コイロ蓋化学受容器に対する二酸化炭素の作用

誌名	日本水産學會誌
ISSN	00215392
巻/号	3610
掲載ページ	p. 1034-1039
発行年月	1970年10月

The Effect of Carbon Dioxide on the Carp Palatal Chemoreceptors

Iwao HIDAKA*

(Received May 21, 1970)

In a previous paper,[1] it was shown that the palatal chemoreceptors of carp are highly sensitive to carbon dioxide. The effect of carbon dioxide dissolved in water differed in several aspects from that of hydrochloric acid or acetic acid, suggesting that carbon dioxide may be sensed by receptors different from acid receptors. In the present study experiments with single or a few fibre preparations have been made in an attempt to characterize further the effect of carbon dioxide on the palatal chemoreceptors of this fish.

Material and Methods

Carp, *Cyprinus carpio*, weighing 0.7 to 1.1 kg, were used. The procedure for obtaining the palatine nerve preparation was principally the same as that described by KONISHI and ZOTTERMAN.[2] To pick up the single or few fibres discharges, a method introduced by MORITA *et al.*[3] was applied. The recording system for the multifibre activity has been described elsewhere.[4] After each application of test solutions for about three seconds, the chemoreceptors were washed with tap water. The procedure for making up the stimuli containing carbon dioxide was as follows: At first a saturated water solution of carbon dioxide was made by bubbling carbon dioxide into distilled water for three to five minutes. The quantity of carbon dioxide dissolved in the water was monitored by measuring the pH of the solution. The saturated solution thus prepared was then diluted with three parts of distilled water or salt solutions for use as taste stimuli. The stimuli thus prepared originally contained about 0.01 M carbon dioxide. The experiments were performed mainly from September to December, at room temperatures ranging from 15 to 25°C. The change of temperature during one experiment was less than one degree.

Results and Discussion

Fourteen out of twenty three single fibres from twenty carp were found to be responsive to carbon dioxide dissolved in water. These fibres responded also to one or more of other kinds of stimuli, 0.001 M hydrochloric acid, 0.02 M acetic acid, 0.5 M sodium chloride, 0.5 M sucrose and 0.01 M quinine hydrochloride, as shown in Table 1. The

* Faculty of Fisheries, Mie Prefectural University, Tsu, Mie Prefecture, Japan. (日高磐夫: 三重県立大学水産学部)

Table 1. Number of impulses per second in the responses of single units during the first 2 seconds after application of stimulus.

Units	CO_2 in water (1/4 sat.)	0.001 N HCl	0.02 N CH_3COOH	0.5 M NaCl	0.5 M sucrose	0.01 M quinine-HCl	Distilled water
J	6–	8–	15–	0–	5–	5–	0–
K	9– 8	1– 0	7– 7	3– 1	0– 0	0– 0	0–
I	2– 0	20– 1	21–11	22– 0	0–	1– 1	3–
A	17– 3	0– 0	15– 6	5–10	1– 0	4– 0	1– 0
D	6– 4	0– 0	5– 5	5– 1	6– 0	1– 0	0– 0
G	3– 3	3– 3	10– 2	1– 2	0– 1	6– 3	2– 0
H	2– 3	0– 1	2– 0	2– 2	2– 0	1– 0	0– 0
E	5– 3	2– 1	5– 4	11– 0	2– 2	0– 0	1– 2
B	13– 6	18– 0	–	7– 2	6– 6	2– 0	1– 1
F	4– 2	0– 0	–	1– 1	3– 2	0– 0	3– 1
C	11– 4	0– 0	–	2– 0	0– 1	0– 8	0– 0
L	9– 4	15– 1	–	1– 1	–	–	0– 0
M	19–13	0– 0	6– 5	6– 6	0– 1	21– 9	0– 0
N	4– 2	26–13	17– 8	14– 0	0– 0	0– 0	0– 0

data in the table indicate that the receptors responsive to carbon dioxide in water lead into nerve units which contain also the fibres from receptors for the conventional four kinds of stimuli. However, no specific correlation in stimulating effectiveness appeared between carbon dioxide and one other taste stimulus (Table 1). This suggests that a mechanism may exist for stimulation of the receptors by carbon dioxide in water different from that leading to responses by other stimuli. Of particular interest in the table is the fact that there were several fibres which did not respond to 0.001 M hydrochloric acid while responding well to the carbon dioxide dissolved in water. In some preparations, even application of 0.01 M hydrochloric acid did not elicit a noticeable response as seen in the example of Fig. 3, reinforcing the evidence that carbon dioxide stimulates receptors different from those responding to hydrochloric acid.

The effect of carbon dioxide in phosphate buffer (0.10 M potassium hydrogen phosphate + 0.15 M disodium hydrogen phosphate) were studied. In Fig. 1, the number of impulses in the responses of nine units (A to I in Table 1) to this mixture (pH 7.0) during the first one second after the beginning of stimulation is illustrated. It is seen from the figure that the fibres which responded well to carbon dioxide dissolved in water tended to respond also to that mixture. If one sums the spike numbers of these nine units in the responses to the mixture of carbon dioxide and phosphate buffer, and subtracts the total for the single phosphate buffer, they amount to about 70 per cent of those for carbon dioxide dissolved in water. The theoretical concentration of carbon dioxide in the mixture, calculated by using the mass law equation for the ionization of carbonic acid and

taking 6.35 for pK_1 at experimental temperature, is 18 per cent of that in water without phosphate buffer (or 4.5 per cent of that in the saturated water solution). According to this value approximate as it may be, it seems unreasonable to ascribe the stimulative effectiveness of the mixture solely to carbon dioxide in it, because such a low concentration of carbon dioxide in the water usually elicited a small summated response[4] of the whole nerve, the magnitude of which being smaller than half of that elicited by the 5.6 times more concentrated solution, as seen in the example shown in Fig. 2. It is possible that bicarbonate ions released in large quantity as the result of dissociation of carbonic acid in the phosphate buffer had an important role in stimulation.

The effects of two kinds of mixture, carbon dioxide and 0.01 M sodium bicarbonate (pH 6.5), and carbon dioxide and 0.01 M disodium hydrogen phosphate (pH 7.4) were studied with several preparations of a few or more fibres. In Fig. 3, a single unit is shown (M in Table 1) which responded to the mixture containing sodium bicarbonate and to

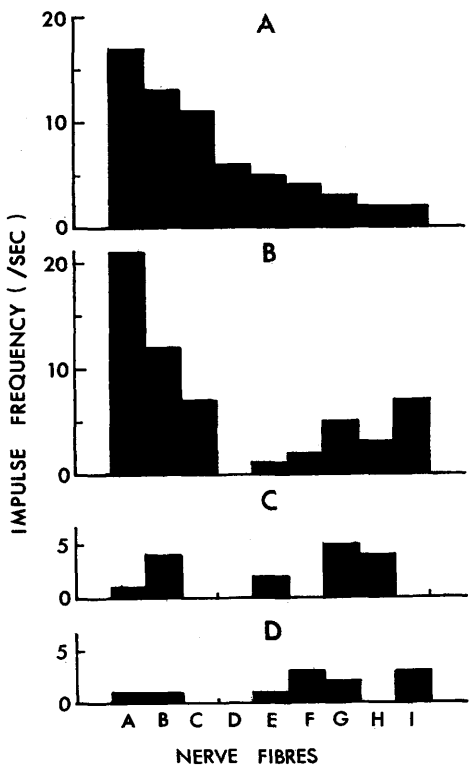

Fig. 1. Histogram showing the number of spikes in the responses of nine single palatine nerve fibres in the first 1 second after the beginning of stimulation. Responses to A: carbon dioxide in water, B: mixture of carbon dioxide and 0.25 M phosphate buffer, C: 0.25 M phosphate buffer (pH 7.1) and D: distilled water.

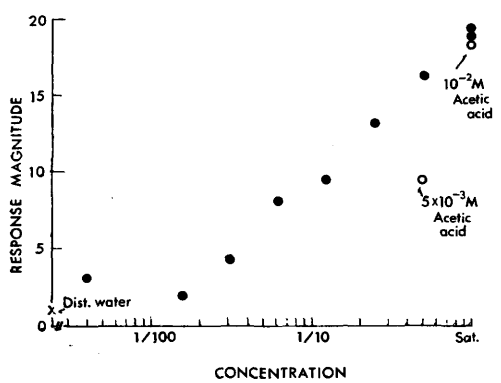

Fig. 2. Magnitudes of responses from the entire nerve bundle to varing concentrations of carbon dioxide in water (●), of acetic acid (○) and to distilled water (×), plotted in arbitrary unit. Abscissa: carbon dioxide concentration expressed relative to that of the saturated solution.

other kinds of stimuli as well. The number of impulses elicited by the mixture in the first second after application of the stimulus was 22 in an average of three recordings. The value is slightly larger than that for carbon dioxide alone in the water (see Table 1, unit M), but apparently does not represent the simple algebraic sum of the latter and the value for 0.01 M sodium bicarbonate (pH 8.4), 14. It is probably safe at present to say that carbon dioxide itself stimulated receptors innervated by this fibre, because it exists in the mixed solution nearly in the same quantities as in the water (pK_1 for carbonic acid at 25°C, 6.35). The mixture containing disodium hydrogen phosphate elicited 17 spikes, and 0.01 M disodium hydrogen phosphate (pH 8.7), eight spikes, on an average of three recordings during the first second after application of each stimulus, whereas it is understood from the pH of the mixture that only a tenth or less amount of the carbon dioxide molecules of the water solution are present in the same volume of the mixture. Thus, the situation is very similar to that of Fig. 1. Therefore, a similar interpretation also may be given for stimulation by this mixture of carbon dioxide and disodium hydrogen phosphate. It is of interest to note that this unit did not respond to 0.01 M sodium chlo-

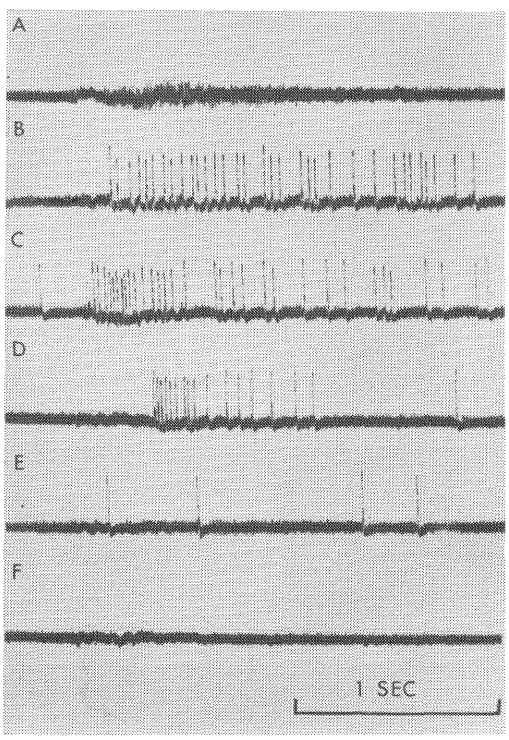

Fig. 3. Responses of a single fibre to A: 0.01 M hydrochloric acid, B: carbon dioxide in water, C: mixture of carbon dioxide and 0.01 M sodium bicarbonate, D: 0.01 M sodium bicarbonate, E: 0.0001 N sodium hydroxide and F: 0.01 M sodium chloride.

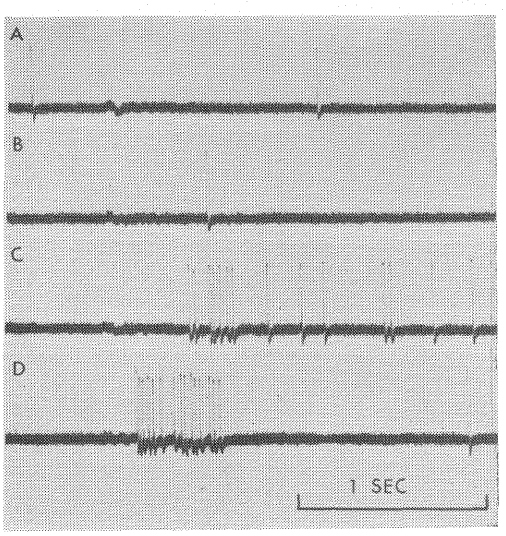

Fig. 4. Responses of a single fibre to A: mixture of carbon dioxide and 0.01 M sodium bicarbonate, B: mixture of carbon dioxide and 0.01 M disodium hydrogen phosphate, C: carbon dioxide in water and D: 0.001 N hydrochloric acid.

ride while responding well to sodium bicarbonate or disodium hydrogen phosphate at the same concentration. Twenty times more enhanced concentration of sodium chloride elicited only four spikes per second. These results are not consistent with the findings with various neutral salts in several vertebrates that sodium salts having various anions do not differ very much in their stimulating effectiveness when compared at the same concentrations, while differences are larger when chloride salts of different cations are used.[5,6,7] The unit in Fig. 3 did not respond to a two thousandth molar sodium ferrocyanide solution or to a washing of the receptors with distilled water immediately after the application of the ferrocyanide solution,[8] suggesting that the receptors in question are different from the specific ones responsive specifically to dilute solutions of electrolytes. Not all the units which are responsive to carbon dioxide dissolved in water responded to either of the two kinds of mixture, carbon dioxide and sodium bicarbonate, or carbon dioxide and disodium hydrogen phosphate (Fig. 4). The unit shown in Fig. 4 responded to neither of the two, while it responded with considerable spike frequency to carbon dioxide dissolved water. Considering the fact that 0.001 M hydrochloric acid elicited a positive response (Fig. 4, D), it seems likely that the hydrogen ion is responsible for the stimulation of this unit by carbon dioxide dissolved water.

ADACHI *et al.*[9] and KAWAMURA and ADACHI[10] studied the effect of soda water on the tongue taste receptors of mammals using a single fibre technique. Their results are in agreement with the present findings on the carp in that carbon dioxide in water stimulates taste receptors other than those for acid.

Summary

The stimulatory effect of carbon dioxide dissolved in water upon carp palatal chemoreceptors were studied by recording the electrical responses from the palatine nerve supplying the chemoreceptors.

There were found taste units responsive to carbon dioxide in water. These units responded also to one of more of the four conventional taste stimuli used. Among them were units which responded well to carbon dioxide in water but not to hydrochloric acid. No marked correlations could be detected between carbon dioxide and the other stimuli in their stimulating effectiveness for these taste units.

The effects of carbon dioxide in phosphate buffer, in disodium hydrogen phosphate and sodium bicarbonate solutions were also studied. From the results with these mixtures, it could be suggested that carbon dioxide itself is responsible for receptor stimulation. It also appeared that the anion is important in stimulation by salts of carp chemoreceptors other than those specifically responsive to dilute electrolyte solutions.

Acknowledgements

The author wishes to thank Professor John E. BARDACH, the University of Michigan, for critical reading of the manuscript. He is also thankful to Dr. Satoru YAMASHITA, Kagoshima University, for helpful advice on preparing the physiological saline solution. This work was supported in part by a research grant from the Ministry of Education.

References

1) J. KONISHI, I. HIDAKA, M. TOYOTA and H. MATSUDA: *Jap. J. Physiol.*, **19**, 327–341 (1969).
2) J. KONISHI and Y. ZOTTERMAN: *Acta Physiol. Scand.*, **52**, 150–161 (1961).
3) H. MORITA, S. DOIRA, K. TAKEDA and M. KUWABARA: *Mem. Fac. Sci., Kyushu Univ., Ser. E. (Biol.)*, **2**, 119–139 (1957).
4) I. HIDAKA and S. YOKOTA: *Jap. J. Physiol.*, **17**, 652–666 (1967).
5) L. M. BEIDLER: *J. Neurophysiol.*, **16**, 595–607 (1953).
6) L. M. BEIDLER, I. Y. FISHMAN and C. W. HARDIMAN: *Amer. J. Physiol.*, **181**, 235–239 (1955).
7) K. KUSANO: *Jap. J. Physiol.*, **10**, 620–633 (1960).
8) J. KONISHI: *J. Gen. Physiol.*, **49**, 1241–1264 (1966).
9) A. ADACHI, M. FUNAKOSHI, Y. KASAWARA and Y. KAWAMURA: *J. Physiol. Soc. Jap.*, **26**, 306–312 (1964) (in Japanese).
10) Y. KAWAMURA and A. ADACHI: in "Olfaction and Taste II" (T. Hayashi, ed.), 431–437, Pergamon Press, Oxford (1967).