Electrical Responses of the Olfactory Tract to Some Chemical Stimulants in Carp

Yasumasa Goh* and Tamotsu Tamura*

(Received May 29, 1978)

Multifiber responses to olfactory stimulation with alcohols, acids and salts were recorded from the olfactory tract of carp.

1) Alcohols were found to be effective to the olfaction of carp, though the responses were small. We found a tendency that alcohols with longer carbon chain were more stimulative.

2) The magnitude of the initial excitatory response to the stimulation with HCl was large, while that to the stimulation with butyric acid was negligible. The pH value lower than 3.5 regardless of the kind of acid, however, induced inhibitory responses, which may probably be nonspecific pharmacological effects rather than olfactory responses.

3) Monovalent cations and monovalent anions induced only the initial excitatory responses, and were not accompanied with the oscillatory responses. Divalent cations induced both the initial excitatory and oscillatory responses, but only inhibitory responses were observed with trivalent cations. The responses to salts were concluded to be largely dependent upon the cations. The salts with monovalent and divalent cations were found to be olfactory stimulants in carp, while those with trivalent cations seemed to be injurious to the olfactory receptors.

Amino acids have been widely known as good olfactory stimulants in many fishes1)-5). In a previous paper6), we reported electrical responses of the olfactory tract to amino acids, which proved the high sensitivity to several amino acids in carp.

On the other hand, some chemicals such as alcohols and fatty acids which were classically believed to be olfactory stimulants to fish, have not been investigated sufficiently by means of electrophysiology.

In this experiment, we investigated olfactory responses to alcohols, acids and salts, and attempted to determine their effectiveness as olfactory stimulants in carp.

Materials and Methods

The experimental procedure and materials were the same as described elsewhere6). The carp (body length 18-25 cm) was immobilized with the injection of Flaxedil (gallamine trethiodide; 2 mg/Kg body weight) and fixed in a lead plate. Then the tract was exposed, and the lateral bundle of the tract was lifted on the platinum hook electrodes.

Continuous flowing of water was given to the olfactory sac, and the stimulating solution (0.1 ml) was injected into the flowing water. The dilution of the injected stimulating solution by the continuous water flow was about one-twentieth. Therefore, we adopted one-twentieth of the lowest concentration of the injected solutions to elicit the reaction as the threshold.

The electrical activity of the tract was amplified, passed through an integrating circuit (time constant 1.0 sec.) and displayed on a pen recorder. For the observation of the oscillatory response, the electrical activity passed through the integrating circuit was further passed through a band-pass filter of 10 Hz, and also displayed on a pen recorder.

Throughout the experiment, the gills of the fish were perfused with water.

Results and Discussions

The olfactory tract was spontaneously active. In general, the discharges increased initially, and then bursted rhythmically at the frequency of 10 Hz when the stimulus was applied on the olfactory mucosa. Consequently, the integrated curve of these discharges shows firstly upward shift (initial excitatory response) and then small up-and-down oscillation (oscillatory response).

It is already known that both the magnitude of

* Fisheries Laboratory, Faculty of Agriculture, Nagoya University, Nagoya, Japan. (Department of Agriculture, Faculty of Agriculture, Nagoya University, Nagoya, Japan.)
the initial excitatory response and the amplitude of the oscillatory response increase linearly with the logarithmic increase of the stimulus concentration of amino acids. Therefore, we gave an attention to the property of the two responses in this experiment.

**Responses to Alcohols**

Five aliphatic alcohols (methyl, ethyl, n-propyl, n-butyl and n-amyl alcohol) were used as stimulants.

With the alcohols, the responses were small and relatively unstable, especially the magnitude of the initial excitatory response was very variable even in one series of the experiment. Therefore, we could not demonstrate the relationship between the magnitude of the initial excitatory response and the stimulus strength, whereas the oscillatory responses were observed rather consistently.

Fig. 1 shows the relation between the amplitude of the oscillatory response and the stimulus intensity of the five alcohols. This figure was made from seven preparations which showed fairly clear responses.

We found a distinct tendency that the amplitude of the oscillatory response became larger with the increase of the carbon number of the alcohol. This tendency was observed in each series of the seven preparations.

N-amyl alcohol (C=5) was most stimulative and the threshold was about $10^{-4}$ M, taking the dilution by the continuous flow into account, and lowest among the alcohols tested. The threshold for n-butyl alcohol (C=4) was about $10^{-3}$ M, that for methyl alcohol (C=1) was about $10^{-3}$ M, whereas in propyl (C=3) and ethyl (C=2) alcohol it was about $10^{-2}$ M. Thus, the threshold also showed a similar tendency to that observed in the amplitude of the oscillatory response and number of the carbons.

Strong stimuli (e.g. $10^{-1}$ M) of the alcohols sometimes induced inhibitory responses which were observed more frequently in alcohols with long carbon chain.

In many teleosts, alcohols have been recognized as good olfactory stimulants. For example, $\beta$-phenylethyl alcohol was very effective to the olfaction of European eel and rainbow trout. BOUDREAU also gave the electrophysiological evidence showing the high effectiveness of butyl alcohol on catfish. He found that even the very low concentration of butyl alcohol ($10^{-15}$ M) changed the multifiber activity of the tract.

On the other hand, the olfactory epithelium of Atlantic salmon did not respond to the same n-aliphatic alcohols as those used here. No response to alcohols were obtained in the bulb of rainbow trout either.

![Fig. 1. Relations between the amplitude of the oscillatory responses and molar concentration of five alcohols (n-amyl, n-butyl, n-propyl, ethyl and methyl alcohol). Ordinate; relative value of the oscillatory response to the standard L-glutamine $10^{-4}$ M. Vertical bars; standard deviations. cont; control.](image-url)
The results obtained here suggest that alcohols are olfactory stimulants in carp, although the responses were considerably small.

**Responses to Acids**

In the responses to either HCl or butyric acid, we observed a strong inhibition at higher concentrations, which is preceded by an excitation (Fig. 2). The preceding excitation to butyric acid is much smaller than that to HCl. Furthermore, the inhibitory response to HCl is followed by a subsequent excitation (Fig. 2 10^-2 M HCl). This phenomenon may be due to the non-rectangular application of the stimulating solution upon the mucosa.

**Fig. 2.** A series of the integrated response to butyric acid (left column) and HCl (right column). All recordings in this figure were obtained from the same preparation. Arrows indicate the inhibitory responses.

Upper part (closed circles) of Fig. 3 shows the relationship between the magnitude of the initial excitatory response and the stimulus concentration of HCl. The response magnitude to HCl increased with the stimulus intensity. And the oscillatory response showed similar tendency to the initial excitatory response. The threshold concentration for HCl was between 10^-4-10^-5 M, taking the dilution by the continuous flow into account.

**Fig. 3.** Relation between the magnitude of the integrated response and the stimulus intensity. Closed circle; HCl. Open circle; butyric acid. Open circle with vertical bar; standard L-glutamine. Ordinate; relative value of the excitatory response to the standard L-glutamine 10^-3 M (upward), and relative value of the inhibitory response (downward). Abscissa; molar concentration. WW; control. Vertical bars; standard deviations. The inhibitory response to control stimulation is the fluctuation of the spontaneous activity.

Upper part (open circles) of Fig. 3 indicates the relation between the magnitude of the initial excitatory response and the stimulus strength of butyric acid. The excitatory response to this acid did not increase in spite of the increase of the stimulus, although only the response to 10^-2 M was slightly larger than that to other concentrations including 10^-1 M, and small oscillatory response was observed at 10^-2 M.

Butyric acid seems to be injurious to the olfactory receptor, since the stimulation with its high concentration resulted in the decrease of the spontaneous activity of the tract.

The small size of the initial excitatory response to butyric acid suggest that this acid may not be so effective as HCl on the olfaction of carp. We cannot say at present whether the small excitatory response to butyric acid was caused by the hydrogen ions or not. However, it is obvious that the stimulatory effectiveness of butyric acid may be very low. This harmonizes well with the results that the rainbow trout^1^ and atlantie salmon^2^...
did not respond to some aliphatic acids.

As the inhibitory responses were always observed at high concentration of both butyric acid and HCl (Fig. 3 lower part), we gave an attention to the relations between pH of the stimulating solution and the appearance of the inhibitory response. In this case, the direct stimulating method which consisted of the direct application of the stimulating solution to the olfactory mucosa was used in the place of the continuous flowing method mentioned earlier.

Clear inhibitory responses always appeared when the pH of the solution of either butyric acid or HCl or acetic acid was lowered to 3.5. A similar phenomenon was also observed in the bulbar response of rainbow trout1). Therefore, we conclude that the inhibitory responses may be the effects of hydrogen ions—probably nonspecific pharmacological effects.

**Responses to Salts**

Fig. 4 shows a series of responses to sodium chloride. The responses to NaCl were characterized by the lack of the oscillatory responses. In short, the responses were composed of only the initial excitatory phase, which increased with the logarithmic increase of stimulus concentration. Under the condition of the present experiment, the threshold for NaCl was about $10^{-4}$-$10^{-5}$ M.

![Fig. 4. Recordings of the integrated responses and the oscillatory responses to NaCl $10^{-1}$-$10^{-4}$ M.](image)

In each recording upper trace indicates the oscillatory response and lower trace integrated response. Numbers indicate the molar concentrations. Note that there are no oscillatory responses even at $10^{-1}$ M.

**Fig. 5. Relation between the magnitude of the integrated response and molar concentration of NaCl.** Response magnitude was represented by relative value to the standard L-glutamine $10^{-3}$ M. Vertical bars indicate the standard deviations.

(Fig. 5).

On the other hand, the responses to barium chloride consisted of both the initial excitatory and the oscillatory responses. Fig. 6 shows the relation of the two responses to the stimulus strength. Each of the two responses increased linearly with the logarithmic increase of stimulus concentration. Under the condition of the present experiment, the threshold for BaCl2 was about $10^{-4}$-$10^{-5}$ M. Thus, the valency of the cation seemed to have
something to do with the oscillatory response, hence we investigated the responses not only to various monovalent and divalent cations but also trivalent ones. These cations were tested in the form of chlorides, while monovalent anions in the form of sodium salts.

The responses to NH$_4^+$, K$^+$, Li$^+$, Cs$^+$, and Choline$^+$ and those to I$^-$, NO$_3^-$, and Br$^-$ were not accompanied with the oscillation as also in the case of NaCl.

These monovalent cations caused a large variation in the magnitude of the initial excitatory responses, whereas the variation of the responses to the monovalent anions was not so remarkable. The sequence of the stimulatory effectiveness of the ions is shown in Fig. 7.

The valency of the cations was concluded to have causal relations to the responses to the salts. Monovalent cations induced only the initial increase of the discharge, whereas the divalent cations induced both the initial increase (initial excitatory response) and the rhythmic bursts (oscillatory response). And only the inhibitory responses were observed with the trivalent cations.

We would like to conclude that the responses to salts are largely dependent upon their cations, because the stimulatory effectiveness of cations was different distinctly from each other (Fig. 7 CaCl$_2$, BaCl$_2$, MgCl$_2$, MnCl$_2$, NaCl, NH$_4$Cl, KCl, LiCl, CsCl, and [Cho]Cl, but the difference of the stimulatory effectiveness of anions was not so remarkable (Fig. 7 NaI, NaCl, NaNO$_3$, and NaBr). TAKAGI$^{10}$ also investigated the effect of the ions on the olfactory bulbar responses in carp, catfish and frog. He found that the stimulatory effectiveness of the anions such as NO$_3^-$, Br$^-$, BrO$_3^-$, and HCO$_3^-$ was different from each other, and concluded that anions are stimulants to the olfaction in carp. OSHIMA and TAKAGI$^{11}$ also obtained the similar results with chum salmon. However, we could not show the difference of the stimulatory effectiveness among anions.

The sequence of the stimulatory effectiveness of the monovalent cations was NH$_4^+ > K^+ > Li^+ > Cs^+$, Na$^+$ > Choline$^+$ and this was similar to the sequences in the bulbar responses of catfish and carp$^{11}$, which are NH$_4^+ > K^+ > Na^+ > Cs^+ > Li^+ >$ Choline$^+$ and K$^+ > Na^+ > NH_4^+ > Cs^+ > Li^+ >$ Choline$^+$. For trivalent cations, the sequence was Al$^{3+}$ > La$^{3+}$ > Ce$^{3+}$ > Pr$^{3+}$ > Nd$^{3+}$ and this was similar to the sequences of the responses to the salts. TAKAGI$^{10}$ also investigated the effect of the ions on the olfactory bulb responses in carp, catfish and frog. He found that the stimulatory effectiveness of the anions such as NO$_3^-$, Br$^-$, BrO$_3^-$, and HCO$_3^-$ was different from each other, and concluded that anions are stimulants to the olfaction in carp. OSHIMA and TAKAGI$^{11}$ also obtained the similar results with chum salmon. However, we could not show the difference of the stimulatory effectiveness among anions.

Classification of the Chemical Stimulants

Judging from the effects to the discharge pattern recorded from the olfactory tract of carp, various chemical stimulants were classified into following three groups.

1. Amino acids$^6$, divalent cations, alcohols and HCl
   - This group induces initial excitatory response and oscillatory response.

2. Monovalent cations
   - This group induces only initial excitatory response.

3. Butyric acid
   - This group induces no response.

Other response pattern (inhibition) was often observed with acids, trivalent cations and alcohols. However, at least the inhibitory response to acids may probably be nonspecific pharmacological effects rather than olfaction.
How the fish feel the chemicals of these groups classified here is very interesting, but we have no clue to discuss the matter at present.

Acknowledgement

We wish to thank Mrs. K. KOGA for typing the manuscript and photographing.

References