, 21, 46). After an intraperitoneal injection of CCK, rat CT responses to NaCl, sucrose, and quinine all increased, albeit at various times (43, 44). Because CCK did not alter the response to acid, it showed that it is tastant specific. In a similar study, it was reported that after two injections of CCK-8 into the rat jugular vein (JV), the tonic CT responses to 0.1 M NaCl and 0.3 M sucrose increased 3 and 8%, respectively (21). Single-unit recordings obtained in the nucleus of the solitary tract (NST) in response to several gustatory stimuli obtained before and after CCK-8 was injected into the rat JV were not significantly changed between 3 and 30 min (20). More recently, CCK has been found in TRCs and CCK-A type receptors have been found on TRCs from rat circumvallate and foliate papillae (27, 38). Moreover, the application of CCK-8 to these TRCs has been shown to increase calcium release from intracellular stores and modulate potassium currents (27, 38). Because of the importance of CCK as a taste modulator, we have reexamined the effects of a nonhy-

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drolyzable CCK analog (CCK-8) on CT neurons by injecting it directly into the tongue via the lingual artery (LA).

SP is a neuropeptide that exhibits many physiological functions throughout the CNS, including the gustatory system (6, 37, 39). In one study it was shown that intraperitoneal injections of SP increased whole nerve rat CT responses to NaCl (43). In addition, SP-NK1 receptors are present on rat TRCs, suggesting that NK1-TRC interactions may play a role in modulating taste responses, perhaps via the release of SP from perigemal neurons (4). For these reasons we investigated the effects of SP on CT responses by injecting it into the LA.

CGRP receptors are distributed throughout the CNS, including perigemal neurons (45). Moreover, intercerebroventricular administration of CGRP has been shown to decrease food intake (36). For these reasons, we examined the direct effect of CGRP on CT responses.

In this study we have delivered neuropeptides into the LA and JV and have shown that within seconds, they can modulate CT response in a peptide-specific manner.

**MATERIALS AND METHODS**

Reagent-grade salts were made up in distilled water. Peptides [(Tyr (SO_3)_27)-CCK fragment 26–33 amide (CCK-8), CGRP, and SP_{4–11}] were obtained from Sigma Chemical (St. Louis, MO). Just before use, the peptides were dissolved in Krebs-Henseleit (KH) buffer (see below).

Subjects were nondeprived adult male Sprague-Dawley rats (230–300 g) obtained from Harlan or Charles River laboratories. As shown in Tables 1 and 2 and in the text, the effects of the neuropeptides were tested in 101 rats: 68 using the LA (28 for CCK-8, 19 for CGRP, and 21 for SP) and 33 using the JV (10 using CCK-8, 9 using CGRP, and 14 using SP). The animals were anesthetized by intraperitoneal injection using the JV (10 using CCK-8, 9 using CGRP, and 14 using SP). The animals were cannulated with PE-10 polyethylene tubing (BPM-8802; Rougemont, NC). The output of the transducer was amplified and led into a DigiPack 1200 (Axon Instruments) and then into a computer, where it was recorded simultaneously with the CT response.

**Injection into the LA: Immediate Perfusion of the Tongue**

For cannulating the LA, the tissue around the trachea was exposed and the hyoid bone was removed. The proximal part of the LA was tied, and a polyethylene tube (PE-10) filled with KH solution (118 mM NaCl, 4.7 mM KCl, 2.5 mM CaCl_2, 1.2 mM MgCl_2, 1.2 mM KH_2PO_4, 25 mM NaHCO_3, 11 mM glucose, pH 7.4) was tied into the peripheral part of the LA (see Fig. 3B). The KH buffer could be immediately replaced with other solutions via a three-way valve (see Fig. 3B).

**Injection into the JV**

The ipsilateral external JV was exposed and cannulated with PE-50 polyethylene tubing filled with KH buffer. A volume of 100 μl KH buffer containing peptide was injected. As above, the cannula was connected to a three-way valve so after KH, other solutions in KH could be immediately replaced with each other without a break in the flow.

**Tastant Stimulation of the Tongue**

The anterior half of the tongue was pulled away from the mouth so that tastants could be easily applied. Solutions were continuously delivered to the tongue by a pressurized system at a flow rate between 20 and 30 ml/min (12). The continuous solution flow over the tongue during the various procedures served to reduce mechanical stimulation and changes in the tongue’s surface temperature (12, 47). The NH_4Cl solutions, used only to test the stability of the recordings, were always applied for 15 s, and the subsequent wash period between tastants was always 45 s. A recording was considered to be stable when the tonic 0.1 M NH_4Cl response magnitudes at the beginning and the end of each stimulation series deviated by no more than 20%. Only responses from these stable recordings were used.

A 5 mM HEPES solution adjusted to pH 7.4 with KOH was used for washing the tongue. We define the activity during this wash phase as the basal activity.

Tastants were applied from 15 to >45 s. The peptides were usually injected −15 s after the taste stimulus was applied. Solutions used for chemical stimuli were 0.1 M NH_4Cl (NH), 0.1 M NaCl (Na), 0.01 M HCl, 3 mM quinine HCl (QHCl), and 0.5 M sucrose (S). The pH of these solutions (except for HCl) was between 5 and 7.4. Unless otherwise stated, each experiment was repeated at least three times in different animals.

**Data Analysis**

As a control, peptides were injected between tastant applications, while the tongue was washed with 5 mM HEPES buffer (see Figs. 2B and 9). For tests, the peptides were injected during the tonic phase of a response to a tastant (see Figs. 1, 2C, and 6). The volume injected was 100 μl unless otherwise mentioned. In a given animal, only data in which the peptides were tested for only a single tastant were used. That is, in each animal only one tastant (besides NH_4Cl) was tested for one peptide. Unless otherwise stated, peptides were only applied a single time.

To test the effects of the peptides, the responses to them were compared with the effects on the responses evoked by injecting KH buffer alone. An example of this analysis is seen in Fig. 1, where successive CT responses to 0.1 NaCl were superimposed. In the initial application, KH buffer was injected into the JV, and in the subsequent response, 5 μg CCK-8 in KH buffer was injected into the JV. We accepted as effects, those changes in the form of the response from the response obtained with only the KH buffer. That is, the peptide-induced response had to be visually larger than the responses obtained by the injection of buffer. To determine whether peptides had an effect on the basal activity, we looked for abrupt changes in the slope that could not be

**Measurement of BP**

In each animal, the BP was measured by cannulating the ipsilateral carotid artery with a Caldwell system pressure transducer (BPM-8802; Rougemont, NC). The output of the transducer was amplified and led into a DigiPack 1200 (Axon Instruments) and then into a computer, where it was recorded simultaneously with the CT response.

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accounted for by the injection itself, which, when it occurred, was very transient (see Fig. 8B). The changes in CT responses are given by the symbols (+/-) for no obvious change, (+) for obviously increased responses, and (-) for obviously decreased responses (see Tables 1 and 2). However, to give the reader a sense of the magnitude in the data we also give a descriptive statistical analysis of the data in which buffer and peptides were injected during the application of a tantant. We refer the reader to Fig. 1 for the methodology. To determine if any breaks occurred in the response, we simply continued the line between the pre- and postinjection activities (see dotted line, Fig. 1). We then measured the changes in the amplitude of the CT activity from the respective baselines to the maximum response evoked by either an injection of KH or the peptide. As a measure of the change that occurred upon the injection of peptides, we took the ratio of the maximum CT change with the injected peptide relative to the change with the injected KH buffer. For the example seen in Fig. 1, the ratio is 1.21/1 (since no change was seen on the injection of KH buffer). Tables 1 and 2 give the means ± SD of the amplitudes of the responses.

RESULTS

LA Perfusion

Controls. EFFECT OF BUFFER ALONE. Figure 2A shows a representative tracing of the CT responses when the ipsilateral LA was cannulated and its proximal portion was clamped. Here NH₄Cl and four tantants were applied to the dorsal tongue for 15 s; between each of the tantants a wash solution of 5 mM HEPES (pH 7.4) was flowed over the tongue for 45 s. These data also show that the CT responses remained stable for an extended period. In this regard, the LA was cannulated before obtaining the CT recording. Figure 2B shows that repeated applications of 0.1 M NaCl give reproducible responses and that an injection of 100 μl of KH buffer between tantant applications does not alter the subsequent tonic responses. Finally, injections of KH buffer during the tonic CT activity to NaCl did not alter the response (Fig. 2C). These data demonstrate that simply injecting a volume of 100 μl of KH buffer, once or repeatedly, does not alter the tonic CT responses to 0.1 M NaCl.

LINGUAL CIRCULATION. During the CT recordings in which the ipsilateral LA was occluded by the cannula (Fig. 3A), it was clear that some area of the ipsilateral tongue must have been receiving a sufficient supply of (oxygenated) blood to sustain the CT responses over periods of hours. However, it was previously shown that clamping the external carotid artery, which feeds the LA, diminished CT responses within 15 min (24).

Table 1. Effects of lingual artery injections of peptides on the basal and tantant-evoked chorda tympani activity

<table>
<thead>
<tr>
<th>Peptides</th>
<th>Basal</th>
<th>0.1 M NaCl</th>
<th>0.1 M HCl</th>
<th>3 mM QHCl</th>
<th>0.5 M Sucrose</th>
</tr>
</thead>
<tbody>
<tr>
<td>CCK-8 (10 μg; n = 28)</td>
<td>NAE (2+/-, 2+/-)</td>
<td>Increase (5+, 1+/-)</td>
<td>Increase (5+, 1+/-)</td>
<td>NAE (6+/-)</td>
<td>NAE (1+, 1-, 4+/-)</td>
</tr>
<tr>
<td></td>
<td>109.0 ± 10.8</td>
<td>122.5 ± 11.2</td>
<td>121.1 ± 11.8</td>
<td>103.6 ± 10.1</td>
<td>101.6 ± 12.7</td>
</tr>
<tr>
<td>CGRP (5 μg; n = 19)</td>
<td>NAE (2+/-, 2+)</td>
<td>Decrease (3-)</td>
<td>Decrease (3-)</td>
<td>NAE (3+/-, 1-)</td>
<td>NAE (2+/-, 2-, 1+)</td>
</tr>
<tr>
<td></td>
<td>112.5 ± 14.2</td>
<td>80.3 ± 5.0</td>
<td>77.6 ± 2.5</td>
<td>94.2 ± 10.4</td>
<td>98.0 ± 18.1</td>
</tr>
<tr>
<td>SP₄₋₁₁ (1 μg; n = 21)</td>
<td>NAE (3+/-, 1+)</td>
<td>NAE (2+, 1+/-, 2-)</td>
<td>NAE (2+/-, 1+, 2-)</td>
<td>NAE (3+/-)</td>
<td>NAE (2+/-, 2-)</td>
</tr>
<tr>
<td></td>
<td>104.0 ± 11.5</td>
<td>103.0 ± 19.1</td>
<td>101.0 ± 16.5</td>
<td>104.0 ± 6.2</td>
<td>96.0 ± 13.6</td>
</tr>
</tbody>
</table>

Values are means ± SD of response to tantant peptide relative to response to tantant (or peptide) alone (=100). CGRP, calcitonin gene-related peptide; SP₄₋₁₁, substance P₄₋₁₁; NAE, no apparent effect; (+), increase; (-), decrease; (+/-), no effect evident.

Table 2. Effects of jugular vein injections of peptides on the basal and 0.1 M NaCl-evoked chorda tympani activity

<table>
<thead>
<tr>
<th>Peptides</th>
<th>Basal Activity</th>
<th>0.1 M NaCl</th>
</tr>
</thead>
<tbody>
<tr>
<td>CCK-8 (5 μg; n = 10)</td>
<td>NAE (4+/-, 1-)</td>
<td>Increase (5+)</td>
</tr>
<tr>
<td></td>
<td>97.6 ± 10.7</td>
<td>121.8 ± 5.7</td>
</tr>
<tr>
<td>CGRP (0.5 μg; n = 6)</td>
<td>NAE (3+/-)</td>
<td>NAE (3+/-)</td>
</tr>
<tr>
<td></td>
<td>96.6 ± 5.0</td>
<td>94.0 ± 3.6</td>
</tr>
<tr>
<td>SP₄₋₁₁ (5 μg; n = 9)</td>
<td>NAE (2+, 2+/-)</td>
<td>NAE (2+, 2-, 1+/-)</td>
</tr>
<tr>
<td></td>
<td>114.7 ± 15.6</td>
<td>105.8 ± 23.8</td>
</tr>
</tbody>
</table>

Values are means ± SD of response to tantant peptide relative to response to tantant (or peptide) alone (=100). For abbreviations, see Table 1.
To better understand why this preparation remained viable for so long, we injected 200 μl of methylene blue (in KH) into the ipsilateral LA (Fig. 3B) and found that the entire ipsilateral side and the tip on the contralateral side turned blue (Fig. 3D), but the rest of the contralateral side remained pink (Fig. 3, C and D). However, when the contralateral LA was clamped and methylene blue (400 μl) in KH buffer was injected in the ipsilateral LA within seconds, the contralateral surface of the tongue also turned blue (Fig. 3E). Upon unclamping the contralateral LA, the blue color of the contralateral tongue faded, implying that the concentration of methylene blue decreased there (not shown).

Hyperosmotic and hyposmotic solutions. To further understand the causes of the effects seen in the LA preparation, we have performed experiments to ascertain whether changes in osmotic pressure in the ipsilateral LA will alter CT responses to 0.1 M NaCl. Osmotic effects were found previously in a rat CT preparation with an intact blood supply (2); such data would serve as a good control for our preparation. The injection of 100 μl of 0.5 M NaCl transiently increased both the basal activity and the tonic CT response to 0.1 M NaCl (Fig. 4A, n = 3). Note that the increase in the tonic response was proportionally larger than the increase in the basal activity. We then tested whether
hypotonic buffers of various compositions will change
the CT response to NaCl and found that 100 µl injec-
tions of 0.01 M solutions of Na glutamate (Fig. 4B, n =
2), CaCl2 (Fig. 4C, n = 2), or KCl (Fig. 4D, n = 3) may
have slightly decreased the basal activity. However, in
the presence of 0.1 M NaCl on the dorsal tongue, the
inhibitory responses of hyposmotic buffers on the CT
responses were much larger than the small changes
seen in the basal activity. Thus changing the osmotic
pressure of the blood supply can modulate gustatory
(CT) responses.

Neuropeptides: effect of CCK-8, CGRP, or SP 4–11 on
the basal CT activity. We next tested whether selected
neuropeptides, at short times, could alter the basal CT
responses. We choose concentrations that would evoke
only small changes in the CT response and yet give
consistent changes in the BP. After testing several
peptide concentrations, we settled on using 10 µg

Fig. 3. Circulation during occlusion of the LA with a cannula. For the purpose of taking these pictures, the tongue
tip is clamped so that the tongue could be extended from the mouth. Note that when experiments are performed
to measure CT responses, the rat is prone. A: dorsal surface of a rat tongue under control conditions where the
blood flow is normal. B: LA perfusion, where the ipsilateral LA was perfused with methylene blue, which turned
much of the ipsilateral tongue blue. The tubing filled with methylene blue, a syringe filled with dye, and a valve
are also shown. Note that when experiments are performed to measure CT responses, the rat is prone. C: lower
magnification of the control tongue (seen in A) after some of the methylene blue that was injected into the LA began
to fade. D: a tongue from a different rat where both sides of the tongue's tip are blue from a unilateral LA injection
of methylene blue. In this experiment, the rat's tongue was extended from its mouth with a hook in its lingual
frenulum so as not to damage the tip. E: same tongue seen in A and C. After clamping the contralateral LA,
methylene blue in KH buffer was reinjected into the ipsilateral LA. The picture shows that the dye is now present
on both sides of the tongue. A, C, and E are from the same animal. B and D are taken from 2 different animals.

Fig. 4. Effects of tonicity on the CT response to 0.1 M NaCl. A: an LA injection of hyperosmotic (0.5 M) NaCl
(pH 7.4) increases both the basal activity (initial arrow) and the tonic re-
sponse to 0.1 M NaCl. LA injections of hyposmotic (0.01 M) solutions of Na
 glutamate (B), CaCl2 (C), or KCl (D) do
not change or slightly decrease basal
activity but markedly reduce the tonic
responses to NaCl.
activity or the responses to 0.1 M NH4Cl. Injecting only changing the BP did not markedly alter the basal these concentrations. These data show that simply decreased by CGRP and SP4

injecting that the peptides were physiologically active at these concentrations, however, the peptides always changed the BP. The BP was increased by CCK-8 and decreased by CGRP and SP4-11, thereby demonstrating that the peptides were physiologically active at these concentrations. These data show that simply changing the BP did not markedly alter the basal activity or the responses to 0.1 M NH4Cl. Injecting only KH buffer did not evoke a change in BP (not shown, but see Fig. 8A).

We did not use higher neuropeptide concentrations to investigate the tonic CT responses because they produced larger responses in the basal CT activity and the BP and often had effects that were irreversible. For example, 10 μg injections of SP4-11 greatly lowered the BP and essentially eliminated the CT responses, and 5 μg SP4-11 injections caused a rapid reduction in the response to NaCl and NH4Cl that did not recover, even after a 10-min wash (data not shown).

Neuropeptides: effects of neuropeptides on tonic responses (LA preparation). We next investigated the effects of these three neuropeptides on the tonic CT responses. This was accomplished by injecting peptides into the LA during the tonic phase of the CT response to a gustatory stimulus.

CCK-8. CCK-8 was tested to determine its effects on the CT responses to four standard taste stimuli (see Table 1). The injection of 10 μg CCK-8 buffer into the LA produced, after a short delay, increases in the tonic CT responses evoked by NaCl (Fig. 6A) and HCl (Fig. 6B). The responses to QHCl (Fig. 6C) or sucrose (Fig. 6D) were not obviously altered by CCK-8 (see Table 1). Note that despite the changes in the CT response, e.g., to 0.1 M NaCl seen in Fig. 6A, the CT response returned to baseline, and the tonic response to NH4Cl was unchanged. These data show that CT responses can be rapidly modulated by the injection of CCK-8.

CGRP. CGRP’s ability to alter CT responses was tested by injecting 5 μg of CGRP (in 100 μl of KH buffer) into the ipsilateral LA (Fig. 7). After the injection, the tonic responses to NaCl (Fig. 7A) and HCl (Fig. 7B) always decreased (see Table 1). In contrast, the responses to QHCl (Fig. 7C) and sucrose (Fig. 7D) were not apparently changed (Table 1). These data show that CGRP modulated the responses to NaCl and HCl, albeit in a different direction than CCK-8.

SP4-11. To test SP’s ability to modulate CT responses, 1 μg SP4-11 in the KH carrier was injected into the LA. At this concentration it did not produce significant changes to any of the four tastants (data not shown, but see Table 1).

Injections of neuropeptides into the JV. Because some researchers that have tested the effects of neuropeptides on gustatory responses have injected them into the JV, we performed JV injections of peptides with NaCl as the tastant.

We initially looked to see how these neuropeptides altered the BP. Again concentrations were identified that would have a small effect on the basal CT activity (Table 2) but yet would give small but consistent changes in BP. As a control, we found that a 100-μl injection of KH buffer in the JV did not produce any changes in CT activity or in BP (Fig. 8A), but injections of 5 μg CCK-8 (in 100 μl KH) produced small transient increases in the basal CT responses, as well as in the BP (Fig. 8B). Injections of 0.5 μg CGRP did not alter the basal CT response but always produced a transient decrease in BP (Fig. 8C). However, injections of 5 μg CGRP evoked a small increase in spontaneous activity (2 +, 1 +/−) and a large decrease in BP that did not recover, even after several minutes of wash (data not shown). Finally, a 100-μl injection of 5 μg of SP4-11 did not produce consistent changes in the basal CT activity but always decreased the BP (Fig. 8D). Injections of 10 μg SP4-11 produced large decreases in BP and irreversible decreases in the CT responses (data not shown).

To test whether these peptides injected into the JV can modulate the CT responses, we repeated the same sequence as we did with the LA perfusion method but only examined the effects for 0.1 M NaCl. As with the LA preparation, we found that injections of CCK-8 increased the response to 0.1 M NaCl (Fig. 1). However, injections of 0.5 μg CGRP did not alter the response to 0.1 M NaCl, despite the fact that it decreased...
the BP (Fig. 9). The responses to JV injections of 5 μg SP4-11 were quite variable and did not evoke marked changes. In this regard, even injections of 10 μg SP4-11 produced variable responses to 0.1 M NaCl (3−, 1+, 1+/−).

**DISCUSSION**

It was shown that neuropeptides injected directly into the LA could induce rapid changes in CT responses in a peptide-specific manner; i.e., CCK increased activity, SP had no apparent effect, and CGRP decreased activity.

The discussion is divided into two parts: the first part consists of a discussion of the LA preparation, and the second part involves a discussion of the effects of the three tested neuropeptides on CT responses.

**LA Preparation**

We have perfused chemicals directly into the tongue via the LA. One advantage of this method (over the intraperitoneal or JV methods) is that the effects of neuropeptides on CT responses are localized to the tongue, rather than involving other systemic effects. Another advantage, at least over the intraperitoneal method, is that the effects can be seen in a few seconds. In some studies, the responses obtained when the LA is occluded by a cannula give results similar to what is obtained with intact LAs. For example, the CT responses to 0.1 M NaCl are modulated in the same manner by CCK-8 with the LA occluded by the cannula (Fig. 6) and in JV experiments where the LA is not occluded (Fig. 1). We also found, as did Bradley (2) who used a preparation with an intact blood supply to the rat tongue, that injections of isotonic saline did not markedly alter CT responses, injections of hypertonic NaCl buffers rapidly increased the CT response to NaCl, and that injections of hypotonic buffers rapidly decreased CT responses to NaCl (Figs. 2 and 4). Moreover, relative to the responses obtained under basal conditions, in the presence of 0.1 M NaCl these osmotic responses are amplified (Fig. 3), as they are with the peptides (see below). We have not explored the cellular physiology underlying the change in CT responses with tonicity but note that these effects follow local changes in osmolality.

In summary, we have demonstrated that the LA preparation is viable and is immediately and distinctly responsive to chemicals introduced into the lingual circulation.

We found it puzzling that the LA preparation with the ipsilateral LA occluded with the cannula remained viable for extended periods (Fig. 2A and 9). This may have occurred because the ipsilateral LA not only supplies blood to the ipsilateral tongue but also to the contralateral tip (Ref. 24 and Fig. 3). That is, clamping the ipsilateral external carotid artery does not prevent
the flow of oxygenated blood from the contralateral LA into the taste buds at the tip on the ipsilateral side (see Fig. 3D). Because the tongue’s tip contains many taste buds (40), it follows that the CT neurons on the ipsilateral side could be maintained by oxygenated blood flow from the contralateral LA. Tongue regions posterior to the tip may also remain viable. In this regard, on unilaterally clamping the lingual and external carotid artery for 1 wk, no differences were observed (at the light microscope level) between the two sides of the rabbit tongue (22). It was suggested that (perhaps through anastomoses) at the tip and posterior tongue, the occluded side received enough blood from the contralateral side to maintain its morphological characteristics. Our experiments are consistent with this possibility.1

It is very interesting that when the contralateral LA is clamped and methylene blue is injected into the ipsilateral LA, the dye is transported readily to both sides of the tongue. Assuming the anastomoses hypothesis, the reason that methylene blue remains predominately on the cannulated ipsilateral side when the contralateral circulation is intact could be that the normal BP from the contralateral side will prevent the dye from penetrating across the midline; this might arise from the high resistance of the anastomoses. In contrast, when the contralateral LA is clamped, the BP on that side will be low enough so that when methylene blue is injected into the ipsilateral LA, it can then be transported (via the BP gradient) into the contralateral tongue (Fig. 3E).

Rapid Modulation of CT Responses by Neuropeptides

Routes of neuropeptides from the bloodstream to TRCs. We have investigated whether the injection of neuropeptides into the LA can rapidly modulate tastant-evoked CT responses. Before discussing these findings, however, the pathways the peptides may take in being transported from the bloodstream to potential sites that could influence CT responses (TRCs, CT, or lingual fibers) will be reviewed. From intravascular taste experiments it appears that small organic molecules, such as saccharine, can rapidly diffuse through the capillary fenestra (32) and/or tight junctions of the endothelium into the extracellular space, where they can bind to receptors on TRCs to produce both a CT response and a sweet (or hedonically positive) taste.

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1The connection of the lingual circulation at the tongue’s tip may explain how peptides injected into the ipsilateral LA are transported into the circulation to change the systemic BP (Fig. 5), although the peptides could also be transported via the intact ipsilateral venous system to cause these changes.
sensation (2, 3, 16). Generalizing from saccharine, once neuropeptides exit the LA, the time for molecules to interact with TRCs will be short. If the distance between the blood supply and the taste receptor cells is even $10^{-6}$ m (25), and if the diffusion constant of small peptides in buffer is $10^{-6}$ cm$^2$/s, then it will take $10^{-1}$ s for peptides to reach the TRCs. So, at least theoretically, if peptides can diffuse out of the circulation, they can reach the TRCs in short times.

How long does it take peptides such as CGRP (mol wt 3,789), CCK-8 (mol wt 1,144), or leptin (176 amino acids) to diffuse from the bloodstream into the extracellular space (or to be released from lingual nerve fibers) where they can possibly interact with TRCs (or CT neurons) to alter their responses? As noted, after an intraperitoneal injection of leptin, which has been shown to bind to receptors on TRCs, the CT responses may be even shorter than for leptin that is synthesized in adipose tissue and thus must reach fungiform papillae through the circulation.

Fig. 8. Effects of jugular vein injections of neuropeptides on the basal CT activity and blood pressure. A: infusion of KH buffer did not alter the basal CT activity, the response to NH$_4$Cl, or the blood pressure. B: infusion of 5 μg CCK-8 induced a small increase in CT activity and a transient increase in blood pressure. C: infusion of 0.5 μg CGRP did not alter CT activity but produced a transient decrease in blood pressure. D: infusion of 5 μg SP$_{4,11}$ induced a small transient increase in CT activity and a biphasic change in the blood pressure. Arrows indicate times when peptides were injected.

Fig. 9. Transient decreases in blood pressure do not alter CT responses to 0.1 M NaCl. This figure shows the CT responses to 10 applications of 0.1 M NaCl that are bracketed by responses to 0.1 M NH$_4$Cl. The corresponding blood pressure responses are seen below. At the arrow, 0.5 μg CGRP in KH buffer was injected in the jugular vein, which produced a transient decrease in the blood pressure but did not markedly alter the CT responses to 0.1 M NaCl. Bars indicate duration of the stimuli.

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GCRP and SP and CCK are present on the terminals of intra- and perigeminal fibers (14, 41). If these neurons are activated and release peptides (perhaps by becoming ischemic, which will result in a lower pH), the times may be even shorter than for leptin that is synthesized in adipose tissue and thus must reach fungiform papillae through the circulation.
to saccharine and sucrose began to decrease in 10 min and reached their maximal decrease in 30 min (33). Given leptin’s comparatively large size and that it was injected intraperitoneally, it follows that neuropeptides having much lower molecular weights would be expected to modulate CT in shorter times, especially if they were injected into the LA. In addition, vasodilators, such as SP and CGRP, that increase arterial permeability (29), should reduce the time it would take peptides to reach TRCs. Therefore, even though we have shown that neuropeptides may modulate CT responses in short times, it is not clear whether they do so by binding to receptors on TRCs, or by several other local mechanisms (see below).

A comment on the peptide-induced variability in some of the CT responses. Variable CT responses were obtained in many of the experiments involving the injection of the three tested neuropeptides. That is, in some experiments some of the responses increased, some decreased, and some did not change (see Tables 1 and 2). Other experiments have shown variability in the responses to the injection of peptides. In a detailed study involving the modulation of NST responses to tastants after a JV injection of CCK-8, the researchers excluded 75% of the data because of its intrinsic variability relative to the magnitude of a response (20).

What is the origin of the variability in the CT responses, especially those involving QHCl and sucrose (Table 1)? It cannot be argued that the time in which we looked for changes during the tonic response was too short, because during this period we have shown that CCK-8 (Fig. 6) and CGRP (Fig. 7) gave reproducible responses to NaCl and HCl (Table 1). We believe that the variability indicates that we were at the limits of our ability to measure these changes for these two tastants. We emphasize that this does not mean that changes did not occur, but rather we were unable to consistently detect them (see Table 1). For this reason we called these responses “No Apparent Effect” to emphasize that we did not have the resolution to determine whether or not an effect occurred.

Effects of peptides on CT responses. Unlike for saccharine and lepin, where the responses were taste specific and lepin receptors were found on TRCs (33), the site(s) where the neuropeptides interact to alter CT responses cannot be readily discerned in the present data. That is, using only information obtained from CT measurements, one cannot distinguish whether changes in the CT responses arise from the interaction of the neuropeptides with taste receptor cells, CT, or trigeminal nerve neurons, a change in tongue’s surface temperature, and/or changes in BP. The contributions to these various possibilities may not be mutually exclusive and may even oppose one another (which may account for some of the variability). In an attempt to reduce two of these possibilities, solutions were continuously flowed over the tongue to reduce mechanosensory and thermal contributions to CT responses.

We have shown that CT responses are peptide specific. For example, unequivocal results were obtained on the modulation of CT responses to 0.1 M NaCl and 0.1 M HCl by CGRP and CCK-8. The CT responses to these two tastants were increased by CCK-8 (Table 1, LA; Table 2 JV) and decreased by CGRP (Table 1, LA). With regard to CCK-8, the increase in the CT response to NaCl is in good agreement with previous studies of the effects of CCK on CT responses to NaCl (21, 44). At high SP concentrations, the responses to NaCl decreased, but so did the basal CT activity, and these decreases were not readily reversible.

The same studies cited above reported that after intraperitoneal injections of CCK or JV injections of CCK-8, the CT response to sucrose was increased (21, 44). In the study that most closely resembled ours, 0.3 M sucrose was applied to rat tongues (with a dropper) for 45 s, and 5 μg CCK-8 was injected into the JV (21). Although no data were shown, the CT responses to sucrose increased 8 and 12% for the first and second CCK-8 injections, respectively (21). In our experiments, we did not find that CCK-8 altered the tonic CT response to sucrose (Fig. 6D and Table 1). In whole nerve CT responses, statistically significant changes at the level of 10% are difficult to obtain, especially when they are weak as they are for sucrose and quinine (see above discussion and Table 1).

CCK. Recent studies have shown that TRCs in rat fungiform papillae, unlike those in circumvallate papillae, do not contain CCK LI (27). If the TRCs in fungiform papillae do not contain CCK or its receptors, the mechanisms of how CCK modulates CT responses have to be indirect. In addition, we do not understand how CCK produces transient increases in the BP, because intraperitoneal injections of CCK usually cause a decrease in BP (30). If CCK-8 gets past the blood-brain barrier, it causes an increase in BP (19). On the other hand, it may be that the small transient BP increases may be a response to the activation of nociceptors by CCK (31).

SP. We also investigated whether SP can alter CT responses. We expected to find some effects of SP4-11 given that NK-1 receptors are on taste cells in fungiform papillae (4). Indeed, in a previous study, intraperitoneal injections of SP (not the nonhydrolyzable analog) were found to increase whole nerve rat CT responses to NaCl (43). In our experiments, however, we found that LA injections of 1 μg SP4-11 did not alter CT responses to any of the tastants but consistently produced a transient and lowering of the BP. It is hard to argue that SP4-11 did not get to particular site(s) during this time because CGRP is a considerably larger peptide and it clearly modulated CT responses. Some of the effects that have been attributed to changes when peptides are injected intraperitoneally could arise from long-term vasoactive mechanisms that could lead to changes in the tongue’s temperature that, in turn, could modulate gustatory responses to tastants in a different manner (5). In this regard, at higher SP4-11 concentrations, CT responses were diminished and the BP was greatly reduced. At higher brain centers, such as the NST, injection of SP has been demonstrated to
modulate single-unit responses to tastants (8).

CGRP. We found that LA injections of CGRP decreased CT responses to NaCl and HCl (Fig. 7). That the effect in the response to CGRP (decrease) is opposite to the effect of CCK-8 (increase) suggests that different mechanisms are involved. One early possibility that we considered to rationalize the CGRP data is that the CT changes may be correlated with the changes in BP, because reducing the BP causes an approximately linear decrease of the CT responses to 0.3 M NaCl (24). Using these data, a decrease in BP of 20 mmHg (say from 100 to 80) decreased the CT response only ~5%. From these data, together with the above discussion, it is unlikely that BP changes can account for the changes that we have seen with CGRP (Figs. 7 and 9) or with CCK-8 (Figs. 1 and 6). However, at larger peptide concentrations the decreases in CT (Figs. 7 and 9) or with CCK-8 (Figs. 1 and 6). However, account for the changes that we have seen with CGRP.

In summary, we have shown that neuropeptides can directly and rapidly modulate gustatory neural activity. These changes could underlie some of the behavioral changes produced by neuropeptides in modulating food intake.

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