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Source: Zoological Science, 15(5) : 691-697

Published By: Zoological Society of Japan

URL: https://doi.org/10.2108/zsj.15.691

ZOOLOGICAL SCIENCE **15**: 691–697 (1998)

Ionic Mechanism of the Carbon Dioxide Reception in the Japanese House Centipede, *Thereuonema hilgendorfi*

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ABSTRACT—The carbon dioxide receptor in the temporal organ of the Japanese house centipede *Thereuonema hilgendorfi* shows spontaneous discharges in carbon dioxide-free air. These responses are decreased or totally suppressed accompanied by a hyperpolarizing receptor potential induced by carbon dioxide stimulation. In the present study the ionic basis of the CO_2 reception has been examined by impulse analysis based upon a known linear relationship between receptor potential and impulse frequency. Even when the temporal organ was perfused with CO_2-free distilled water, receptor cells showed spontaneous discharges, and these discharges were also decreased due to perfusion of CO_2-containing solution. The spontaneous impulse frequency in the CO_2-free solution increased with increasing Na^+ and K^+ concentration, and decreased with increasing Ca^{2+} concentration. Other ions such as Mg^{2+}, Li^+, choline$^+$ and Cl^- had little effect on the receptor cell discharges. In the CO_2 containing perfusate, the effects of increasing Na^+ or Ca^{2+} concentration on the receptor cell discharges disappeared, whereas that of increasing K^+ concentration remained. Response amplitudes to CO_2 stimulation depended largely on Na^+ and Ca^{2+} concentration, but less on K^+ concentration in the perfusate. These results suggest that Na^+ ions are major current carriers for the generation of receptor potential in response to CO_2 stimulation.

INTRODUCTION

It has been reported that in several animals generation of receptor potential in the chemoreceptor cells was accompanied by changes in membrane conductance (Ozeki, 1971; Akaike *et al*., 1976; Suzuki, 1977; Sato and Beidler, 1982; Trotier and MacLeod, 1983; Tonosaki and Funakoshi, 1984; Anderson and Ache, 1985). Some of these reports described effects of extracellular cations: Na^+ and Ca^{2+} ions might play important roles in the frog taste cells in response to salt stimulation (Sato *et al*., 1982), whereas Ca^{2+} ions were reported to be inward current carriers in the lamprey olfactory receptors in response to amino acid stimulation (Suzuki,1978). Effects of extracellular cations were also reported in insect sugar receptor cells: Na^+ and/or K^+ ions were thought to be involved in the generation of taste responses (labellar sugar receptors, Morita *et al*., 1966; tarsal sugar receptors, Broyles and Hanson,1976).

Receptor cells of the temporal organ of the Japanese house centipede *Thereuonema hilgendorfi* responded sensitively to CO_2, though they showed some additional responses to other air-borne chemicals. Based on physiological and morphological studies, the temporal organ is thought to be a CO_2 receptor (Yamana *et al*., 1986; Yamana and Toh, 1987, 1990). The receptor cell responded to CO_2 with hyperpolarizing receptor potentials, which caused a decrease of spontaneous impulse discharges (Yamana and Toh, 1987). The hyperpolarizing receptor potential was accompanied by an increase in the membrane resistance, but it is still unclear which kinds of ions contribute to the generation of the hyperpolarizing receptor potential. In order to answer this basic question, the effects of external ionic environments on CO_2 responses have been examined in the temporal organ of *T. hilgendorfi*.

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MATERIALS AND METHODS

Adult Japanese house centipedes *T. hilgendorfi* of both sexes were used throughout this study. Animals were collected in the field around Kyushu University. Because the methods for recordings and perfusion experiments have been described in detail in the previous report (Yamana *et al*., 1986), they are dealt with here only briefly. After the animal was immobilized by cooling it with ice, its head was fixed in the chamber (1 ml in volume), and perfused with control and test solutions at a rate of 4 ml/min (details in RESULTS). An indifferent electrode (tungsten wire of 0.1 mm diameter) was inserted into the antenna and a recording electrode (glass-coated sharpened tungsten wire) was inserted into the temporal organ in order to record impulse discharges.

Carbon dioxide-free distilled water was prepared by boiling the distilled water for decarboxylation. The boiled water was cooled down while supplying carbon dioxide free air passed through NaOH solution as a CO_2 trap. The solution containing CO_2 was prepared by bubbling air containing CO_2 into distilled water. The solution containing CO_2 in equilibrium with air containing 5% CO_2 was used through-

out the present experiment, and it is referred to in convenience as "CO_2 containing solution". Details for solution containing CO_2 were described previously (Yoshii *et al*.,1980; Yamana *et al*., 1986). Salts used in the present study were dissolved in CO_2-free distilled water or in distilled water that contained CO_2.

RESULTS

Effect of perfusion

Receptor cells of the temporal organ discharged at a rate of about 20 impulses/sec in the CO_2-free air. When the temporal organ was perfused with CO_2-free distilled water (control solution), the impulse frequency decreased to 10 impulses/sec (Fig. 1). Impulse discharges in the air and in the control solution are referred to as spontaneous discharges in the present study. Replacement of the CO_2-free distilled water with the solution containing CO_2 resulted in a decrease in the discharge frequency of the receptor cells. Even in a perfused specimen, the receptor cell of the temporal organ could respond to a CO_2 stimulation.

Effects of salt solutions on the spontaneous activities of the receptor cells

The activities of receptor cells were changed by replacement of the CO_2-free perfusate with test solutions, which were perfusates containing CO_2, NaCl, KCl or $CaCl_2$ (Fig. 1). Receptor cells were first adapted to the control perfusate for more than 5 min, and then the perfusate was replaced by test solutions. The effect of changing solutions appeared about 10 sec after the replacement of the solution. This latency is thought to be due to a slow exchange of the solutions around the receptor region.

The receptor cell responded to the solution containing CO_2 in a phasic-tonic manner: the impulse frequency rapidly decreased and it recovered to a steady level within a few minutes. On the other hand, changes of the impulse frequencies in response to perfusion of salt solutions appeared to be rather tonic.

The change in the impulse frequency of the receptor cell by perfusion of solution containing Ca^{2+} appeared with a delay that was longer by about 10 sec than those caused by other salt solutions and solution containing CO_2 (n = 7). Because the change of the impulse frequency reached a steady state about 120 sec after the beginning of the perfusion, the impulse frequency around 180 sec after initiation of the perfusion was analyzed. The activities of receptor cell adapted to six kinds of salt solutions under the CO_2 free condition were recorded and compared with each other. The spontaneous activities of the receptor cell increased when the temporal organ was perfused with NaCl or KCl solution, but decreased when perfused with $CaCl_2$ solution. On the other hand, $MgCl_2$, LiCl and choline chloride solutions had little effect upon the activity of the receptor cell (Fig. 2, n = 5).

The activity of the receptor cell increased as NaCl concentration increased, and did not reach a saturated level even at 100 mM NaCl in the concentration-activity curve (Fig. 2). The activity also increased as the concentration of KCl solution increased, and the impulse frequency was 1.9 times higher in 10 mM KCl than in KCl-free distilled water. The activities became irregular at concentrations greater than 100 mM KCl. On the other hand, $CaCl_2$ solution depressed the activities of the receptor cell (Fig. 3), and the depressing effect appeared at a lower concentration than the concentration in which the elevating effect of NaCl appeared (e.g., 0.5 mM, n = 4).

Effects of salts on the response to carbon dioxide

The amplitude of the receptor cell responses to CO_2 application in several salt solutions was measured. Time courses of the change in the impulse frequency caused by CO_2 application in salt solution appeared similar to those in distilled water (the response to distilled water in Fig. 4).

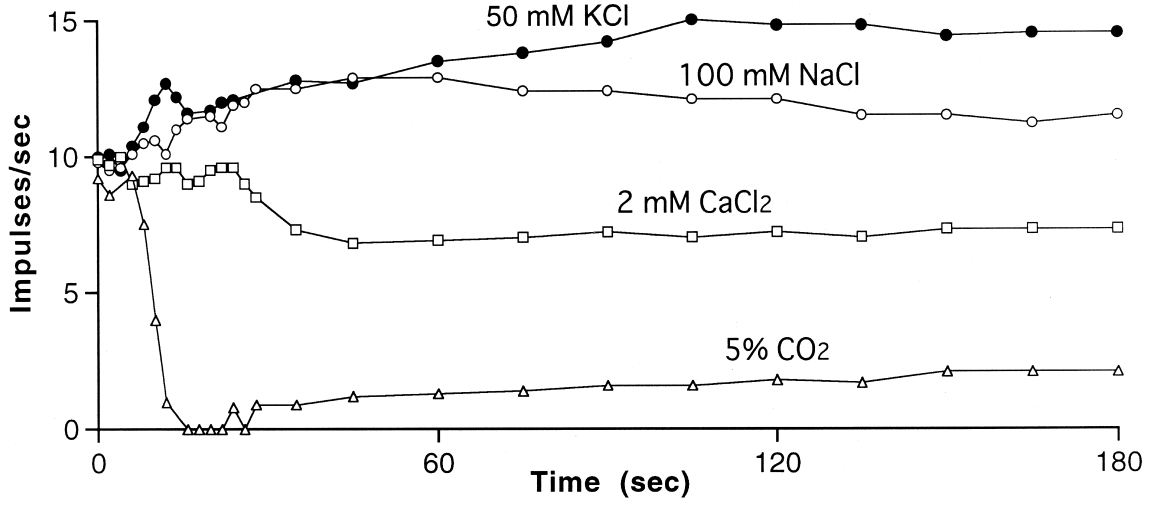

Fig. 1. Time courses of the changes in impulse frequencies of the receptor cells after perfusates were changed from distilled water to CO_2-free salt solutions (100 mM NaCl, open circles; 50 mM KCl, closed circles ; 2 mM $CaCl_2$, open squares) and distilled water in equilibrium with 5% CO_2 (open triangles). All recordings were taken from the same receptor cell.

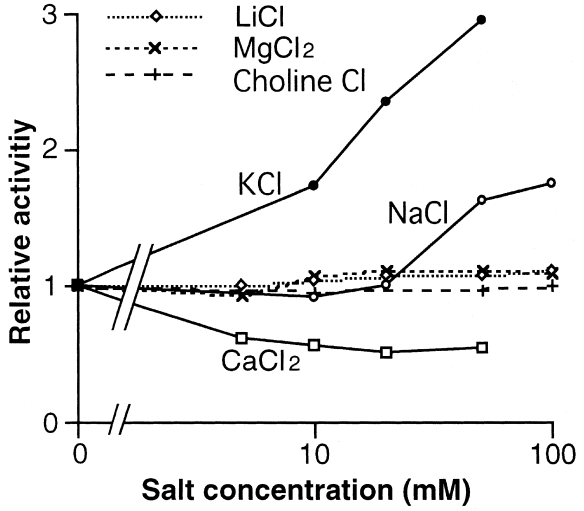

Fig. 2. Relation between the concentration of salt solutions and receptor cell activities: NaCl (open circles), KCl (closed circles), LiCl (open diamonds), $MgCl_2$ (x), $CaCl_2$ (open squares) and choline chloride (+). Relative impulse frequencies were measured 180 sec after replacement of perfusates from the distilled water to each salt solution in the CO_2-free condition. The impulse frequency adapted to CO_2-free distilled water is taken as 1.0. Recordings of NaCl, KCl, $CaCl_2$ and choline chloride were from the same receptor cell and those of $MgCl_2$ and LiCl were from another cell.

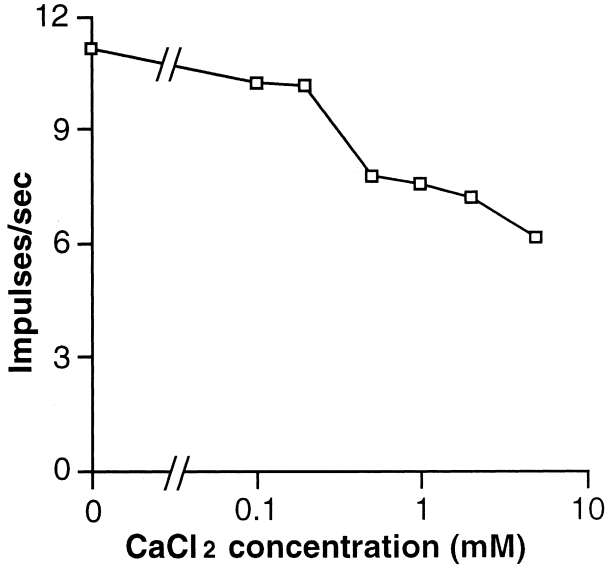

Fig. 3. Effects of low concentration of $CaCl_2$ on impulse frequencies after adaptation to distilled water. Dose-dependent reduction of impulse frequencies was the largest in the receptor cell shown in this figure (among four cells analyzed). The average activity of the receptor cells in 1 mM $CaCl_2$ was 7.9 ± 0.11 impulses/sec (mean ± S.E., n = 4).

The effects of NaCl concentration on receptor responses were examined. The temporal organ was first perfused with CO_2-free NaCl solutions (20, 50 and 100 mM). After adaptation to each concentration of NaCl solutions, the perfusion was changed to NaCl solutions of the same concentration with CO_2 (Fig. 4A). The impulse frequency depended on the concentration of NaCl under CO_2-free condition: it is higher in the higher concentration of NaCl, as shown at time 0 in Fig. 4A. After the application of CO_2 the impulse frequencies were reduced, but they were almost independent of the Na^+ concentration, falling to the same level in the three different NaCl concentrations (n = 4).

The effects of KCl on the responses were also examined in the same manner as those of NaCl (Fig. 4B). The receptor cells showed higher activities in the higher concentrations of KCl in CO_2-free solution as shown at time 0 in Fig. 4B. The impulse frequency was reduced by the application of CO_2, but the effects of KCl still remained at the examined concentrations (10, 20 and 50 mM), the impulse frequency being higher in the higher concentration of KCl (Fig. 4, n = 3).

Perfusion of the $CaCl_2$ solution led to effects on the receptor activities that were different from those of NaCl and KCl. The receptor activities adapted to $CaCl_2$ solution were lower than those in Ca^{2+}-free solution, as shown at time 0 in Fig. 4C. Application of CO_2 reduced impulse frequencies to zero within 20 sec in both solution containing Ca^{2+} and Ca^{2+}-free solution. The impulse frequencies recovered to steady state levels 180 sec after the onset of CO_2 perfusion. The impulse frequency at the steady state was almost the same between Ca^{2+}-free solution and solution containing Ca^{2+} (30-50% of the initial frequencies), but the recovery appeared faster in solution containing Ca^{2+} than Ca^{2+}-free solution (observed in 5 cells out of 8 experiments).

The effects of different concentrations of the salt solution on the CO_2 responses were examined (Fig. 5). In both Ca^{2+}-free solution and solution containing Ca^{2+}, impulse frequencies increased when the KCl concentration was increased (Fig. 5A). Impulse frequencies also increased when Na^+ concentration in CO_2-free solutions was increased, but they slightly increased (Fig. 5A) or did not change in solutions containing CO_2 (Fig. 5B). In summary the different effects of Na^+ and K^+ mean that an increase in the concentration of KCl resulted in an increase in the impulse frequency of the receptor cell, regardless of presence or absence of CO_2 in the perfusate, whereas an increase in the concentration of NaCl affected the impulse frequency only during the absence of CO_2.

The impulse frequencies of receptor cells declined when the Ca^{2+} concentration was increased in CO_2-free perfusate, whereas the cells kept their low frequency levels regardless of an increase in Ca^{2+} concentration (Fig. 5B).

The activities of receptor cells differed among different salt solutions as shown in Fig. 1. The way in which the impulse frequencies were reduced by CO_2 application is presented for each of 50 mM NaCl, 20 mM KCl and 2 mM $CaCl_2$ solution (Fig. 6). The amplitude of the responses, and the reduction of the impulse frequencies induced by CO_2 application were strengthened in NaCl perfusion, weakened by $CaCl_2$ perfusion, and little changed by KCl perfusion, as compared with responses to CO_2 measured for distilled water without any kind of salt.

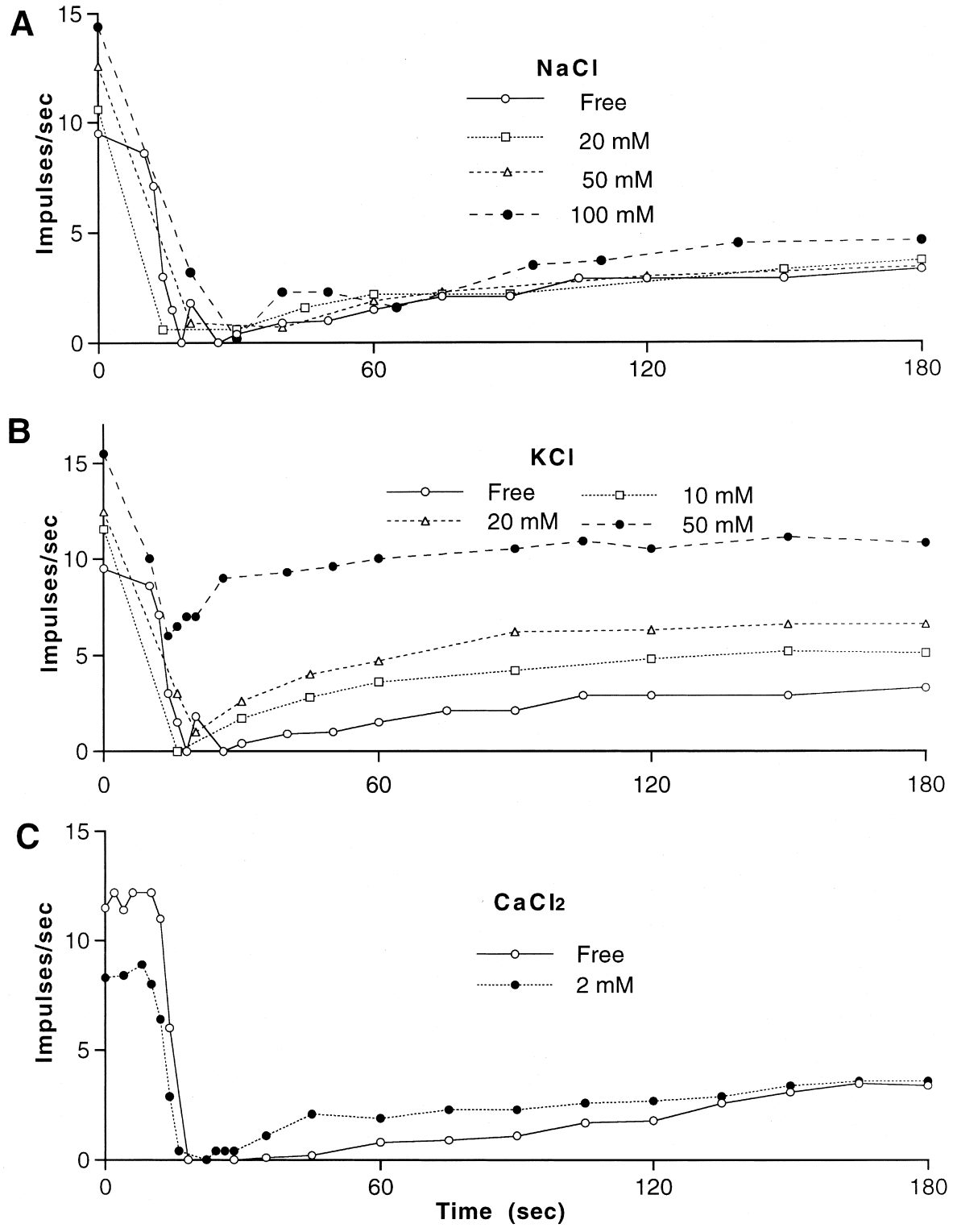

Fig. 4. Time courses of changes in the impulse frequencies after perfusion with CO_2 solution. After adaptation to a given CO_2-free salt solution, the same solution but in equilibrium with air containing 5% CO_2 was perfused. (**A**) Effects of CO_2 in 20 mM, 50 mM and 100 mM NaCl solutions. (**B**) Effects of CO_2 in 10 mM, 20 mM and 50 mM KCl solutions. (**C**) Effects of CO_2 in 2 mM $CaCl_2$ solution. Recordings shown in **A**, **B** and **C** were from different cells.

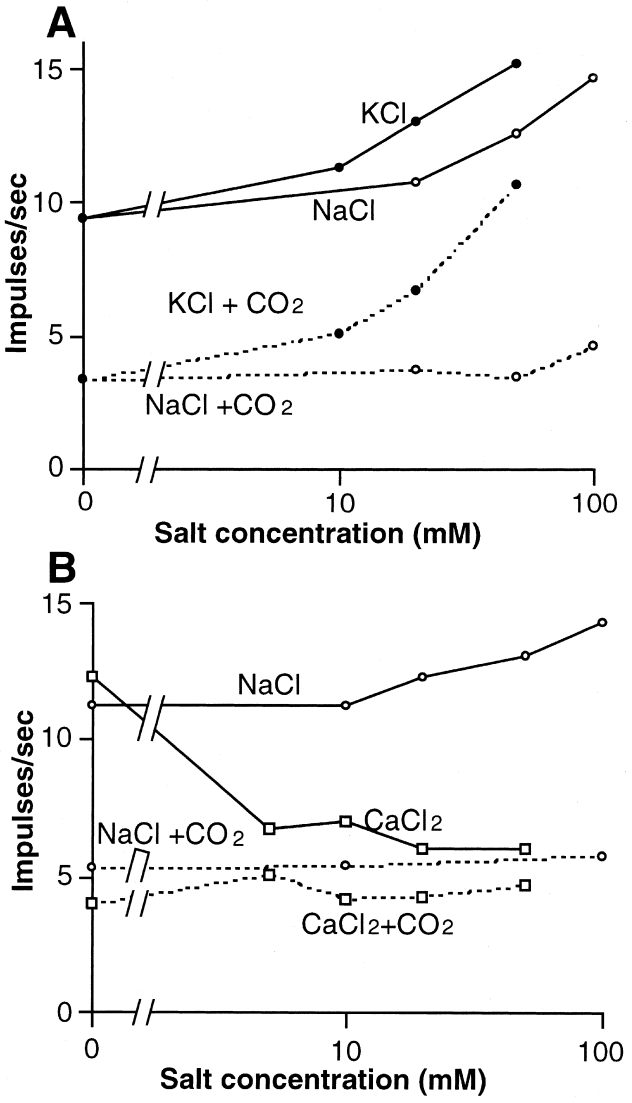

Fig. 5. Relationship between receptor cell activities and the concentration of salt solutions in the presence and absence of CO_2. Impulse frequencies adapted to salt solutions with (broken lines) or without (solid lines) CO_2 are plotted. (**A**) NaCl (open circles) and KCl (closed circles). (**B**) NaCl (open circles) and $CaCl_2$ (open squares). Recordings of **A** and **B** were from different cells.

DISCUSSION

It has been reported that in the temporal organ of the Japanese house centipede *T. hilgendorfi*, the hyperpolarizing receptor potential was accompanied with a decrease in the membrane conductance of the receptor cell, and the decrease in impulse frequency was proportional to the amplitude of the hyperpolarizing receptor potential (Yamana and Toh, 1987). Although only impulse frequencies were recorded in the present study, they can be used for the interpretation of conductance changes of the receptor membrane, owing to the above-mentioned relationship among impulse frequencies, amplitude of receptor potentials and membrane conductance.

In the present study, Na^+, K^+ and Ca^{2+} ions in the perfusate influenced the activity of the receptor cells to varying degrees depending upon their concentration. Other ions such as Mg^{2+}, Li^+ and choline$^+$ had no effects on the activity of the receptor cell (Figs. 1 and 2). This lack of effects suggests that these cations would hardly permeate the receptor cell membrane.

The impulse frequency of the receptor cell increased with an increase in the concentration of K^+ ions in both CO_2-free and solution containing CO_2 (Figs. 4B and 5A). These results suggest that the permeability of the receptor cell membrane to K^+ ions is not changed by carbon dioxide stimulation and that K^+ ions may contribute little to the generation of receptor potential to CO_2 stimulation.

An increase in the concentration of Na^+ ions resulted in an increase of the receptor cell activity in CO_2-free solution, but this effect of Na^+ ions disappeared in solution containing CO_2 (Fig. 5). Moreover, the response amplitude of the receptor cell resulting from CO_2 application, which is expressed as a reduction of impulse frequency by CO_2 perfusion, was reinforced in 50 mM NaCl (Fig. 6). These results suggest that the decrease of Na^+ conductance of the receptor cell may be involved in the generation of the hyperpolarizing receptor potentials. If the hyperpolarizing receptor potential to CO_2 stimulus observed in the previous work (Yamana and Toh, 1987) was caused by a decrease in permeability of receptor membrane to Na^+ ions, then the ionic mechanism of CO_2 reception in the temporal organ would appear to be quite similar to that of vertebrate photoreceptor cells (Korenbrot and Cone, 1972).

Some of the data obtained in the present work appear contradictory. Perfusion of distilled water should largely reduce or abolish impulse discharges of the receptor cell, because Na^+ ions, supposedly inward current carriers, do not exist even if sodium-specific channels remain open under unstimulated conditions. The perfusion of distilled water may also result in a lack of differences in impulse frequency between the presence and absence of CO_2 in perfusates. In the present study the impulse frequency declined by half in the distilled water as compared with the frequency in CO_2-free air, but it was not totally suppressed (Fig. 1). Moreover, the receptor cell could further reduce impulse frequency in response to CO_2 even in the distilled water. This conflicting phenomenon may be due to structurtal complexity of the temporal organ. During perfusion with distilled water some Na^+ ions in the external environment remained in the mucous or constantly released from supporting cells. The ultrastructural study supports this view, because there are many closely packed supporting cell processes around receptor cell dendrites (Yamana and Toh, 1990). The decreased activity of the receptor cell in distilled water may be explained by the fact that the ionic environment of the mucous is diluted with distilled water during the perfusion with distilled water.

The effects of Ca^{2+} ions on the receptor cell response to CO_2 were complex. The impulse frequency decreased as Ca^{2+} ion concentration was increased in CO_2-free solution. This effect disappeared in the perfusate containing CO_2 (Fig. 5B). Moreover, the response amplitude to CO_2 application of the

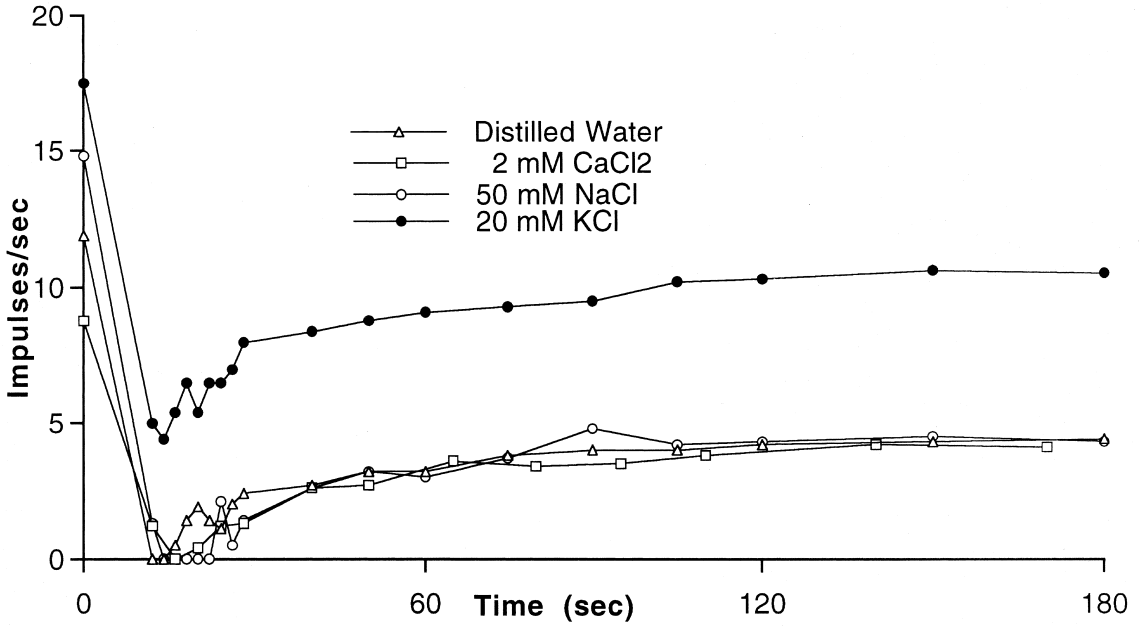

Fig. 6. Time courses of reduction of impulse frequencies by CO_2. Receptor cells are first adapted to CO_2-free solutions of 50 mM NaCl (open circles), 20 mM KCl (closed circles), 2 mM $CaCl_2$ (open squares) and distilled water (open triangles). These perfusates are switched to the same solutions, but are in equilibrium with air containing 5% CO_2. The reduction of impulse frequency is the largest in the NaCl solution. The time course and degree of reduction are similar between distilled water and KCl solution. All recordings were from the same receptor cell.

receptor cell, reduction of impulse frequency by CO_2 perfusion, was small in $CaCl_2$ solution (Fig. 6). These results suggest that Ca^{2+} ions may participate in the primary process of CO_2 reception.

Changes in Ca^{2+} conductance in the chemoreceptor cell by chemical stimuli have been reported in the channel catfish (Restrepo *et al.*, 1990), where amino acid stimuli triggered an influx of Ca^{2+} into the receptor cells via inositol-1,4,5-trisphosphate activated channels. This Ca^{2+} influx is proposed to be one of the mechanisms of the olfactory transduction. The influx of Ca^{2+} ions through non-selective cation channels has been reported to be regulated by stimulants (Nakamura and Gold, 1987).

It is unlikely that a change in Ca^{2+} permeability in the receptor cell of the temporal organ will result in the generation of a hyperpolarizing receptor potential, because to increase the concentration of extracellular Ca^{2+} would lead to an increase in Ca^{2+} influx, and influx of cation must result in the depolarization of the receptor cell. However, in the present study, Ca^{2+} ions decreased the impulse frequency of the receptor cell. It is more likely that Ca^{2+} may be involved in or regulate intracellular signal transmission in the chemo-electric transduction as is proposed to occur in other olfactory systems (Pace *et al.*, 1985; Sklar *et al.*, 1986; Nakamura and Gold, 1987; Matthews, 1991).

Many questions remain to be answered by more elaborate experiments, but the involvement of the sodium ion in the generation of hyperpolarizing receptor potential, and some indirect effects of calcium ions are proposed to be involved in the primary process of the carbon dioxide reception in the temporal organ of *T. hilgendorfi*.

ACKNOWLEDGMENTS

The authors wish to express their thanks to Ms S. Emsley (Halifax, NS) for revision of the English. This study was supported in part by a Grant-in-Aid for Scientific Research from the Ministry of Education, Science, Sports and Culture, Japan (No. 08454270) to Y. T.

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(Received April 2, 1997 / Accepted June 24, 1998)