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ELECTROPHYSIOLOGICAL STUDIES ON THE TEMPORAL ORGAN OF THE JAPANESE HOUSE CENTIPEDE, *THEREUONEMA HILGENDORFI*

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SUMMARY

The response of the temporal organ (organ of Tömösváry) has been studied electrophysiologically in the Japanese house centipede (*Thereuonema hilgendorfi*: Chilopoda).

1. Receptor cells of the temporal organ show spontaneous discharges (15–40 impulses s^{-1}) which are depressed in a phasic–tonic manner by carbon dioxide stimuli. Perfusion of the presumed receptor site with a carbon dioxide-containing solution also changes the impulse frequency.

2. The impulse frequency of a receptor cell decreases linearly with a logarithmic increase in carbon dioxide concentration ranging from 0·001 % to 0·1 % in the phasic state and from 0·05 % to 5·0 % in the tonic state. These results suggest that the temporal organ of the house centipede functions as a carbon dioxide receptor, though it responds to other stimuli as described below.

3. Changing the pH of a carbon dioxide-containing solution changes the impulse frequency, probably by changing the molar fraction of CO_2 molecules in the lymph surrounding the receptor site. pH also directly influences the impulse frequency of the receptor cell, but the effect is smaller than that of CO_2 molecules.

4. Receptor cells respond to humidity changes, but the response is smaller than that to carbon dioxide. When receptor cells are stimulated by moist air (100 % relative humidity, RH) after adaptation to dry air (0 % RH), the impulse frequency increases by a factor of $1·29 \pm 0·37$ (mean ± S.D. measured for the first second, $N = 29$), and $1·16 \pm 0·28$ (measured after adaptation to moist air, $N = 29$).

5. Air-borne chemicals also affect the impulse frequency of a receptor cell; it is increased by amines and decreased by fatty acids and aldehydes. Effects of these chemicals are discussed in relation to changing the pH of lymph bathing the receptor site.

INTRODUCTION

Some soil arthropods such as the Chilopoda, Diplopoda, Pauropoda, Symphyla and Apterygota possess a pair of sensory organs on the head behind the insertion of

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Key words: temporal organ, house centipede, carbon dioxide receptor.

the antenna. This organ is referred to as a 'temporal organ' (see Haupt, 1979). There have been several ultrastructural accounts of the temporal organs. Based upon these morphological data, the temporal organs are thought to function in several ways: as chemo- or hygroreceptors (Collembola, Karuhize, 1971; Symphyla, Haupt, 1971) or as chemo-, hygro- and/or thermoreceptors (Protura, Haupt, 1972; Pauropoda, Haupt, 1973; Collembola, Altner & Thies, 1976). Behavioural experiments have suggested that the temporal organ of *Lithobius* (organ of Tömösváry) functions as a hygroreceptor (Tichy, 1972, 1973). Electrophysiological studies which would be important in determining the adequate stimulus of the sensory organ are lacking.

In the present study, the temporal organ of the Japanese house centipede *Thereuonema hilgendorfi* (Chilopoda) has been studied electrophysiologically. The main aim of the present study is to advance our understanding of the temporal organ by identifying its adequate stimulus and physiological properties.

MATERIALS AND METHODS

Materials

Adult Japanese house centipedes, *Thereuonema hilgendorfi*, of either sex were used throughout this study. Animals were collected in the field around Kyushu University, Fukuoka, Japan.

Preparation and recordings

After immobilization by cooling with ice, the animal was fixed to an acrylic platform. Spike activities were recorded extracellularly from a receptor cell by impaling an active electrode about 50 μm deep into the temporal organ from the side of the domed cuticle. The active electrode was a sharpened tungsten wire insulated with lacquer (tip diameter was about 1·0 μm). Glass-coated tungsten electrodes were also used for recording from the perfused temporal organ. Stable, single unit recordings could be made for over 3 h by using these electrodes.

Carbon dioxide stimulation in air

Air of a desired carbon dioxide concentration was prepared as follows (Fig. 1). During a series of carbon dioxide stimuli, we used dry deodorized air in order to minimize the effects of humidity and air-borne chemicals. Air was dried through concentrated sulphuric acid solution and then decarboxylated through potassium hydroxide pellets. After passing through active carbon, the air flow was divided into two parts by a T-tube. One part was used for a carbon dioxide-free conditioning stream. Another part was used for stepwise dilutions of pure carbon dioxide supplied from a tank. The stimulus concentration of carbon dioxide was changed by manipulating six valves, and determined by air flux using five flow meters (Fig. 1). The head of the animal was always exposed to the carbon dioxide-free conditioning stream (4·5 l min^{-1}), and stimulation was performed by adding the second stream (0·5 l min^{-1}) containing a known concentration of carbon dioxide to the conditioning

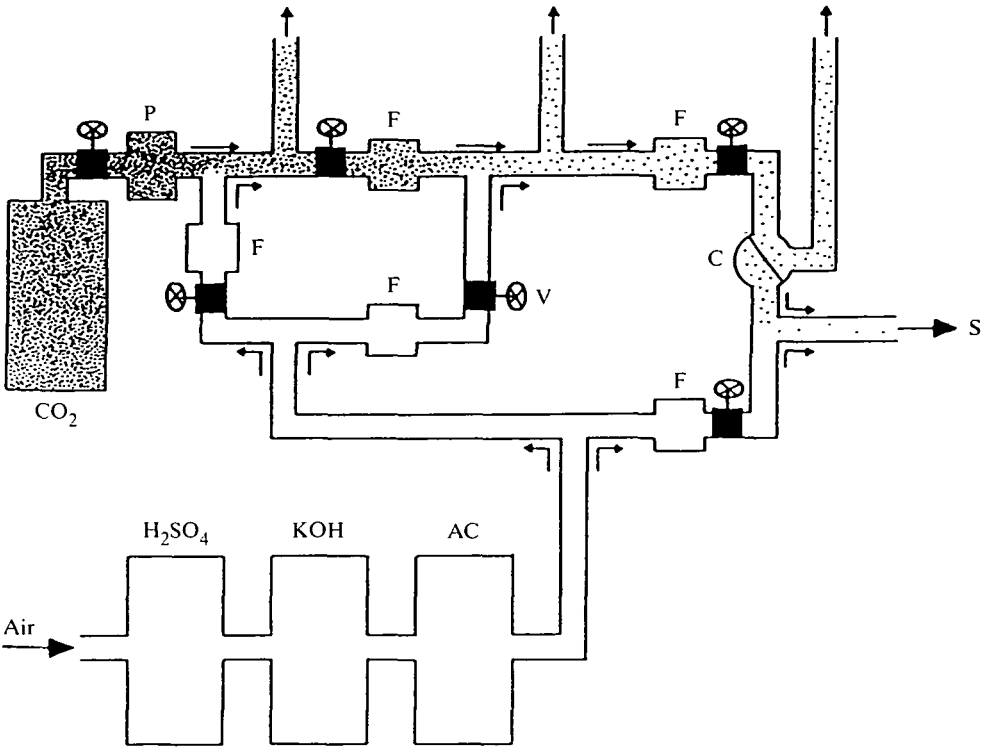

Fig. 1. A schematic diagram of the stimulation arrangement. AC, active carbon; C, flow-way changer; F, flowmeter; P, pressure gauge; S, stimulus flow; V, valve. The three upper exits are air escapes. For explanation, see text.

stream. A flow-direction changer was operated manually. The outlet of the changer (8 mm in diameter) was set about 5–10 mm from the head, and the flow rate of the air stream was about 150–165 cm s^{-1}. This rate had no effect on the spontaneous activity of the receptor cells. Stimulus concentrations of carbon dioxide between 0·05 % and 10 % were prepared by using this apparatus. Stimulus duration was 120 s at intervals of 180 s.

Disposable polyethylene syringes were used to observe the phasic responses to lower concentrations (less than 0·05 %) of carbon dioxide and to abrupt changes of carbon dioxide concentration. The syringe was filled with carbon dioxide of a known concentration, which was injected into the stimulus stream through a vinyl tube (1·0 mm in diameter). The syringe was driven for 2 s by a constant speed syringe pump.

Perfusion of the receptor site

The domed cuticle directly covering the temporal organ was partially removed to allow the perfusate to spread over the presumed receptor site. The head of an intact animal was fixed in a chamber (1·0 ml in volume), and perfused at a rate of 4·0 ml min^{-1}. Carbon dioxide solutions of varying concentrations were prepared in

the manner of Yoshii, Kashiwayanagi, Kurihara & Kobatake (1980). To examine the effect of pH, we used 25 mmol l^{-1} phosphate buffer (pH 5–8), 25 mmol l^{-1} Tris-HCl buffer (pH 7·2–9·0) and distilled water whose pH was adjusted with 0·1 mol l^{-1} HCl and 0·1 mol l^{-1} NaOH. Perfusion experiments were carried out at 25 °C.

Humidity, temperature and chemical stimulation

Humidity stimulation was carried out as described by Yokohari & Tateda (1976), but the flow rate of the air stream was modified to 100 cm s^{-1}. Heat emission from a tungsten lamp was used as a source of temperature stimulation.

An examination was made of the effects of 10 air-borne chemicals, listed in Table 1, upon impulse frequencies of receptor cells. Each chemical was diluted to 1 % (volume/volume) in paraffin oil. A syringe containing a piece of filter paper immersed in each stimulant solution was used for stimulation. The outlet of the syringe was about 2·0 mm in diameter, and positioned 10 mm from the temporal organ. The flow rate was about 6·4 m s^{-1}. Stimulation began 2 min after filling a syringe with air, for periods of 2 s at 2-min intervals. To examine responses to odours of prey animals and house centipedes themselves, pieces of filter paper were used on which faeces of German cockroaches or house centipedes had been absorbed.

RESULTS

Structure

The temporal organ of the house centipede is a paired structure located on the head between the base of the antenna and the pseudo-compound eye (Fig. 2). The organ appears externally as a small protuberance with a central opening (Fig. 3): like a volcano with a deep crater on its summit. The basal diameter of the protuberance is about 40 μm, and the opening of the pit is about 5 μm in diameter. There are several receptor cells together with about 100 supporting cells under the protuberance. The

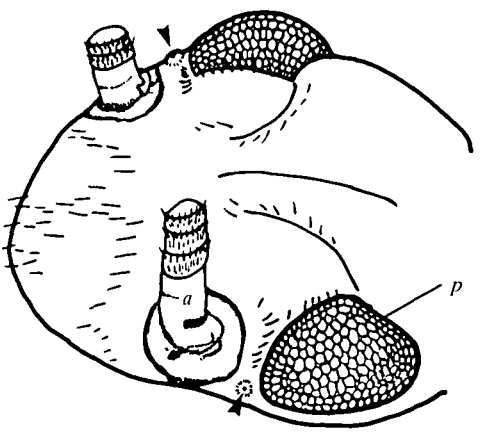

Fig. 2. A schematic drawing of the head of a Japanese house centipede. Arrowheads, temporal organ; *a*, antenna, distal part of which is omitted; *p*, pseudo-compound eye.

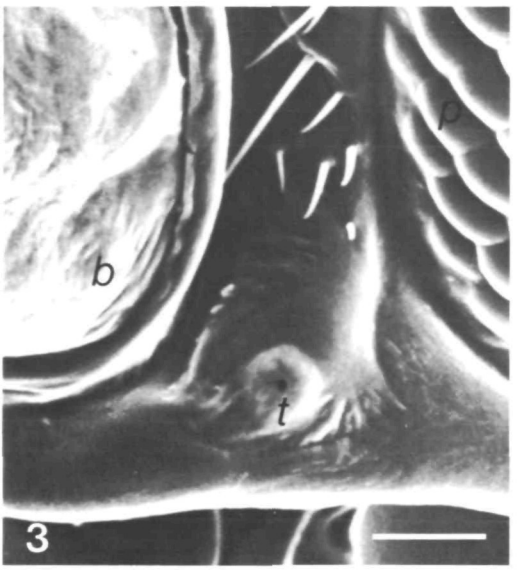

Fig. 3. A scanning electron micrograph of the temporal organ (*t*). *b*, base of an antenna. *p*, pseudo-compound eye. Scale bar, 50 μm.

internal morphology of the temporal organ will be reported elsewhere (K. Yamana & Y. Toh, in preparation).

Electrophysiological responses of the temporal organ

Responses to carbon dioxide in air

Time course of the response. Single unit recordings were obtained from about 100 receptor cells of the temporal organ. They showed spontaneous discharges of 15–40 impulses s^{-1} (Fig. 4) which were depressed by carbon dioxide application: the time course was phasic–tonic (Fig. 5). The receptor cell in Fig. 5, spontaneously discharging at about 16 impulses s^{-1} in carbon dioxide-free air, was completely inhibited for the first 20 s of 1·0% carbon dioxide application. In the subsequent 70 s the impulse frequency slowly increased to a steady discharge level (about 5 impulses s^{-1}) during continued stimulation. Switching the 1·0% carbon dioxide to carbon dioxide-free air resulted in an abrupt increase in impulse frequency (off response: about 35 impulses s^{-1}) beyond the pre-stimulus level, followed by a gradual recovery to the pre-stimulus level (in about 90 s for this specimen). It usually took 1–3 min for a receptor cell to recover its full responsiveness after the cessation of stimulation. When a non-insulated electrode was inserted into the temporal organ, several units could be recorded simultaneously. In such conditions, frequencies of all units decreased when carbon dioxide concentration was raised and increased when carbon dioxide concentration decreased. All receptor cells examined in the present study responded to carbon dioxide with a pattern similar to that shown in Fig. 5.

Relationship between carbon dioxide concentration and response. The receptor was stimulated by air of various carbon dioxide concentrations following adaptation

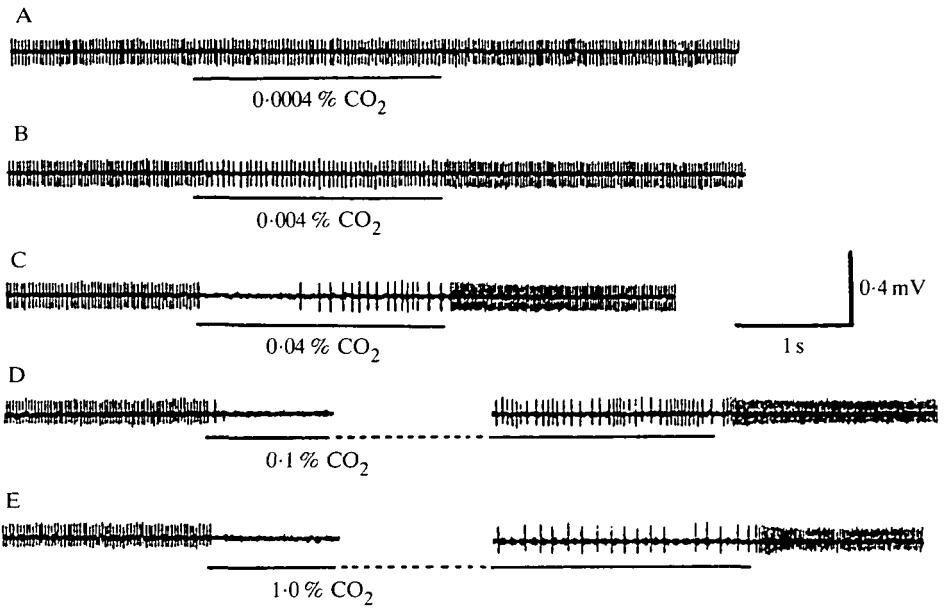

Fig. 4. Electrical activities recorded from a temporal organ. During adaptation to carbon dioxide-free air, a receptor cell shows spontaneous discharges, which are depressed by carbon dioxide stimuli. (A–C) Stimulation for 2 s; (D,E) stimulation for 120 s. The bar under the record indicates stimulation.

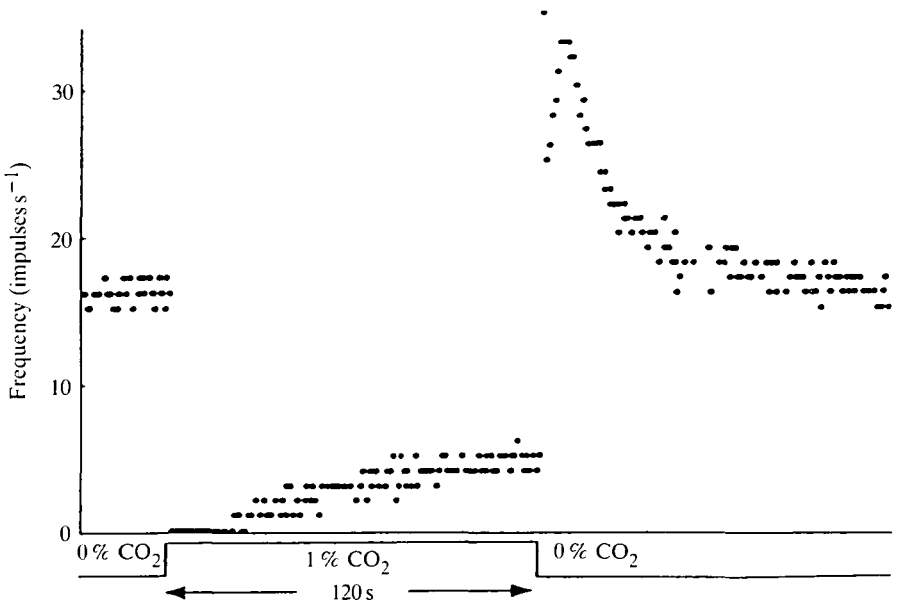

Fig. 5. Time course of impulse frequency. A receptor cell responds to a 1 % carbon dioxide stimulus in a phasic–tonic manner. Each point indicates the impulse frequency measured every second. It takes about 90 s to reach a steady discharge level. The cessation of carbon dioxide stimulation causes an off response, whose impulse frequency is increased beyond the pre-stimulus level and reduced gradually to the pre-stimulus level.

to carbon dioxide-free air. The impulse frequency decreased linearly with the logarithm of carbon dioxide concentration from 0·001 % to 0·1 % in the phasic state and from 0·05 % to 5·0 % in the tonic state (Fig. 6). The magnitude of the off response also depended upon the stimulus concentration (Fig. 7). Stimulation by air containing carbon dioxide after adaptation to carbon dioxide-free air is unlikely in natural environments. Therefore, responses to changes of carbon dioxide concentration were examined after the animal had been fully adapted to given concentrations of carbon dioxide. It was found that slight increases and decreases of carbon dioxide concentration from a given adapting level resulted in large changes in the impulse frequency (Fig. 8). For instance, steady-state impulse discharges (18 impulses s^{-1}) in 0·1 % carbon dioxide were rapidly decreased to 50 % (9 impulses s^{-1}) in 0·13 % carbon dioxide in Fig. 8. On average, the impulse frequency in 0·13 % carbon dioxide, after adaptation to 0·1 %, decreased to 60 ± 9 % ($N = 9$).

Perfusion experiments

Responses to carbon dioxide solutions. To examine whether receptor cells of the temporal organ may be stimulated by carbon dioxide which is dissolved in the surrounding receptor lymph, changes of impulse frequency were recorded while perfusing carbon dioxide solution over the receptor site. The receptor cells were first

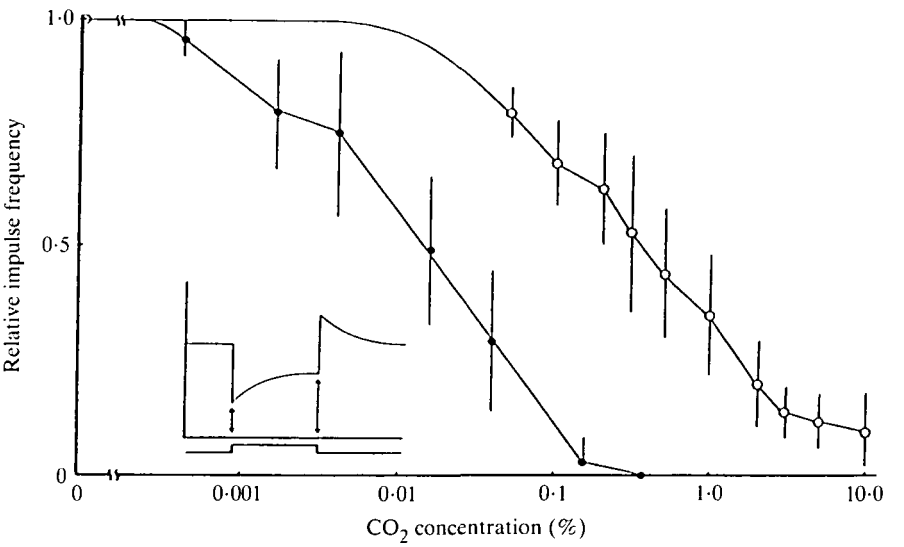

Fig. 6. Relationships between relative impulse frequencies and the logarithmic carbon dioxide concentration. A receptor cell was stimulated after adaptation to carbon dioxide-free air. Closed circles represent average impulse frequencies during the phasic state (measured for the first second after the stimulus onset, $N = 13$). Open circles represent average impulse frequencies in the tonic state (measured from 118–120 s after the stimulus onset, $N = 23$). The impulse frequency in response to carbon dioxide-free air (pre-stimulus level) is taken as 1·0. Inset: a schematic time course of the response, two vertical markers indicating amplitudes of phasic and tonic responses measured here.

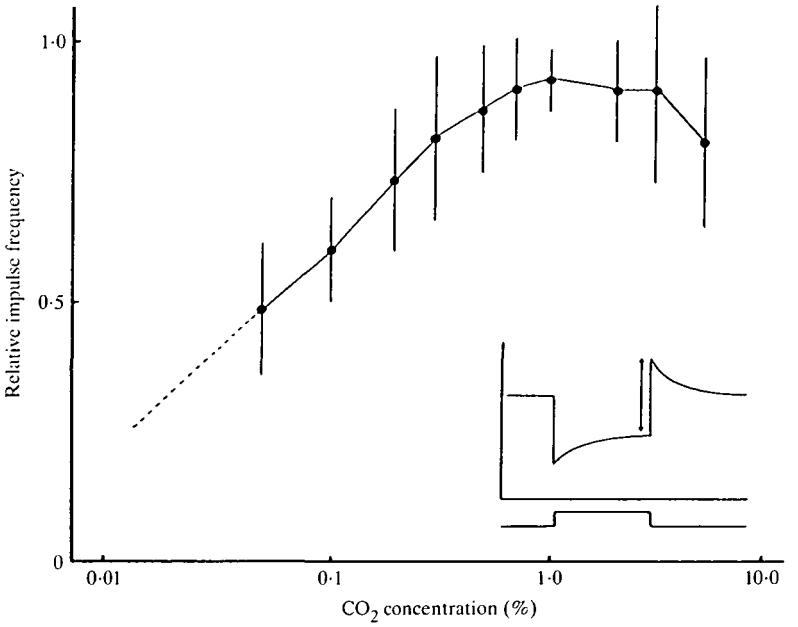

Fig. 7. Relative amplitudes of the off responses plotted against the logarithmic concentration of carbon dioxide. Differences in the impulse frequencies between the adaptation level to a given concentration and the first second after cessation of stimulation were measured. Each point indicates an average of 18 experiments. The maximum difference is taken as 1·0. Inset: a vertical marker indicates difference in the impulse frequencies measured here.

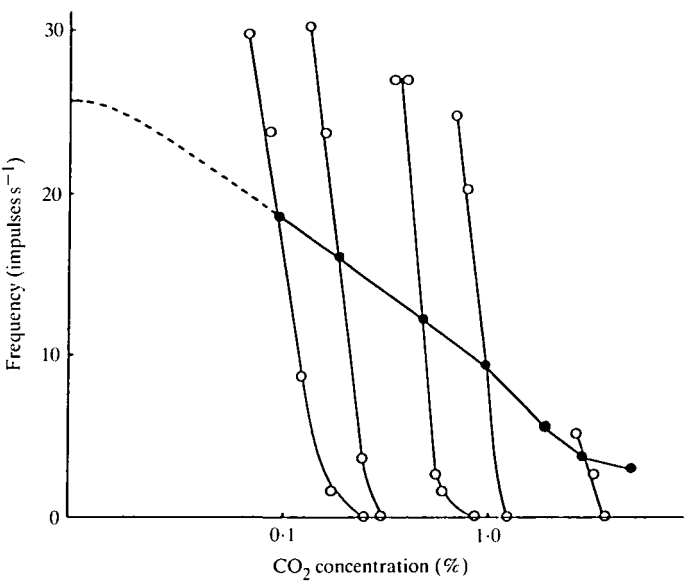

Fig. 8. Semi-logarithmic plots of typical phasic responses. Steady-state activities to a given concentration of carbon dioxide (filled circles) were abruptly changed by slight changes of the carbon dioxide concentration (open circles). Open circles are impulse frequencies for the first second after changing the carbon dioxide concentration.

adapted to a perfusate of carbon dioxide-free distilled water before replacing the perfusate with carbon dioxide-containing distilled water. The impulse frequency was unchanged for the first 15 s after this replacement, and then decreased gradually (Fig. 9). The time course of the response to carbon dioxide solution differed from the response to gaseous carbon dioxide, the phasic state being gradual with a smaller peak depression.

As carbon dioxide concentration was increased, impulse frequencies in both phasic and tonic states decreased (Fig. 10A). The dynamic range of the response in the tonic state was as wide as that obtained to gaseous carbon dioxide stimuli.

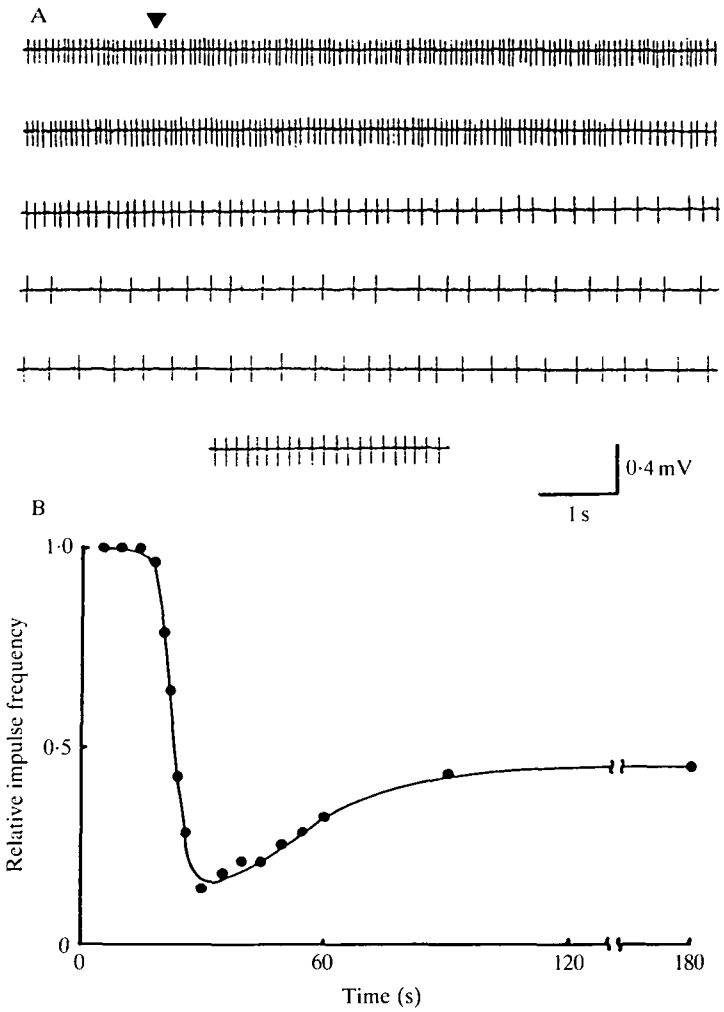

Fig. 9. Recordings from a perfused temporal organ. (A) Upper five traces are continuous recordings. A carbon dioxide solution in equilibrium with air containing 2% carbon dioxide was superfused at the triangle following adaptation to distilled water. The lowest trace is a recording of the last 3 s of the perfusion. (B) The time course of the response shown in Fig. 9A. The impulse frequency in response to distilled water (pre-stimulus level) is taken as 1·0.

Effects of pH. Dissolving carbon dioxide in an aqueous solution is accompanied by a change of pH. To examine the effect of a lowered pH on the response, the receptor site of the temporal organ was perfused with 25 mmol l^{-1} phosphate buffer solutions of varying pH after adaptation to phosphate buffer of pH 7·5. When the pH was lowered, the impulse frequency in the phasic state decreased, but the depression was smaller than that caused by carbon dioxide solution (Fig. 10B). A carbon dioxide solution in equilibrium with air containing 1·0% carbon dioxide has a pH of about 5·0, and it was found to depress the impulse frequency to 10% of the pre-stimulus level in the phasic state (open circles in Fig. 10A) whereas the buffer solution at

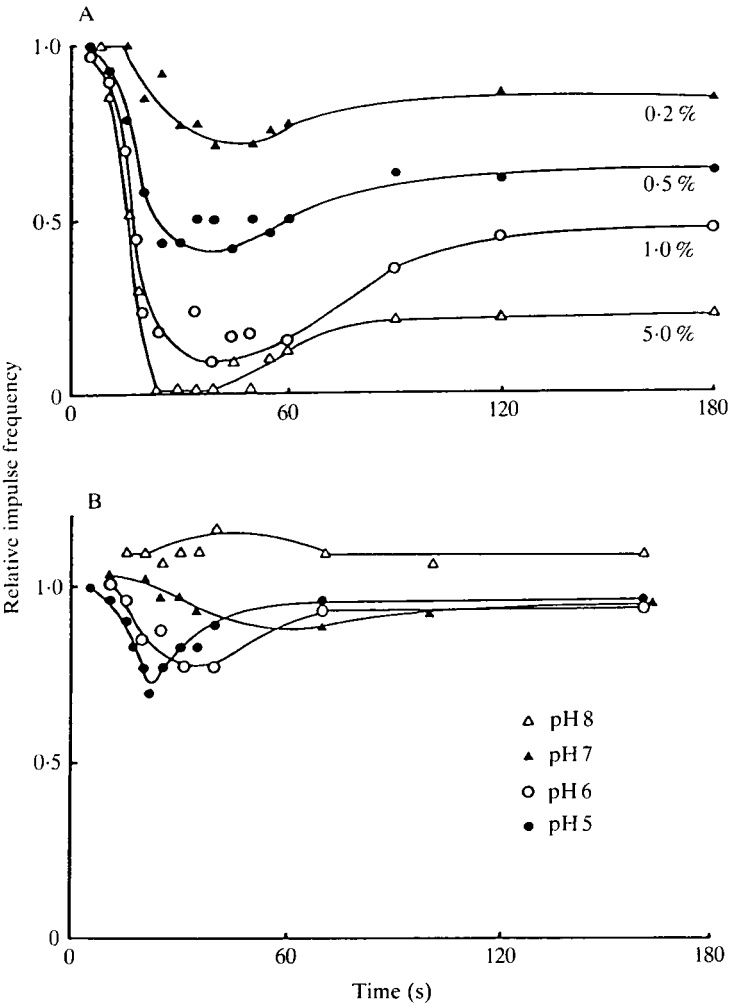

Fig. 10. (A) Time courses of responses to carbon dioxide solutions of different concentrations. Each solution was superfused after adaptation to distilled water. The impulse frequency in response to distilled water (carbon dioxide-free solution) is taken as 1·0. (B) Responses to 25 mmol l^{-1} phosphate buffer solutions at different pH values. Each solution was superfused after adaptation to phosphate buffer of pH 7·5. The impulse frequency at pH 7·5 is taken as 1·0.

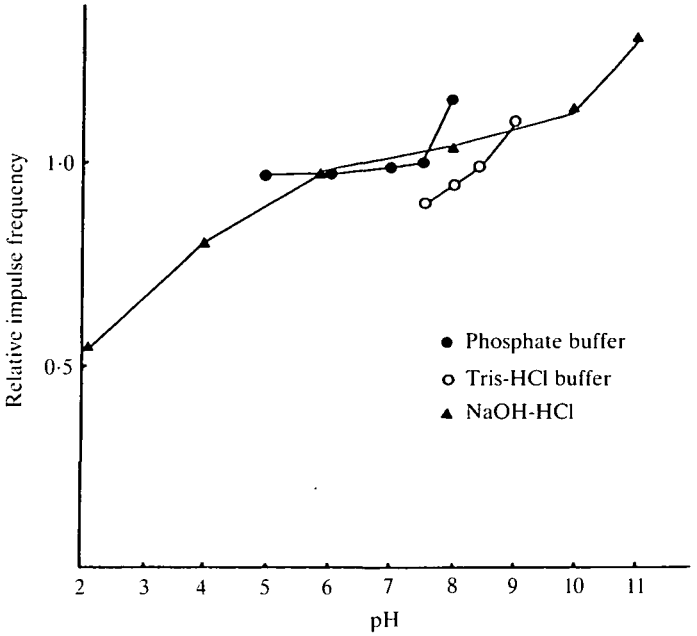

Fig. 11. pH dependency of the impulse frequency in the steady state (180 s after the beginning of stimulation). Each point indicates the relative impulse frequency, the impulse frequency in response to phosphate buffer of pH 7·5 being taken as 1·0.

pH 5·0 depressed the impulse frequency to 70% of the pre-stimulus level (filled circles in Fig. 10B). Moreover, in the tonic state (180 s after the stimulus onset), impulse frequencies to buffer solutions of pH 5, 6 and 7 were near the pre-stimulus level (Fig. 10B). When the pH was raised with phosphate buffer solution to 8·0, the impulse frequency increased to about 115% of the pre-stimulus level, and was maintained during continued stimulation.

To observe the effects of pH over a wider range, we examined the pH-response relationships with applications of 25 mmol l^{-1} phosphate buffer, 25 mmol l^{-1} Tris-HCl buffer and distilled water whose pH was adjusted with 0·1 mol l^{-1} NaOH and 0·1 mol l^{-1} HCl (Fig. 11). A solution was perfused after adaptation to phosphate buffer of pH 7·5. In all three kinds of solutions, when the pH was raised there was an increase in impulse frequency, whereas when the pH was lowered, impulse frequencies decreased. However, the maximum response amplitude (reduction of impulse frequency), found at pH 2 was smaller than the response to 1·0% carbon dioxide either in air or in solution. Stimulation with a solution of pH 2 does not seem to be physiological.

Effect of ionization of carbon dioxide. When carbon dioxide is dissolved in solution, CO_2, H_2CO_3, HCO_3^- and CO_3^{2-} coexist in the solution (Fig. 12A). The degree of hydrolysis of the carbon dioxide is determined by the pH of the solution. Since concentration of H_2CO_3 is always less than 0·4% of that of CO_2 ($K = 2{\cdot}6 \times 10^{-3}$), $[H_2CO_3]$ is negligible. Molar fractions of CO_2, HCO_3^- and CO_3^{2-} depend upon the pH of the solution (Kitano *et al.* 1976) as shown in Fig. 12A. If

the solution becomes acidic, the molar fraction of CO_2 increases (broken line in Fig. 12A). For example, more than 95 % of the total carbon dioxide content in the solution exists in the form of CO_2 molecules at pH 5·0. Because of the properties of carbon dioxide in solution, the following experiment was carried out to determine which molecules or ions stimulate the receptor cell of the temporal organ.

Carbon dioxide solutions in equilibrium with air containing 2 % carbon dioxide were pH adjusted with phosphate buffer or NaOH, so as to stimulate the receptor cell with a solution of known composition. As pH was lowered over the range pH 5–10, thus altering the molar fraction of CO_2 in solution, there was a decrease in

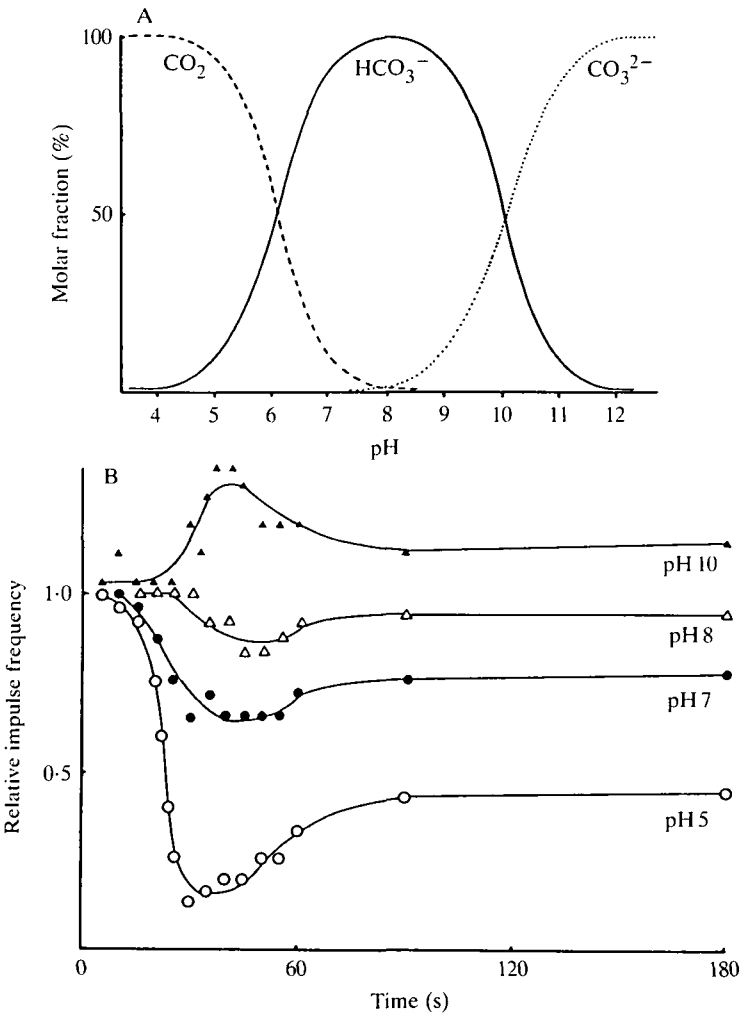

Fig. 12. (A) Molar fractions of CO_2 (including H_2CO_3), HCO_3^- and CO_3^{2-} as a function of pH (Kitano *et al.* 1976). (B) Responses to carbon dioxide solutions of various pH values. The pH of a solution in equilibrium with air containing 2 % carbon dioxide was adjusted with phosphate buffer and NaOH. When the molar fraction of CO_2 molecules was increased by lowering the pH, larger responses were obtained.

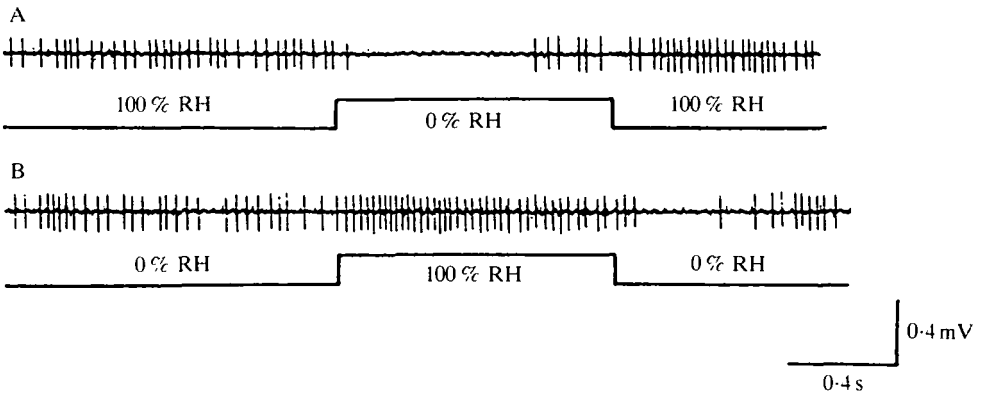

Fig. 13. (A) Response of a receptor cell to 0% relative humidity (RH) air after adaptation to 100% RH air. (B) Response to 100% RH air after adaptation to 0% RH air. The responses shown in A and B were obtained from the same cell, which showed the largest response to humidity stimulation of 29 cells tested.

impulse frequency (Fig. 12B). These results, together with those in Fig. 10A, suggest that the impulse frequency of a receptor cell decreases with increase in concentration of CO_2 molecules around the receptor site.

Responses to humidity, temperature and air-borne chemicals

The temporal organ was found to respond to changes in humidity, by stimulation with moist and dry air (100% and 0% RH, respectively). When the relative humidity was changed from 100% to 0%, the impulse frequency decreased (Fig. 13A). Conversely, when the humidity was changed from 0% to 100%, the impulse frequency increased (Fig. 13B). The recordings shown in Fig. 13A,B were obtained from the same cell. This cell showed the largest responses to humidity stimuli of 29 cells examined. On average, relative impulse frequency was increased by a factor of $1{\cdot}29 \pm 0{\cdot}37$ (mean $\pm$ S.D., $N = 29$) when the humidity was raised from 0% to 100% (measured for the first second after the beginning of stimulation). The impulse frequency during adaptation to 100% RH was also higher than that during adaptation to 0% RH by a factor of $1{\cdot}16 \pm 0{\cdot}28$ ($N = 29$).

In response to aldehydes and acids, a decrease in impulse frequency was observed, an increase was observed with amines. Alcohols, the odour of house centipedes and the odour of German cockroaches have little or no effects on the impulse frequencies (Table 1). For all these chemicals, the responses of the recorded cells had quite identical spectra.

Spontaneous activity was not altered by changing the temperature (not shown).

DISCUSSION

Adequate stimulus of the temporal organ of the Japanese house centipede

The temporal organ of arthropods is thought to have different functions in different animals; namely, hygroreception, thermoreception or olfaction (see

Table 1. *Spontaneous impulse frequencies, and impulse frequencies to 10 air-borne chemicals and two natural odours recorded from 14 receptor cells*

	Frequency (impulses s^{-1})													
Cell no.	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Spontaneous	21	26	17	16	23	21	20	20	22	15	21	18	20	20
Butyl alcohol	21	25	18	16	22	20	22	20	20	15	20	19	20	21
Hexyl alcohol	21	24	16	17	22	20	18	20	21	15	19	18	20	21
Butyl aldehyde	0	0	0	4	3	0	0	0	0	0	0	3	5	2
Caprylic aldehyde	0	0	4	2	0	0	0	0	0	0	0	0	3	0
Acetic acid	0	0	0	5	2	0	0	1	4	2	2	0	4	5
Butyric acid	0	0	0	0	0	0	0	0	0	0	0	0	0	1
Caproic acid	16	12	13	16	19	11	13	16	15	11	13	14	18	16
Butylamine	26	36	25	28	28	48	40	36	40	26	36	25	27	25
Amylamine	38	60	56	40	64	43	34	48	50	31	44	26	29	27
Hexylamine	31	40	36	32	33	32	30	44	40	30	29	24	26	25
Cockroach	20	24	16	16	22	23	22	21	22	16	20	17	20	20
House centipede	21	19	18	15	21	21	21	22	23	15	19	18	20	19

The impulse frequency is measured between 1 and 2 s after stimulus onset.

Introduction). In the present study, the temporal organ of the house centipede is shown to act primarily as a CO_2 receptor.

Humidity stimuli could result in changes in the impulse frequency of a receptor cell (Fig. 13). Impulse frequency was changed by a factor of $1{\cdot}29 \pm 0{\cdot}37$ in the phasic state, and $1{\cdot}16 \pm 0{\cdot}28$ in the steady state when the humidity was increased from 0% to 100%. These responses are less than those generally observed in insect hygroreceptors: a factor of 6 between 70% and 0% RH (locust; Waldow, 1970), a factor of 3 between 100% and 0% RH (cockroach; Yokohari & Tateda, 1976) and a factor of 2 between 90% and 10% RH (honey-bee; Yokohari, Tominaga & Tateda, 1982). The locust moisture receptor responds to a rise in relative humidity from 0% to 70% with a rise in impulse frequency from 10 to 175 impulses s^{-1} (measured for the first second, Waldow, 1970). Moreover, hygroreceptive sensilla of insects have a hygroscopic cap structure upon which sensory cilia with mechanoreceptive structures impinge (Yokohari, 1981, 1983), whereas the sensory cilia in the temporal organ of the house centipede do not possess such structures (K. Yamana & Y. Toh, in preparation). These physiological and morphological data suggest that the temporal organ of the house centipede may not be a functional hygroreceptor. Changes in the impulse frequency may be secondary effects due to transient changes in the ionic composition of the receptor lymph.

Receptor cells of the temporal organ also respond to some air-borne chemicals: stimulation with aldehydes and fatty acids decreases the activities of receptor cells, whereas stimulation with three amines increases them. This does not indicate that the temporal organ is an olfactory organ, however, for the following reasons. (1) In general, olfactory organs contain many types of receptor cells of different spectra. Outputs from such different cells are thought to be decoded in the central nervous system for olfactory discrimination (Boeckh, 1980). However, as shown in Table 1, the temporal organ appears to contain receptor cells with identical spectra. (2) Receptor cells of the temporal organ did not respond to two natural olfactory stimuli examined, the odours of the German cockroach and the house centipede. (3) The house centipede possesses a pair of long, well-developed antennae, which function as an olfactory organ. It is more likely that the observed effects of the air-borne stimuli tested here are indirect, influencing the pH and/or carbon dioxide concentration at the receptor site.

Since spontaneous activities were not affected by temperature changes, the temporal organ does not appear to be a thermoreceptor.

The steady-state activity of these receptor cells is reduced by an increase in carbon dioxide concentration between 0·05% and 5·0% (Fig. 6). In addition, there is a response to slight changes in carbon dioxide concentration (Fig. 8). Carbon dioxide concentration in air is about 0·03%. House centipedes are soil animals whose habitat usually contains a carbon dioxide concentration exceeding that of normal air by 10–100 times (Aoki, 1973). Thus it appears that the temporal organ of the house centipede functions as a carbon dioxide receptor. These receptors are unusual in that they show an inhibitory response to carbon dioxide, since those in other arthropods

show excitatory responses (Lacher, 1964; Kellogg, 1970; Stange & Diesendorf, 1973; Stange, 1974).

Mechanism of carbon dioxide reception

Electrophysiological responses of carbon dioxide receptors have been recorded in honey-bees (Lacher, 1964; Stange & Diesendorf, 1973; Stange, 1974), mosquitoes (Kellogg, 1970) and blowflies (Stange, 1975). Although multiple steps are proposed between carbon dioxide stimulation and spike generation in insect carbon dioxide receptors (Stange, 1974, 1975), Fig. 12 suggests that the amplitude of the response (reduction of impulse frequency) appears to depend primarily upon the concentration of CO_2 molecules at the receptor site. Similar results have been reported for fish chemoreceptors which respond specifically to CO_2 molecules (Yoshii *et al.* 1980).

The effects of pH upon impulse frequency (Figs 10B, 11) suggest that changes of pH around the possible receptor site may be involved in the carbon dioxide receptor process. This is unlikely, however, because changes in impulse frequency caused by changing pH are smaller than those caused by carbon dioxide either in air or in solution. Moreover, there are few differences in the steady-state activities in perfusates of pH 5, 6 and 7 (Figs 10B, 11). Several possible explanations may be proposed for these observed effects of pH. First, the activity of the receptor cell could be directly influenced by pH; this might or might not be related to carbon dioxide reception. Similar effects of pH upon chemoreceptors have been reported in insect contact chemoreceptors (Shiraishi & Morita, 1969). Second, even in perfusion experiments, a small amount of haemolymph containing HCO_3^- and CO_3^{2-} still remains around the receptor site, and decreasing pH could result in an increase in CO_2 molecules, which in turn could decrease the impulse frequency of the receptor cells. The latter explanation cannot account for the increasing impulse frequency above pH 8–11 (Fig. 11) or the higher impulse frequency at pH 10 than that at pH 8 (Fig. 12B). Because the concentration of CO_2 is near zero at a pH greater than 8, the observed pH effect may be intrinsic to the receptor cell. Acidic chemicals (fatty acids) and basic chemicals (amines) may somehow affect this intrinsic nature of the receptor cell. Aldehydes are also easily oxidized in solution and become acids. The delayed responses observed in the perfusion experiments (Figs 9, 10, 12B) may be related to a greater diffusion time of CO_2 molecules over the receptor site.

Possible function

It has been demonstrated that carbon dioxide is the most important stimulus involved in host-finding in mosquitoes (see Gillies, 1980), and that carbon dioxide receptors in mosquitoes can detect an increase in carbon dioxide concentration from 0·04% to 0·05% (Kellogg, 1970). It is also known in several insects that the rhythmic movements of the respiratory system are regulated by the external concentration of carbon dioxide (e.g. Wigglesworth, 1935). Thus, two roles can be proposed for the temporal organ; it may serve when an animal seeks its prey, and/or it may be involved in respiratory regulation. Receptor cells of the temporal organ are

sufficiently sensitive to carbon dioxide to play either role. Further studies are necessary to understand the function of the temporal organ.

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