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IS SEROTONIN A CHEMICAL TRANSMITTER IN THE FROG TASTE ORGAN ?

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Summary

By artificially perfusing the frog tongue with serotonin (5HT) and its antagonists, the possibility of 5HT as a chemical transmitter from taste cells to nerve terminals in frog taste organ was examined. Although serotonin creatinine sulfate, when perfused through the lingual artery, produced impulse discharges in the glossopharyngeal nerve, creatinine sulfate elicited a similar response. Neural responses to taste stimuli were depressed by perfusion with 5HT. Among many antiserotonergic drugs perfused through the lingual artery, **LSD was** the only one which modified responses to taste stimuli. LSD suppressed taste responses to NaCl, $CaCl_2$ and water, while LSD at a high concentration (10^{-5} g/ml) enhanced responses to quinine and HCl. When PCPA (DL-p-chlorophenylalanine) was injected intraperitoneally in conbination with reserpine, the agent did not significantly change taste responses. The above results possibly suggest that 5HT would not be a chemical mediator from taste cells to nerve terminals.

DeHan and Graziadei (1,2) reported the presence of synaptic granular vesicles containing an adrenergic substance in the sensory (taste) cells of the fungiform papilla of the frog, and suggested that a catecholamine is a possible chemical transmitter from taste cells to sensory nerve terminals. Subsequently, Morimoto and Sato (3) carried out experiments, in which drugs were applied via the lingual artery of the bullfrog and glossopharyngeal nerve responses were recorded, and presented evidence which favours the hypothesis that noradrenaline is a likely substance involved in the transmission from taste cells to sensory nerve terminals in the frog. However, Goosens and Vandenberghe (4) demonstrated yellow fluorescence characteristic for serotonin (5HT) in the basal cells of the fungiform papilla of the common frog, and Hirata and Nada (5) also showed yellow fluorescence at the most basal region of the sensory epithelium in the fungiform papilla of the bullfrog. From its peak emission spectrum and its reduction following the treatment by reserpine and 5,6 dihydroxy-

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tryptamine (5,6 DHT), they regarded the yellow fluorescence as representing 5HT. They further considered 5HT being contained in the cored vesicles of the taste cell and a chemical substance which takes part in gustatory processes. Nomura, Muneoka and Kanno (6) also mentioned the presence of fluorescence possibly originated from 5HT in the basal region of the sensory disc in the fungiform papilla of the common frog.

The present experiments were conducted to examine the effect of 5HT and its antagonistic drugs on the taste responses in the frog by applying them via the lingual artery. Effects of 5HT depleting agents were also examined.

Materials and Methods

American bullfrogs (Rana Catesbeiana) weighing 250-400 g were anesthethized with intraperitoneal injection of 20 % urethane solution, and a polyethylene tube was cannulated into the lingual artery for artificial perfusion, its procedure being almost the same as that adopted by Rapuzzi (7). Experimental methods were the same as those described in the preceding paper by Morimoto and Sato (3). One of the glossopharyngeal nerves was isolated from the connective tissue, and cut at the proximal potion. The nerve was suspended with a pair of silver wire electrodes and immersed in liquid paraffin to prevent from drying of the nerve. Impulse discharges in the whole nerve were led to an amplifier connected with an integrator of a time constant of 0.5 sec (8), and recorded with a pen-writing recorder.

For stimulation of gustatory receptors, a taste solution of about 30 ml was applied through a tube of 5 mm in diameter over the surface of the tongue. For adaptation and rinse of the tongue 0.01 M NaCl solution was used. Varying concentrations of NaCl, KCl, $CaCl_2$, quinine hydrochloride and HCl were used as test stimuli. Quinine, HCl and $CaCl_2$ were dissolved in 0.01 M NaCl solution, while NaCl and KCl in deionized water.

The glossopharyngeal nerve response to light touch of the tongue surface with a plastic rod, its tip diameter being 5 x 4 mm, was recorded and used as a control response. The rod was fixed on the arm of an electromagnetic relay, and was driven by rectangular electric pulses of 50 msec in duration at a rate of 4/sec for 20 sec.

For perfusing the lingual artery serotonin creatinine sulfate and several antagonists of a serotonergic transmitter was employed. In addition, creatinine sulfate was used as a control to identify the effect of serotonin. As antagonists of a serotonergic transmitter, D-lysergic acid diethylamine tartarate (LSD), gramine, phenformin and 5-hydroxy-tryptophan (both D- and L-forms) were employed. These agents, except gramine, were dissolved in Ringer solution just prior to the experiment. Gramine was used after it being dissolved in Ringer solution containing 0.2 % alchohol.

To deplete 5HT from the taste organ DL-p-chlorophenylalanine (PCPA) was used alone or in combination with reserpine. PCPA was injected intraperitoneally in one series of experiments, and in another series reserpine combined with PCPA was injected every day during 5 to 10 days preceding the electrophysiological experiment. PCPA was dissolved in Ringer solution which was adjusted at pH 10

with 1 N NaOH. Its pH was readjusted to 7.0 by 1 N HCl just prior to injection.

Results

Afferent impulse discharges produced by 5HT

Perfusion of the lingual artery with 5HT elicited impulse discharges in the glossopharyngeal nerve (Fig.1). The impulse discharge was augmented with increase in 5HT concentration

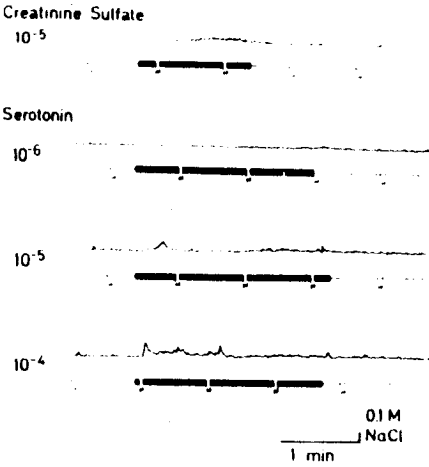

Fig.1

Glossopharyngeal nerve responses to intravascular administration of 5HT and creatinine sulfate in a frog. The period of perfusion of drugs is shown by holizontal thick lines. Concentration of the drug (g/ml) is shown at the left side of each trace. Time calibration and the magnitude of response to 1.0 M NaCl are shown at the bottom of the figure.

(Fig.1), although the magnitude of the glossopharyngeal nerve response to 5HT varied from preparation to preparation. The dose-response relation for 5HT is shown in Fig.2. The threshold concentration was approximately 10^{-6} g/ml. The magnitude of response to 10^{-4} g/ml 5HT was almost the same as that of the response to tactile stimulation of the tongue or to stimulation by 0.1 M NaCl. Since 5HT used in the present experiment was a creatinine sulfate compound, the effect of creatinine sulfate on the glossopharyngeal nerve response was examined. Similar to the effect of 5HT, perfusion of the tongue with creatinine sulfate through the lingual artery produced impulse discharges in the glossopharyngeal nerve. the threshold concentration of creatinine sulfate was 10^{-6} g/ml which was the same as that of 5HT. However, the discharge differed slightly from that of 5HT (Figs.1 and 3); responses to creatinine sulfate usually occurred with a long latency and gradually increased. On the other hand, responses to 5HT showed an initial phasic component with a short latency.

Responses in the glossopharyngeal nerve to taste stimuli were

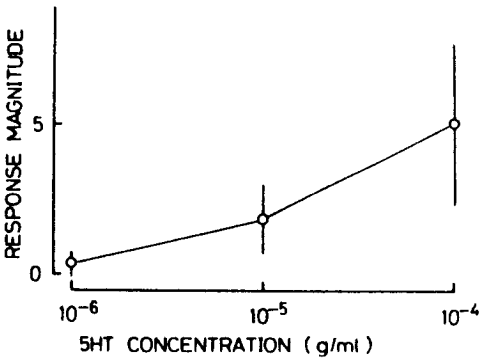

Fig.2

Dose-response relation for perfused 5HT. The vertical line at each point represents ± S.D. The response magnitude is the maximum integrated response magnitude expressed in an arbitrary unit.

depressed shortly after the onset of perfusion with high concentrations of 5HT, while creatinine sulfate showed no effect on the taste responses (Fig.3). Although in Fig.3 response to KCl appears to be enhanced slightly after the perfusion with creatinine sulfate, this is not due to the proper effect of creatinine sulfate, because such enhancement of taste response was usually observed during the early stage of artificial perfusion with Ringer solution.

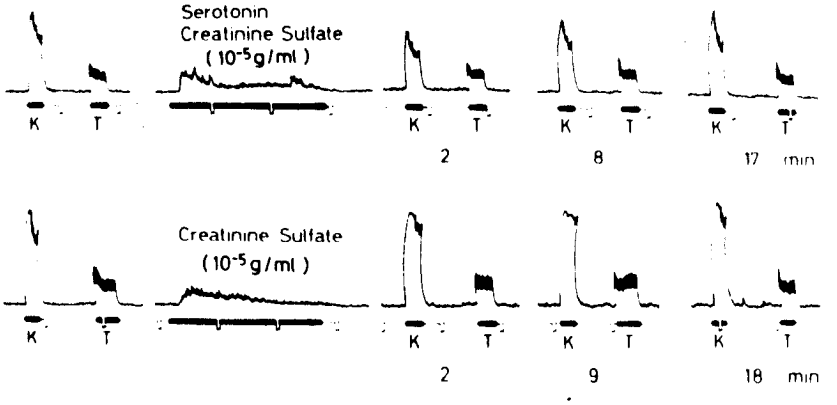

Fig.3

Effects of 10^{-5} g/ml 5HT and creatinine sulfate applied intravascularly on taste responses to 0.3 M KCl (K) and on tactile responses (T). Depression in the KCl response was observed after 5HT perfusion but not in the tactile response. Numeral under each trace indicate the time elapsed after the end of perfusion of the drug to application of KCl.

A high concentration of 5HT caused contraction of muscles in the tongue and evoked rythmic firing in mechanosensitive fibers. On the other hand, such muscular contraction was not observed when a high concentration of creatinine sulfate was perfused.

Effect-of-anti-serotonergic drugs on taste responses.

Each anti-serotonergic drug was applied through the lingual artery, and the magnitudes of neural responses to taste stimuli obtained after the perfusion were compared with the control ones.

LSD: LSD of 10^{-7}g/ml did not cause significant change in taste responses, but LSD in concentration above 10^{-6}g/ml significantly altered the responses. The effect of LSD differed according to difference in the kind of taste stimuli. Responses to NaCl, KCl and $CaCl_2$ diminished to 50 % of the control values after perfusion with 10^{-6}g/ml LSD for 15 min. On the other hand, under the same condition the initial phase of response to quinine remained unchanged, while the stationary state of responses to

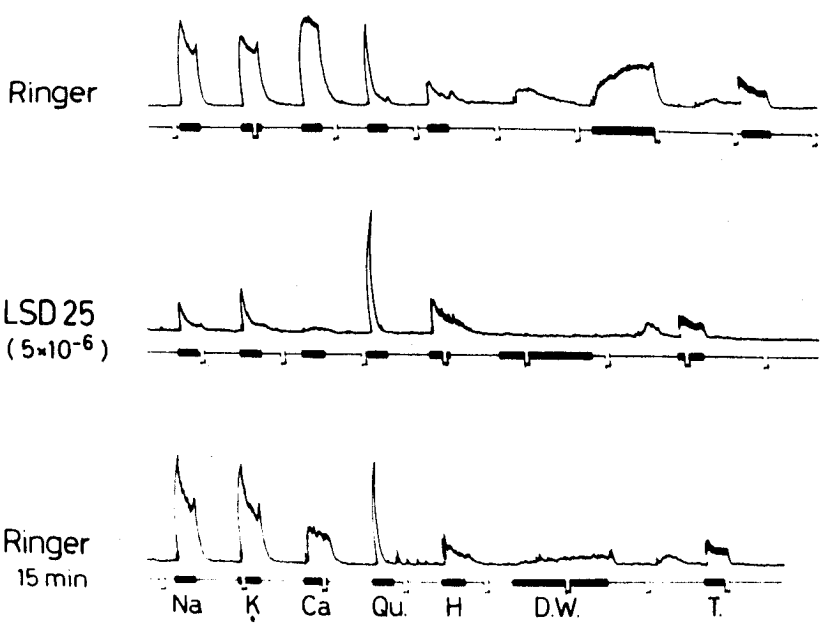

Fig.4

Effects of 5 x 10^{-6}g/ml LSD perfusion on taste responses. The upper trace shows taste responses to 0.3 M KCl, 0.3 mM $CaCl_2$, 0.1 mM quinine, 1.0 mM HCl, deionized water and tactile responses recorded just before LSD perfusion. The middle trace shows the responses during the perfusion of LSD. The bottom trace shows the responses obtained after reperfusion of Ringer solution for 15 min. Taste substances and tactile stimuli were applied at the holizontal black bar on the tongue.

quinine was significantly depressed (Fig.4). The response to HCl was not altered by LSD of a similar concentration. LSD at a high concentration sometimes evoked a rhythmic contraction of the tongue muscle as did 5HT, and produced a burst-like firing in the glossopharyngeal nerve synchronously with tongue movement, which is probably originated from mechanosensitive units (Fig.5). As shown in Table 1, depression of responses to KCl, NaCl and $CaCl_2$ by 10^{-5} g/ml LSD was almost the same as that by 5×10^{-6} g/ml LSD shown in Fig.4. The stationary state of responses to quinine and HCl were significantly enhanced by the perfusion with 10^{-5} g/ml LSD (Table 1).

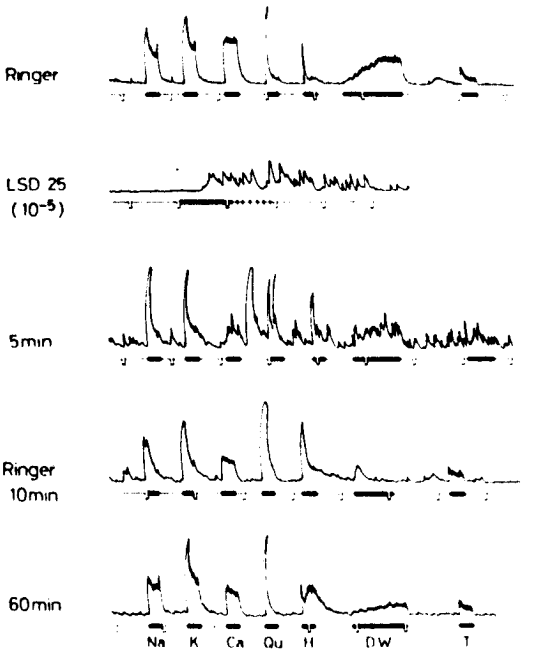

Fig.5

Effects produced by the perfusion of 10^{-5} g/ml LSD. The top trace indicates control responses to taste and tactile stimuli. LSD was perfused for 20 min. The point of initiation of the drug perfusion is shown by the thick horizontal line in the second trace. the third, fourth and fifth traces show the responses 5 min after perfusion of LSD, 10 min and 60 min after reperfusion of Ringer. Stimuli were the same as those shown in Fig.4.

The effect of LSD of 5×10^{-6} g/ml and 10^{-5} g/ml on the response to deionized water was also shown in Figs.4 and 5 and Table 1. water responses were strongly depressed by the perfusion with LSD. Although the initial phase of responses to tactile stimulation was slightly depressed, the stationary phase was not significantly altered.

Gramine: First, Ringer solution containing 2 % ethyl alcohol was perfused through the lingual artery. Taste responses were depressed to about 50 % of the original value, and they remained at this level for several hours during the perfusion. Then, the artery was perfused with gramine dissolved in Ringer solution containing 2 % ethyl alcohol. Gramine of 10^{-4}g/ml further depressed both taste and tactile responses, but responses to both stimuli were scarecely affected by gramine of 10^{-5} g/ml.

Table 1

Taste Response Magnitude after Perfusion of LSD (10^{-5}g/ml)

	Initial Response	Stationary Response
NaCl	0.74 ± 0.46	0.26 ± 0.18
KCl	0.66 ± 0.32	0.38 ± 0.25
$CaCl_2$	0.12 ± 0.18	0.11 ± 0.16
Quinine	0.97 ± 0.14	1.62 ± 0.98
HCl	0.91 ± 0.16	2.20 ± 0.85
D.W.	----------	0.17 ± 0.29
Tactile	0.77 ± 0.20	0.87 ± 0.34

*Neumerals represent the magnitude of responses expressed relative to that of the control responses.

5-hydroxy-tryptophan: Even a high concentration of 5-hydroxy-tryptophan (10^{-3} g/ml), perfused through the frog lingual artery, altered neither taste nor tactile responses.

Phenformin: When phenformin at a concentration higher than 10^{-4} g/ml was perfused through the lingual artery, it elicited impulse discharges in the glossopharyngeal nerve. However, both taste and tactile responses were depressed when 10^{-4}g/ml phenformin was perfused through the lingual artery, although less concentrated solutions did not cause significant change in either response.

Effect of serotonin-depleting agents.

PCPA was dissolved in Ringer solution at pH 10. Since this Ringer solution caused significant depression in taste responses when it was injected intraperitoneally (Fig.6), pH of the PCPA solution had to be adjusted to 7.0 just before injection of the drug.

A single dose injection of 300 mg/kg PCPA caused no alternation in taste responses (Fig.6). Therefore, 100 mg/kg PCPA was injected every day for 6 to 9 days depending on the condition of the frog. Even though extraordinarily high doses such as 600-1,000 mg/kg were injected, taste responses in the frog did not change as shown in Fig.7.

PCPA caused a diminution in the amount of 5HT at the serotonergic nerve and cell by inhibiting synthesis of 5HT (9). If a very large amount of 5HT is stored in the frog taste organ, PCPA would not cause significant effect in the amount of 5HT and consequently in taste responses. To deplete 5HT in the taste organ drastically, both PCPA and reserpin were injected intraperitoneally in seven frogs during 4 to 7 days. The daily

injection dose of PCPA was 100 mg/kg and that of reserpin 10 mg/kg. Three frogs among the seven died within 5 days, and the remaining four frogs were subjected to electrophysiological experiments. Although behaviour of all the four frogs appeared to be affected heavily by the drugs, taste responses were not significantly altered. Only a slight depression was observed in responses to NaCl, KCl, and $CaCl_2$ of highest concentrations as shown in Fig.7.

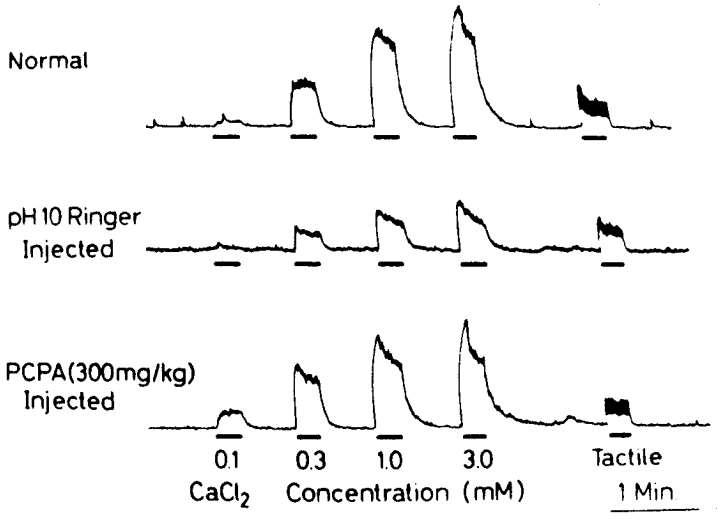

Fig.6

Effects of injection of PCPA on responses to $CaCl_2$ solutions of varying concentrations. The recording of responses was made 1 day after administration of 300 mg/kg PCPA which was dissolved in pH 10-Ringer solution or the same amount of pH 10-Ringer solution. The upper recording shows taste responses to $CaCl_2$ solutions in an untreated normal frog.

Discussion

In the present study, it has been shown that 5HT-creatinine sulphate compound evoked spike discharges in the glossopharyngeal nerve when the agent was applied through the lingual artery, the discharge frequency increasing dose-dependently, and the threshold concentration was 10^{-6} g/ml. However, creatinine sulfate *per se* also produced a similar action to that of 5HT compound and its threshold concentration was the same as that of 5HT-compound. Consequently, there is a possibility that the evoked response by 5HT-creatinine sulfate compound would have been due to the action of creatinine sulfate moiety. However, this seems unlikely since the stimulating effect of creatinine sulfate differed from that of 5HT-creatinine sulfate compound. Furthermore, the discharge caused by 5HT-creatinine sulfate compound showed a phasic pattern. When we consider the fact that 5HT has a self-antagonistic effect on many 5HT sensitive organs, the phasic excitation observed in

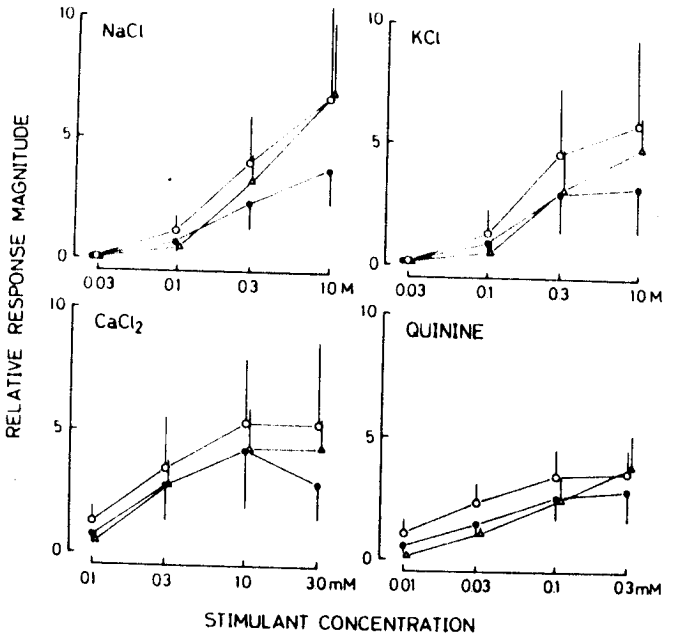

Fig.7

Relations between the magnitude of glossopharyngeal nerve responses and concentrations of taste stimuli. Five frogs were treated with PCPA (triangles) and 4 frogs were treated with PCPA and reserpine (solid circles). Control responses were obtained from 3 frogs which did not receive any treatment (empty circles). All taste responses were obtained under artificial perfusion of the tongue with Ringer. The vertical line shows S.D. The magnitude of response is expressed by the ratio of taste to tactile response.

the present experiment may be caused by this nature of 5HT moiety (12, 13). Therefore, it is concluded that the response induced by 5HT-creatinine sulfate compound was induced, at least, in part by 5HT moiety per se. 5HT depressed the taste responses while creatinine sulfate did not. If we assume that 5HT is a chemical transmitter from taste cell, then the depressing effect could have been explained by a self-antagonistic effect of 5HT. However, an other possible explanation for this can been offered : 5HT possibly depolarized the afferent nerve endings of the glossopharyngeal nerve, and excess depolarization would cause the reduction of the responses to successively applied stimuli, since 5HT excites the non-medulated nerve endings nonspecifically through its depolalizing effect (14).

Among the drugs known as bloking agents of serotonergic receptors (15) only LSD specifically altered taste responses, but gramin, phenformin and 5-hydroxy-tryptophane did not. LSD

depressed the glossopharyngeal nerve responses to NaCl, KCl, $CaCl_2$ and deionized water, while it enhanced those to quinine and HCl. Since in the frog there has neither been demonstrated a taste cell specifically sensitive to one kind of stimulus, nor two kinds of morphologically defferent synapses on taste cells, it is very likely that taste responses to all kinds of stimuli should be mediated by one and the same chemical transmitter substance, if present (Dale's principle; 17, 18). Therefore, the differential action of LSD on taste stimulus-qualities is puzzling if 5HT is a chemical transmitter from taste cells to nerve terminals because almost all taste cells in the bullfrog are responsive to all the taste stimuli used in the present experiment (16). Therefore, it is rather plausible that LSD, not acting as a specific serotonergic receptor blocking agent, may exert non-specific effects on the taste receptors, although at this moment, we have no direct evidence of the exact site of its action at hand.

PCPA is a potent and selecteve depletor of 5HT in the brain and peripheral nerves in mice, rats and dogs, but it dose not produce any significant decrease in catecholamine content (9). If the 5HT-like monoamine is a chemical transmitter in taste cells, application of PCPA should decrease 5HT content in taste cells, and consequently taste responses. Therefore, we applied the 5HT-depleting agent and examined the taste responses to various chemicals after the treatment. However we could not observe any significant decrease in taste responses after treatment with high doses of PCPA. Even when PCPA in conbination with reserpin were injected intraperitonealy, taste responses did not significantly decrease. Since a quantitative estimate of the degree of depletion of 5HT by the treatment with those 5HT-depleting agents has not been made, exact amount of the decrease in the 5HT contents by PCPA and reserpin in the taste receptor is not clear in the present experiments. However, large doses of these agents produce a considerable decrease in 5HT content in other tissues, and consequently it is reasonable to consider that the content of 5HT in the taste receptors would have been reduced significantly. Furthermore, Hirata and Nada (5) observed depletion of a significant amount of serotonin by intraperitoneal injection of 5,6DHT in the frog taste organ. In our preliminary experiment (unpublished), 5,6DHT scarcely altered taste responses in the frog when it had been injected intraperitonealy in the same manner as that adopted by Hirata and Nada (5).

On the other hand, we demonstrated in our previous paper: i) catecholamines applied to the taste organ through the lingual artery enhanced the spike frequency in the glossopharyngeal nerve; ii) α-adrenergic bloking agents, phentolamin and phenoxybenzamine, depressed the taste responses; iii) α-methyl-p-tyrosine, one of a catecholamine depleting agents also depressed the taste responses, when it had been injected intraperitonealy in conbination with reserpin. When these results were compared with those obtained in present experiments, we have to conclude catecholamine is a more likely candidate for a chemical transmitter in frog taste organ than 5HT.

Then what is a role of 5HT present in a considerable amount in the basal region of the fungiform papillae of the frog? Since Düring and Andres (19) demonstrated many Merkel cells at the base of the taste disc in the frog fungiform papillae, one possi-

bility is that 5HT may be contained in these cells and may play a subtle functional role although it is not yet known. Alternatively, 5HT, contained in the basal cells or other parts of the taste organ may have no significant role or have some modifying role for the taste discrimination in the frog.

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