Norepinephrine as a Possible Transmitter Involved in Synaptic Transmission in Frog Taste Organs and Ca Dependence of Its Release

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ABSTRACT In order to explore the role of catecholamine and Ca$^{2+}$ in the synaptic transmission from taste cells to sensory nerve terminals, the effects of various agents added to an artificial solution perfusing the lingual artery on the frog taste nerve responses were examined. (a) The injection of reserpine or guanetidine, which are catecholamine-depleting agents, led to a great reduction of the frog taste nerve responses. The addition of catecholamines to the perfusing solution did not practically enhance the spontaneous impulse discharges, but did recover the response to all the taste stimuli examined. Norepinephrine was most effective and is the most likely candidate for the transmitter. (b) The enhancement of the responses by norepinephrine was suppressed by desipramine, cocaine, or imipramine, which suggests that the enhancement was brought about by incorporation of norepinephrine into taste cells. (c) In a previous paper (Nagahama, S., Y. Kobatake, and K. Kurihara, 1982. J. Gen. Physiol. 80:785), we showed that the responses to the stimuli of one group depended on Ca$^{2+}$, cGMP, and cAMP added to the perfusing solution and those to the stimuli of another group did not depend on these agents. After the injection or addition of reserpine to the lingual artery, which probably modified the permeability of the artery, the responses to the stimuli of the latter group also came to exhibit dependences on these agents, which indicates that the responses to all the taste stimuli have dependences on Ca$^{2+}$, cGMP, and cAMP.

INTRODUCTION

Stimulus information received at taste cells is transmitted to the sensory nerve terminals via chemical synapses (Nomura et al., 1975; Uga and Hama, 1967; DeHan and Graziadei, 1971; Graziadei and DeHan, 1971; During and Andres, 1976). It was shown (DeHan and Graziadei, 1971; Graziadei and DeHan, 1971) that the specific catecholamine fluorescence in frog taste cells was significantly reduced by the injection of reserpine into the frog, while the injection of...
norepinephrine (NE) increased the fluorescence. Based on these observations, it was suggested that NE is the transmitter involved in the synaptic transmission from the taste cells to the sensory nerve terminals.

In order to test the possibility that the catecholamine is the transmitter, Morimoto and Sato (1975, 1982) added catecholamines and their antagonists to a solution perfusing the frog lingual artery and showed that addition of NE enhanced both the spontaneous impulse discharges in the glossopharyngeal nerve and the nerve responses to taste stimuli and that addition of $\beta$-blocker suppressed the taste nerve responses. These results are interesting, but it is uncertain whether or not the agents added to the lingual artery penetrate the narrow synaptic cleft between the taste cell and the nerve terminal and by which mechanism NE enhances the taste responses. It will be necessary to examine the action of the agents added to the artery in more detail.

On the other hand, we reported in an earlier paper (Nagahama et al., 1982) that the frog taste nerve responses to chemical stimuli of group 1 (CaCl$_2$, NaCl, distilled water, $\alpha$-galactose, and L-threonine) are greatly decreased by a decrease in the Ca$^{2+}$ concentration in a solution perfusing the lingual artery, enhanced by cyclic GMP (cGMP), and suppressed by cyclic AMP (cAMP), while the responses to chemical stimuli of group 2 (quinine HCl, theophylline, ethanol, and HCl) are practically unaffected by Ca$^{2+}$, cGMP, and cAMP.

In the present study, we have examined the suppression of the frog taste responses by injection of catecholamine-depleting agents (reserpine and guanethidine) and the recovery of the responses by addition of catecholamines to a solution perfusing the lingual artery. It is shown that catecholamines added to the lingual artery have little effect on the spontaneous nerve discharges but greatly enhance the nerve responses to taste stimuli by being incorporated into taste cells. It is also shown that the responses to chemical stimuli of group 2 come to show dependence on Ca$^{2+}$, cGMP, and cAMP after reserpine treatment, probably because the permeability of the lingual artery to these agents is modified by the action of reserpine.

**MATERIALS AND METHODS**

Adult bullfrogs, *Rana catesbeiana*, weighing between 180 and 250 g, were used in the present experiments. The dissection of the glossopharyngeal nerve was carried out as described previously (Kashiwagura et al., 1980). The nerve impulses were amplified with an AC amplifier and integrated with an electronic integrator with a time constant of 0.3 s. The tongue was bathed in Ringer's solution when not subjected to stimulation. Stimulating solutions were applied to the tongue at a flow rate of 1.7 ml/s.

Perfusion of the lingual artery with artificial solution was carried out as described previously (Nagahama et al., 1982). In brief, a polyethylene tube was cannulated into the lingual artery, and Ringer's solution (112 mM NaCl, 3.4 mM KCl, 3.6 mM MgSO$_4$, 0.2 mM CaCl$_2$, 2.5 mM NaHCO$_3$, pH 7.2) was perfused through the tube into the artery by using a peristaltic pump at a rate of 0.01 ml/s. The perfused solution was flowed out from the vein at the bottom of the tongue.

The treatment of the frog with reserpine (0.5 mg/100 g body weight) or guanethidine (2 mg/100 g body weight) was carried out by injecting the agents intraperitoneally. Stimulating solutions were prepared as described previously (Nagahama et al., 1982). All experiments were carried out at 20°C.
**RESULTS**

First, the effect of NE added to a solution perfusing the lingual artery on the frog taste nerve responses was examined. Fig. 1 shows the summated responses of the glossopharyngeal nerve to CaCl₂, distilled water, D-galactose, L-threonine, quinine HCl, HCl, ethanol, and theophylline before and after the addition of $10^{-4}$ M NE. All the responses to taste stimuli examined were enhanced by the addition of NE. The spontaneous discharges were not practically enhanced by the addition of NE.

To explore the role of catecholamines in taste cells in more detail, reserpine and guanetidine, which are catecholamine-depleting agents (Paton and Vizi, 1969; Vizi and Knoll, 1971), were injected intraperitoneally into the frog. Fig. 2 shows the magnitude of the response (peak height of the summated response) to 1 mM CaCl₂ as a function of time after injection of the agents. The response is decreased to a level near the spontaneous one ~10 h after the injection of reserpine and guanetidine. The response is recovered by addition of $10^{-4}$ M NE to a solution perfusing the lingual artery. The response is slightly increased 9 h after the reserpine injection and reaches near the spontaneous level ~18 h after the injection. Addition of NE recovers the response to ~70% of the original level. This value of recovery has no absolute meaning since no control response is available during the recording of the time course of the response. The nerve activity must be weakened during the lengthy recording of the responses.

24 h after the reserpine injection, various concentrations of NE were added to a solution perfusing the lingual artery and the responses to 1 mM CaCl₂, 0.1 mM HCl, and 0.1 mM quinine HCl were recorded 1.5 h after the addition of NE. The responses were increased by addition of NE and reached stationary levels 1.5 h after addition of NE. In the experiments shown in Fig. 2, it took longer for the responses to reach a stationary level, probably because the preparations used for the experiments were subjected many times to chemical stimulation before the addition of NE and hence the nerve activity was weakened. Fig. 3 shows the relative magnitude of the responses to these stimuli as a function
of NE concentration, where the magnitude of the response to each stimulus before the addition of NE is taken as unit. The responses to all the stimuli examined are increased with an increase in NE concentration.

The effects of other species of monoamines on the responses to various stimuli were also examined. Various concentrations of monoamines were added to the perfusing solution 24 h after the reserpine treatment. Fig. 4 shows the relative magnitude of the responses to various chemical stimuli after addition of the monoamines. The responses to the stimuli were increased with increasing concentrations of dopamine and L-dopa. Epinephrine exhibits little effect on the responses even when the concentration is increased to $10^{-3}$ M. An increase in the concentration of 5-hydroxytryptamine (5-HT, serotonin) leads to a suppression of the responses. Fig. 5A compares the relative magnitude of the responses when various monoamines ($10^{-4}$ M) are added. The magnitudes of the responses decrease in the following order: NE > dopamine > L-dopa > epinephrine > 5-HT. The addition of $10^{-4}$ M glutamate, substance P, and carbachol did not practically affect the responses (data not shown).

There is a possibility that the enhancing effects of the monoamines are brought about by incorporation of the amines into the taste cells. In order to test this possibility, desipramine (DP), cocaine, and imipramine, which inhibit the uptake of NE into cells (Horn et al., 1971; Hughes, 1972), were added to the perfusing solution and the effects of these agents on the responses to 1 mM CaCl$_2$, 0.1 mM HCl, and 0.1 mM quinine HCl were examined. The results obtained with use of DP and cocaine are shown in Fig. 5, B and C. The addition of $10^{-4}$ M NE in the presence of $10^{-8}$ M DP or cocaine had no enhancing effect on the responses to the stimuli. Similar results were obtained with imipramine (data not shown).
FIGURE 3. Effects of various concentrations of NE on the responses to various stimuli. 24 h after the reserpine injection, the lingual artery was perfused with Ringer's solution and the control responses to various stimuli were recorded. Then NE was added to the perfusing solution. The magnitudes of the responses 1.5 h after addition of NE (R) are plotted in the figure, where magnitude of the control response to each stimulus is taken as unit. Each point in the figure shows the mean value of data obtained from 10 frogs. O, 1 mM CaCl₂; •, 0.1 mM quinine HCl; ▲, 0.1 mM HCl.

FIGURE 4. Effects of various concentrations of dopamine (A), L-dopa (B), epinephrine (C) and 5-HT (D) on the responses to various stimuli. The experimental procedures are the same as those for Fig. 3. The magnitude of the control response to each stimulus is taken as unit in the figure. Each point in the figure represents the mean value of data obtained from three preparations. O, 1 mM CaCl₂; •, 0.1 mM quinine HCl; ▲, 0.1 mM HCl.
Note that DP, cocaine, and imipramine, at the concentrations used, did not exhibit any suppressive effect on the responses to the stimuli in the absence of NE. The elimination of these agents from the perfusing solution led to an increase of the responses, which was brought about by uptake of NE into the taste cells.

As described in the Introduction, the responses to chemical stimuli of group 2 were not decreased by a decrease in the Ca\(^{2+}\) concentration in the solution perfusing the lingual artery, while the responses to group 1 exhibited Ca dependence (Nagahama et al., 1982). During the course of the experiments, we noticed that the taste responses of the reserpine-treated frog to the stimuli of group 2 exhibited the Ca dependence. Fig. 6A shows the relative magnitude of the responses in the reserpine-treated frog to various stimuli as a function of Ca\(^{2+}\) concentration in the perfusing solution. Here the magnitude of the response to each stimulus at 0.2 mM CaCl\(_2\) is taken as unit. The responses of the stimuli

![Figure 5.](image-url)
of group 2 (quinine HCl, HCl, and ethanol) were reduced greatly with a decrease in Ca$^{2+}$ concentration and increased with an increase in Ca concentration. A further increase in Ca concentration led to a decrease in the responses, which was studied in detail elsewhere (Nagahama and Kurihara, 1984). Thus, the responses to group 2 exhibited a distinct Ca dependence after the reserpine treatment. The Ca dependence of the responses to the stimuli of group 1 (CaCl$_2$, distilled water, and L-threonine) was similar to that in the untreated frog (Nagahama et al., 1982). The concentration at the abscissa represents the Ca concentration in the perfusing solution, and thus the Ca concentration in the artery or the fluid between taste cells would be higher than that in the perfusing solution, especially at low concentrations. Fig. 6B shows the Ca dependence of

**Figure 6.** Dependences of the responses to various stimuli on the Ca concentration in a solution perfusing the lingual artery after the injection of reserpine (A) and guanethidine (B). $10^{-4}$ M NE was added to the Ringer's solution (containing 0.2 mM CaCl$_2$) perfusing the lingual artery 24 h after the reserpine injection, and the control response to each stimulus was recorded after the response reached a stationary level. The magnitude of the control response to each stimulus is taken as unit in the figure. The lingual artery was perfused with solutions containing various concentrations of Ca$^{2+}$. The magnitudes of the responses recorded after the responses reached stationary levels (~40 min) are plotted in the figure. Ca$^{2+}$ concentrations were varied with use of EGTA and calculated on the basis of its binding constant to Ca$^{2+}$. Each point in the figure represents the mean value of data obtained from five preparations. ○, 1 mM CaCl$_2$; Δ, distilled water; ×, 0.05 M threonine; ●, 0.1 mM quinine HCl; ▲, 0.1 mM HCl; ■, 0.4 M ethanol.
the responses in the frog treated with guanetidine, another catecholamine-depleting agent. The Ca dependence of each response is quite similar to that of the corresponding response in the untreated frog, which suggests that the modification of the Ca dependence of the responses to group 2 by the reserpine injection was not brought about by its catecholamine-depleting action. As will be discussed later, the reserpine treatment seems to lead to an increase in the permeability of the lingual artery, which brings about the modification of the Ca dependence.

To test this possibility, the effect of reserpine added directly to a solution perfusing the lingual artery on the taste responses was examined. Fig. 7 shows the effect of reserpine added to the perfusing solution on the responses to the stimuli. Reserpine below $10^{-6}$ has no effect on the responses, while reserpine at a higher concentration has a suppressive effect. In the following experiments, $10^{-9}$ M reserpine was added to the perfusing solution. The Ca dependence of the responses obtained under these conditions is shown in Fig. 8. The responses to the stimuli of group 2 exhibit Ca dependence when reserpine is added to the perfusing solution. The curve for the Ca dependence of the responses to the stimuli of group 2 is slightly shifted to a lower concentration region compared with that in the reserpine-injected frog (Fig. 6A).

In an earlier paper (Nagahama et al., 1982), we showed that the responses of the stimuli of group 2 are not affected by the addition of cGMP and cAMP to the perfusing solution, while those of group 1 are increased by cGMP and decreased by cAMP. Fig. 9 shows the effects of cGMP and cAMP on the responses to various stimuli when $10^{-9}$ M reserpine is added to the perfusing solution. All the responses increased with time after the addition of cGMP and decreased after the addition of cAMP. Thus, the responses to the stimuli of group 2...
FIGURE 8. Dependences of the responses to various stimuli on the Ca concentration in a solution perfusing the lingual artery when reserpine is added to the perfusing solution. The lingual artery was perfused with Ringer's solution containing 0.2 mM CaCl₂ and 10⁻⁹ M reserpine, and the control response to each stimulus was recorded. The magnitude of control response to each stimulus is taken as unit in the figure. The lingual artery was perfused with solutions containing 10⁻⁹ M reserpine and various concentrations of Ca²⁺. The magnitudes of the responses plotted in the figure (R) are those recorded after the responses reached stationary levels (~40 min). Each point in the figure represents the mean value of data obtained from three preparations. O, 1 mM CaCl₂; A, 0.1 mM HCl; ●, 0.1 mM quinine HCl.

FIGURE 9. Effects of cGMP (A) and cAMP (B) added to a solution perfusing the lingual artery on the responses to various stimuli when reserpine is added to the perfusing solution. The lingual artery was perfused with Ringer's solution containing 10⁻⁹ M reserpine, and the control response to each stimulus was recorded. The magnitude of the control response to each stimulus is taken as unit in the figure. After the recordings of the control responses, 0.5 mM cGMP or 5 mM cAMP was added to the perfusing solution. The figure represents the time course of changes in magnitude of the responses to various stimuli after addition of the nucleotides. Each point in the figure represents the mean value of data obtained from three preparations. O, 1 mM CaCl₂; △, distilled water; □, 0.8 M galactose; ×, 0.05 M threonine; ●, 0.1 mM quinine HCl; A, 0.1 mM HCl; ■, 0.4 M ethanol; O, 2 mM theophylline.
DISCUSSION

Morimoto and Sato (1975, 1982) stressed that the addition of NE to a lingual-artery perfusing solution leads to an increase in the impulse discharges of the glossopharyngeal nerve. The present results, however, show that the spontaneous impulse discharges in response to NE are not practically enhanced even when a relatively high concentration of NE is added to the solution. This suggests that NE added to a lingual-artery perfusing solution does not penetrate the narrow synaptic cleft between the taste cell and the sensory nerve terminal. In general, the catecholamines have both hormonal and neurotransmitter actions and their receptors are differentiated (Ariens, 1981). For certain nervous systems, it seems that the catecholamines in blood do not act on the postsynaptic membrane since the nerve activity is protected from changes in the concentration of catecholamines in blood.

The present results show that NE added to the lingual-artery perfusing solution is incorporated into taste cells and hence the taste responses are enhanced. This was clearly proved by the fact that desipramine, cocaine, and imipramine suppress the enhancement of the responses. Of the various monoamines examined, NE was the most effective. It is less likely that L-dopa and dopamine act as neurotransmitters in peripheral systems such as taste cells. The present results suggest that NE is the most likely candidate for the transmitter involved in the synaptic transmission from the taste cells to the sensory nerve terminals. Thus, the present results support those obtained from previous histological (DeHan and Graziadei, 1971; Graziadei and DeHan, 1973) and physiological (Morimoto and Sato, 1975; 1982) studies. However, further study is needed to prove that NE is a true transmitter in the taste cells.

The present results show that the Ca dependence of the responses to the stimuli of group 2 is modified by the reserpine injection. The direct addition of reserpine to the lingual artery also leads to a modification of the Ca dependence. Furthermore, the responses to the stimuli of group 2 come to exhibit dependence on cGMP and cAMP when reserpine is added to the lingual artery. It is known that reserpine directly affects certain tissues independently of its catecholamine-depleting action (Nayler, 1962; Droogmans et al., 1977; Login et al., 1983; Casteels and Login, 1983). For example, reserpine acts on the smooth muscle of rabbit ear artery and inhibits the contractile system (Casteels and Login, 1983). These results, together with the present results, suggest that reserpine modifies the permeability of the lingual artery.

It was confirmed again in the present study that the responses to the stimuli of group 2 in a non-reserpine-treated frog do not depend on the addition of Ca\(^{2+}\), cGMP, and cAMP to the lingual artery. These results suggest that the agents added to the lingual artery of a non-reserpine-treated frog do not penetrate some area responsible for the generation of the responses to the stimuli of group 2. It is unknown at present whether the taste cells responsible for the generation of the responses to the stimuli of group 2 are different from the responses to the stimuli of group 1, whether the synapses responsible for both
responses are different, or whether other factors are attributable to the difference in penetration of the agents. This is an important problem to be elucidated in future studies.

There are voltage-dependent Ca channels in taste cell membranes (Kashiwayanagi et al., 1983; Roper, 1983). The application of chemical stimuli to the tongue depolarizes taste cells, which leads to activation of the voltage-dependent Ca channels. The present results suggest that Ca influx through these channels is needed for release of the transmitter from taste cells, irrespective of species of taste stimuli. As suggested previously (Nagahama et al., 1982), cyclic nucleotides do not seem to act as a second messenger since the nucleotides themselves do not affect the spontaneous discharges but rather act as a modulator for taste responses.

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REFERENCES

Ariens, E. J. 1981. The classification of beta-adrenoceptors. Trends Pharmacol. Sci. 2:170–172.
Casteels, R., and I. S. Login. 1983. Reserpine is a direct calcium antagonist in smooth muscle of rabbit ear artery. J. Physiol. (Lond.). 340:403–414.
Dehan, R. S., and P. P. C. Graziadei. 1971. Functional anatomy of frog’s taste organs. Experientia. 27:823–836.
Droogmans, G., L. Raeymaekers, and R. Casteels. 1977. Electro- and pharmacomechanical coupling in the smooth muscle cells of the rabbit ear artery. J. Gen. Physiol. 70:129–148.
During, M. V., and K. H. Andres. 1976. The ultrastructure of taste and touch receptors of the frog’s taste organ. Cell Tissue Res. 165:185–198.
Graziadei, P. P. C., and R. S. DeHan. 1971. The ultrastructure of frog’s taste organs. Acta Anat. (Basel). 80:663–603.
Graziadei, P. P. C., and R. S. DeHan. 1973. The innervation of frog’s taste organ: a histochemical study. Life Sci. 13:1435–1449.
Horn, A. S., J. T. Coyle, and S. H. Snyder. 1971. Catecholamine uptake by synaptosomes from rat brain. Structure-activity relationships of drugs with differential effects on dopamine and norepinephrine neurons. Mol. Pharmacol. 7:66–80.
Hughes, J. 1972. Evaluation of mechanisms controlling the release and inactivation of the adrenergic transmitter in the rabbit portal vein and vas deferens. Br. J. Pharmacol. 44:472–491.
Kashiwagura, M., N. Kamo, K. Kurihara, and Y. Kobatake. 1980. Interpretation by theoretical model of dynamic and steady components in frog gustatory response. Am. J. Physiol. 238:G445–G452.
Kashiwayanagi, M., M. Miyake, and K. Kurihara. 1983. Voltage-dependent Ca²⁺ channel and Na⁺ channel in frog taste cells. Am. J. Physiol. 244:C82–C88.
Login, I. S., M. S. Cronin, S. W. J. Lamberts, C. A. Valdenegro, and R. M. MacLeod. 1983. Reserpine inhibits rat anterior pituitary hormone secretion in vitro: effects on prolactin and ACTH and ultrastructural observations. Brain Res. 260:99–106.
Morimoto, K., and M. Sato. 1975. Noradrenaline as a chemical transmitter from taste cells to sensory nerve terminals in frog. Proc. Jpn. Acad. 51:347–352.

Morimoto, K., and M. Sato. 1983. Role of monoamines in afferent synaptic transmission in frog taste organ. Jpn. J. Physiol. 32:855–871.

Nagahama, S., Y. Kobatake, and K. Kurihara. 1983. Effect of Ca²⁺, cyclic GMP, and cyclic AMP added to artificial solution perfusing lingual artery on frog gustatory nerve responses. J. Gen. Physiol. 80:785–800.

Nagahama, S., and K. Kurihara. 1984. Effects of respiratory inhibitors and ionophore A23187 on frog gustatory nerve responses. Comp. Biochem. Physiol. 79A:431–435.

Nayler, W. G. 1962. A direct effect of reserpine on ventricular contractility. J. Pharmacol. Exp. Ther. 139:222–229.

Nomura, S., Y. Muneoka, and Y. Kanno. 1975. The ultrastructure of taste organs of a frog (Rana catesbeiana). Jpn. J. Oral. Biol. 17:371–384.

Paton, W. D. M., and E. S. Vizi. 1969. The inhibitory action of noradrenaline and adrenaline on acetylcholine output by guinea-pig ileum longitudinal muscle strip. Br. J. Pharmacol. 35:10–28.

Roper, S. 1983. Regenerative impulses in taste cells. Science (Wash. DC). 220:1311–1312.

Uga, S., and K. Hama. 1967. Electron microscopic studies on the synaptic region of the taste organ of carp and frogs: three types of synapse between taste cells. J. Electron Microsc. (Tokyo). 16:269–276.

Vizi, E. S., and J. Knoll. 1971. The effects of sympathetic nerve stimulation and guanetidine on parasympathetic neuroeffector transmission; the inhibition of acetylcholine release. J. Pharm. Pharmacol. 23:918–925.