

コイ側線器官(孔器)の炭酸ガス応答

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Carbon Dioxide Responses of the Lateral Line Organ in the Carp *Cyprinus carpio*

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The responsiveness of free neuromasts (pit organs), innervated by a buccal branch of the anterior lateral line nerve, the *ramus buccalis*, in the carp *Cyprinus carpio* to carbon dioxide dissolved in distilled water was studied using whole nerve and single unit recording techniques.

The stimulation of the carp pit organ with CO₂ solution produced a large phasic response followed by a long period (60-90s) of depression in impulse discharges. These temporal patterns of integrated responses were CO₂-specific and differed from those of various acids, in which an initial phasic response was followed by a plateau level of neural activity sustained during the stimulation.

The magnitude of the CO₂ responses was slightly pH-dependent, suggesting that dissolved free CO₂ may have a facilitating effect on the receptor cell.

All the single units tested responded to CO₂ and other acids with response patterns similar to those in whole nerve recordings. No specific single units responding to CO₂ alone were detected, showing the receptor cells to be 'generalist' in nature.

High sensitivity of palatal gustatory receptors to carbon dioxide (CO₂) has been observed electrophysiologically in some fresh-water fish,¹⁻³ indicating that the gustatory receptors may play a role as a CO₂-detector in respiratory regulation. On the other hand, the free neuromast (pit organ), one of the ordinary lateral line organs, responds to various salts and acids, suggesting that it may serve as a primitive chemoreceptor or as an ion-sensitive organ besides its main function as a mechanoreceptor.⁴ In addition, all the single fibers obtained from the buccal nerve branch innervating pit organs in the snout region of the carp *Cyprinus carpio* have recently been found to respond uniformly to various inorganic salts and amino acids; there are no dose-specific single units.^{5,6}

The aim of the present experiments is to elucidate electrophysiologically whether the pit organs respond to CO₂ as in gustatory receptors and further to examine whether there are CO₂-specific single units.

Material and Methods

Adult carp *Cyprinus carpio*, 20-25 cm in body length, were used in the present experiments. The

fish were paralyzed with Flaxedil (gallamine triethiodide, 2 mg/kg b. wt., i.m.). After removing the eye ball, the *ramus buccalis* (the buccal nerve branch) which innervates pit organs in the snout region was cut off centrally in the orbit.

For whole nerve recordings, the buccal nerve branch was hooked on a pair of Ag-AgCl electrodes connected to an R-C coupled amplifier. Neural activity was processed through an electric integrator circuit with a time constant of 0.5 s and recorded on an ink-writing recorder. The height from the spontaneous discharge level was used as the response measure. For single fiber recordings, the peripheral end of the nerve branch was dissected into one or few fibers and placed on a pair of Ag-AgCl electrodes as in the whole nerve recording. Single fiber activity was displayed on an oscilloscope, monitored by means of a loudspeaker, and stored on a magnetic tape for subsequent photography.

Test solutions made from reagent grade chemicals in distilled water were delivered to the snout region for 10 s and thereafter the stimulated region was continuously rinsed with distilled water. Saturated CO₂ solution was prepared by bubbling pure CO₂ gas (99.99 vol. %) into distilled water just before the stimulation. It was then diluted

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four times to use as the test solution. The pH of the test solution was 4.40 at 20°C. The concentration of CO₂ calculated from the total inorganic carbon (measured by gas chromatography⁷⁾) was approximately 0.0036 M. All experiments were performed at room temperature (20±2°C). Test solutions and rinsing water were also given at the same temperature.

Results

The carp pit organs produced fairly large responses to the CO₂ solution, despite its relatively high pH (pH 4.4). The time course of the response consisted of a rapid rise to a peak followed by an abrupt decline to below the spontaneous discharge level (Fig. 1e). Inhibition of spontaneous discharges began within the first 5 s of stimu-

lation and recovered gradually to the initial spontaneous discharge level by 60–90 s after rinsing with distilled water. Each pit organ also responded well to all thirteen acids tested. In contrast to CO₂ responses, acid responses exhibited initial phasic and following tonic components, returning readily to the initial spontaneous discharge level after distilled water rinse, without any abrupt inhibition of impulse discharges during stimulation (Fig. 1a-d).

Gaseous CO₂ readily reacts with water to form carbonic acid, H₂CO₃ which partly dissociates to produce H⁺ and HCO₃⁻. Of this aqueous CO₂, however, more than 99% exists as dissolved gas (free CO₂) and only less than 1% as H₂CO₃ at low pH.⁸⁾ The concentrations of free CO₂ and H₂CO₃ which were dissolved in a fourth saturated CO₂ solution (pH 4.4 at 20°C) tested in the present ex-



Fig. 1. Integrated responses of the carp pit organ to fumaric (a), tartaric (b), succinic (c) and acetic (d) acids of 0.005 M and a fourth saturated CO₂ solution (e), when recorded from the whole nerve preparation of the buccal nerve branch. Horizontal bars at the bottom of each recording indicate the stimulation period.

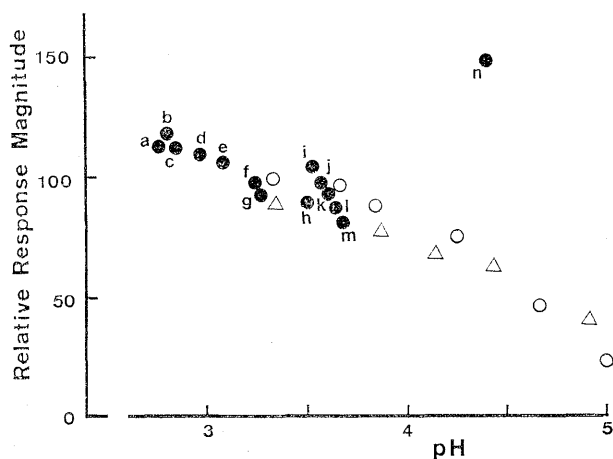


Fig. 2. Magnitudes of responses to 13 organic acids of 0.005 M, and a fourth saturated CO₂ solution, together with those to various concentrations of HCl (open circle) and acetic acid (open triangle), when plotted as a function of their pH values. Magnitudes of responses are expressed relative the magnitude of the phasic response to 0.0005 N HCl. Means of seven experiments.

a=tartaric acid, b=fumaric acid, c=citric acid, d=malic acid, e=formic acid, f=succinic acid, g=aspartic acid, h=glutamic acid, i=valeric acid, j=butyric acid, k=propionic acid, l=acetic acid, m=caproic acid, n=CO₂ solution.

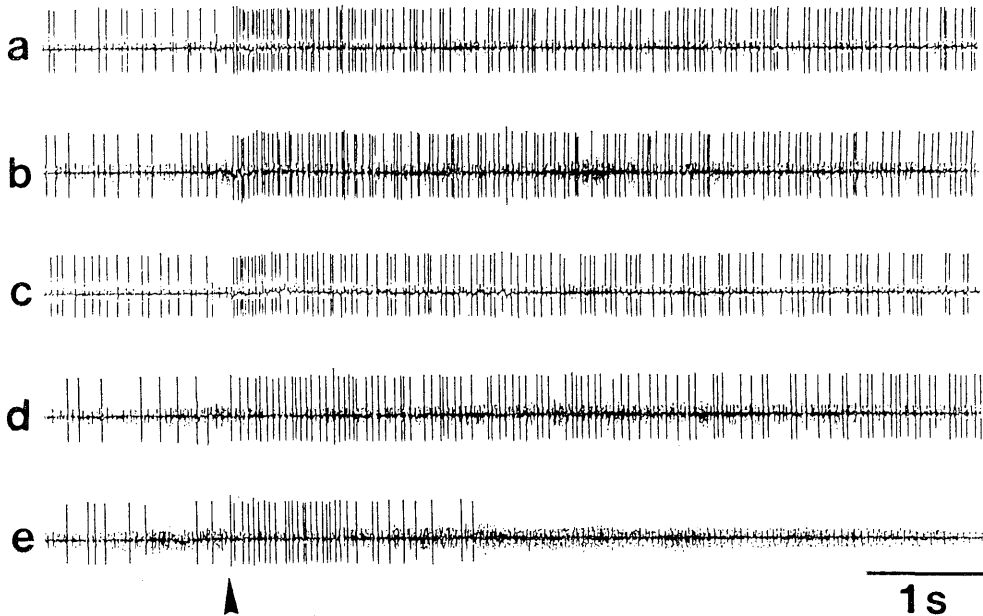


Fig. 3. Single unit responses of the buccal nerve elicited by the stimulation with fumaric (a), tartaric (b), succinic (c) and acetic (d) acids of 0.005 M and a fourth saturated CO₂ solution (e). The arrow head under the lowest trace indicates the onset of stimulation.

periments were 3.5×10^{-3} M and 5.0×10^{-6} M, respectively. Because the pit organs responded well to various acids, we examined the effect of hydrogen ion dissociated from H₂CO₃ on the receptors. Responses to CO₂ and various organic acids of 0.005 M, (higher than CO₂ concentration in the test solution) together with those to hydrochloric and acetic acids of various concentrations are shown as a function of pH in Fig. 2. The response magnitudes for organic acids tend to decrease with increasing pH ($r = -0.751$, $p < 0.01$) and this is also true for the response-pH relation for hydrochloric and acetic acids. The mean of response magnitudes for CO₂, however, is far larger than any other acid responses.

To clarify whether any specific single units were predominantly sensitive to CO₂, single fiber responses of the buccal nerve to thirteen acids of 0.005 M and CO₂ solution were observed. All the responses to acids tested consisted of an initial increase in impulse discharges, followed by a plateau level sustained at high impulse frequency. On the other hand CO₂ caused a burst of impulses for the first few seconds (1–3.5 s) during stimulation, and then the impulses completely disappeared (Fig. 3). Each of the fifteen single units tested responded to all the acids and CO₂ with temporal response patterns similar to those in

Fig. 3, showing the receptor cell to be 'generalist' in nature.

Discussion

The palatal gustatory receptors of the carp and eel are highly sensitive to CO₂, and the integrated responses consist of a rapid rise at the onset of the stimulation and a subsequent gradual decline.^{1,2} These response features are also true for CO₂ responses of gustatory receptors in the lip of sea catfish¹ and rainbow trout.* Such inhibition of the response below spontaneous discharge level as observed in the present experiments has not been detected. Thus, a considerable difference in the temporal pattern for CO₂ responses is evident between the intrinsic gustatory organ and the pit organ.

According to Hidaka,² single units obtained from the carp palatine nerve respond not only to CO₂ but also to one or more other conventional gustatory stimuli, but there are no significant correlations in stimulating effectiveness between pairs of CO₂ and one of other stimuli, suggesting that there is an intrinsic difference between the mechanisms of response to CO₂ and other stimuli. In addition, it has recently been demonstrated that no single units responding to CO₂ responded

* S. Yamashita, R. E. Evans, and T. J. Hara: *Proc. Jpn. Symp. Taste & Smell*, **20**, 101–104 (1986).

to other gustatory stimuli in the palatal chemoreceptors of the rainbow trout *Salmo gairdneri*.^{*} No specific single units responding to CO₂ alone or predominantly to one or more of the other stimuli were detected from the present experiments. On the basis of this difference in the responsiveness of single units it seems probable that sensory receptors of the pit organ have not differentiated as chemoreceptors as highly as gustatory receptors.

Though no CO₂ sensors have been documented, detecting changes in the proportion of CO₂ in water is important for fish to live. It is unlikely that sensory cells of the pit organ are specific CO₂ sensors, but responses of the pit organ to CO₂ may serve as one of sensory signals for CO₂ to the central nervous system.

The specific response pattern, an initial phase and a subsequent long period of depression, and the considerably large response to CO₂ at relatively high pH, which resulted from the present experiments, indicate that CO₂ responses may be caused partly by the stimulation of the receptor cell with H⁺ and/or HCO₃⁻ partly dissociated from H₂CO₃ and also that direct or indirect action of free CO₂, which comprises most fractions of aqueous CO₂, may be involved in or on the receptor membrane. It is well established that free CO₂ rapidly enters gnat neurons and muscle fibers of invertebrates and temporarily allows the cell membrane to depolarize.^{9,10)} It is also known that vertebrate cortical cells produce a transient phase of sharp excitation, followed by a long depression during the application of moderate dose of CO₂.¹¹⁾ Thus, it is supposed that in the pit organ the responses produced by ionic components which were dissociated from H₂CO₃ can be accelerated, provided that the cell membrane is temporarily depolarized by free CO₂ when it invades into the cell; but a direct action of CO₂ on the membrane can not be excluded.

The well-known effect of CO₂ entering the cell is a fall in the intracellular pH, corresponding to an increase in H⁺ dissociated from hydrated CO₂ after the entry into the cell.^{12,13)} A possible explanation for the depressant effect rapidly accelerated in response to CO₂ in the present experiments is that a fall in internal pH may gradual-

ly increase the membrane potential, for example, by reducing the membrane permeability to Na⁺, although the mechanism by which this occurs is not known. There is general agreement that an increase in CO₂ concentration tends to reduce the excitability of vertebrate nerve fibers; a metabolic mechanism has been suggested.¹⁴⁾

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