

INTRACELLULAR RECORDING FROM RECEPTOR CELLS OF THE TEMPORAL ORGAN OF THE JAPANESE HOUSE CENTIPEDE, *THEREUONEMA HILGENDORFI*: RECEPTOR POTENTIAL AND CONDUCTANCE CHANGES

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SUMMARY

The primary process of carbon dioxide reception in the temporal organ of the Japanese house centipede *Thereuonema hilgendorfi* has been studied by means of intracellular recording.

1. During adaptation to air containing no carbon dioxide, the membrane potential of receptor cells in the temporal organ ranged from -25 to -59 mV with an average of -39 mV ($N = 21$). Input resistance ranged from 10 to 46 M Ω with an average of 22.6 M Ω ($N = 16$).

2. Receptor cells responded to a carbon dioxide stimulus with a graded hyperpolarizing receptor potential, which reduced impulse frequency. The decrease in steady-state frequency was proportional to the amplitude of the receptor potential.

3. During the receptor potential, the input resistance increased. Injection of hyperpolarizing current resulted in an increase in amplitude of receptor potential, while depolarizing current caused a decrease. These results suggest that the receptor potential is caused by a decrease in membrane permeability to ions whose equilibrium potential is more positive than the membrane potential adapted to 0% carbon dioxide.

INTRODUCTION

It has been shown in chemoreceptors of several vertebrates that membrane conductance of receptor cells changes during chemical stimulation, and this must result in the generation of receptor potentials (Ozeki, 1971; Akaike, Noma & Sato, 1976; Suzuki, 1977; Sato & Beidler, 1982; Trotier & MacLeod, 1983; Tonosaki & Funakoshi, 1984). For arthropod chemoreceptors many studies have been made with extracellular electrodes, and elaborate models for the primary process of chemoreception have been proposed (e.g. Morita, 1972). Recently, membrane properties of

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lobster olfactory cells have been revealed by patch-clamp techniques (Anderson & Ache, 1985), but conventional microelectrode recordings remain necessary for an analysis of the primary process of arthropod chemoreception.

The temporal organ of the Japanese house centipede *Thereuonema hilgendorfi* is sensitive to carbon dioxide stimulation (Yamana, Toh & Tateda, 1986). Morphological studies show that this sense organ contains about 10 receptor cells 15 μm in diameter (K. Yamana & Y. Toh, unpublished data). In the present study, receptor potential, impulse frequency and membrane conductance during exposure to carbon dioxide have been recorded using intracellular microelectrodes. Results are discussed by analogy with electrical parameters of vertebrate photoreceptor cells, which have been well documented (e.g. Tomita, 1965).

MATERIALS AND METHODS

Adult house centipedes, *Thereuonema hilgendorfi*, were collected around Kyushu University.

An animal was immobilized by cooling with ice, then fixed on an acrylic platform. Responses of the receptor cell of the temporal organ were recorded using conventional microelectrode techniques. Glass microelectrodes were filled with 2 mol l⁻¹ potassium acetate and had resistances of 100–150 M Ω ; the tip diameters were less than 0.1 μm . A small hole was opened on the surface cuticle near the temporal organ with a sharpened tungsten wire, and a microelectrode was inserted into the temporal organ through the hole. An indifferent electrode of tungsten wire, 0.1 mm in diameter, was inserted into the base of the antenna. The electrical events were amplified with a d.c. amplifier, displayed on an oscilloscope and photographed.

Carbon dioxide stimuli, with concentrations ranging from 0.01 to 10%, were prepared by stepwise dilution of pure carbon dioxide taken from a container. CO₂-free air was prepared by passing air through potassium hydroxide pellets. The stimulation system was as described by Yamana *et al.* (1986).

The membrane resistance of a receptor cell was determined by applying a train of hyperpolarizing constant-current pulses (100 ms in duration) through the recording microelectrode connected to a Wheatstone bridge and by measuring the voltage drop across the cell membrane.

RESULTS

The data presented here were obtained from 21 receptor cells in which recordings were made continuously for over 10 min.

Membrane potential and spontaneous impulse frequency

During adaptation to CO₂-free air, membrane potentials of the 21 receptor cells ranged from -25 to -59 mV with a mean value of -39 ± 9 mV (means \pm S.D.,

Fig. 1A). Spontaneous impulses were recorded, with frequencies ranging from 9 to 35 impulses s^{-1} , and they appeared to be negatively correlated to the resting membrane potential (Fig. 1B).

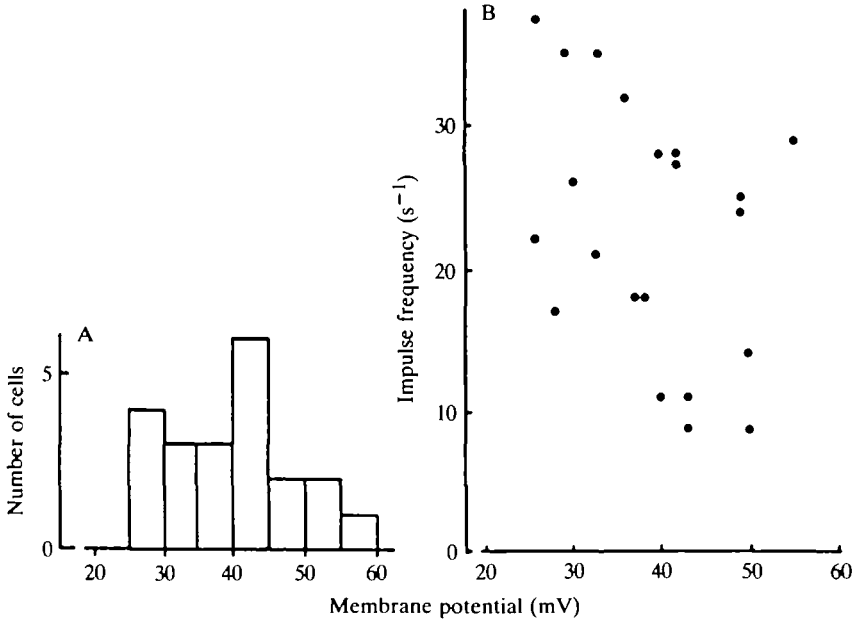


Fig. 1. (A) A distribution histogram of resting membrane potentials (adapted to CO_2 -free air) of receptor cells in the temporal organ. (B) Relationship between resting membrane potentials and spontaneous impulse frequencies.

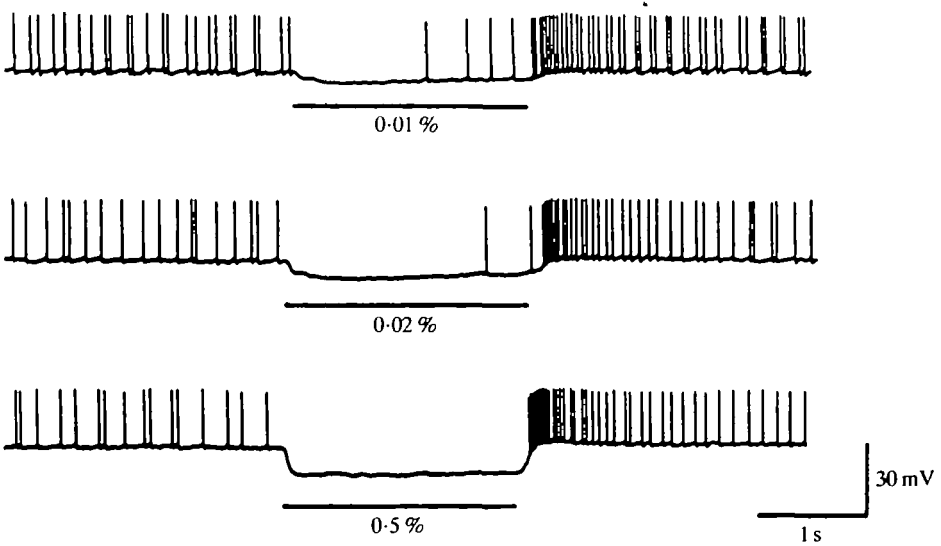


Fig. 2. Intracellular recordings from the receptor cell of the temporal organ during carbon dioxide stimulation, concentrations being shown under the stimulus markers. The resting potential was -40 mV.

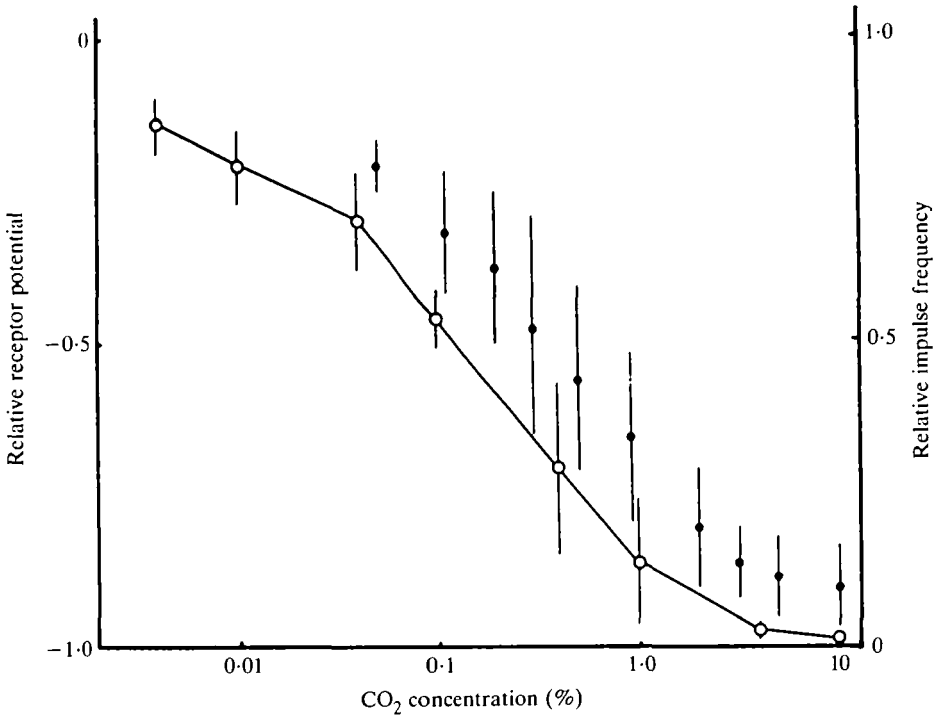


Fig. 3. Effect of carbon dioxide concentrations upon relative amplitude of receptor potential (○) and upon relative frequency of impulses (●). Mean values from seven preparations. Vertical bars are standard deviations. For receptor potentials the response to CO₂-free air and that to 10% carbon dioxide are taken as 0 and -1, respectively. For impulse frequencies the response to CO₂-free air is taken as 1.0. Measurements were made in the steady state, 1–2 s after stimulation had begun.

Receptor potential in response to carbon dioxide stimulation

Carbon dioxide stimulation produced a graded hyperpolarization in the receptor cell, and depressed the spontaneous discharge of impulses (Fig. 2). Fig. 2 shows an 11 mV hyperpolarizing receptor potential, given in response to 0.5% carbon dioxide, which completely inhibited impulse activity for 2 s. After cessation of stimulation, the membrane potential returned to the pre-stimulus level. When the stimulus concentration was high, the membrane potential depolarized beyond the pre-stimulus level (overshoot) by 2–3 mV for 0.2–0.3 s after the cessation of stimulation. Impulse frequency had a maximum just after the stimulus was cut off, during the return phase of the membrane potential, or during the overshoot. These observations about impulse activities are consistent with the results obtained from extracellular recordings (Yamana *et al.* 1986).

The effect of stimulus concentration upon relative receptor potential amplitude (open circles, Fig. 3) and upon relative impulse frequency (closed circles, Fig. 3; and also Yamana *et al.* 1986) showed linear relationships with the logarithm of CO₂ concentration in the range of 0.04 to 1.0%. This parallelism is due to a linear

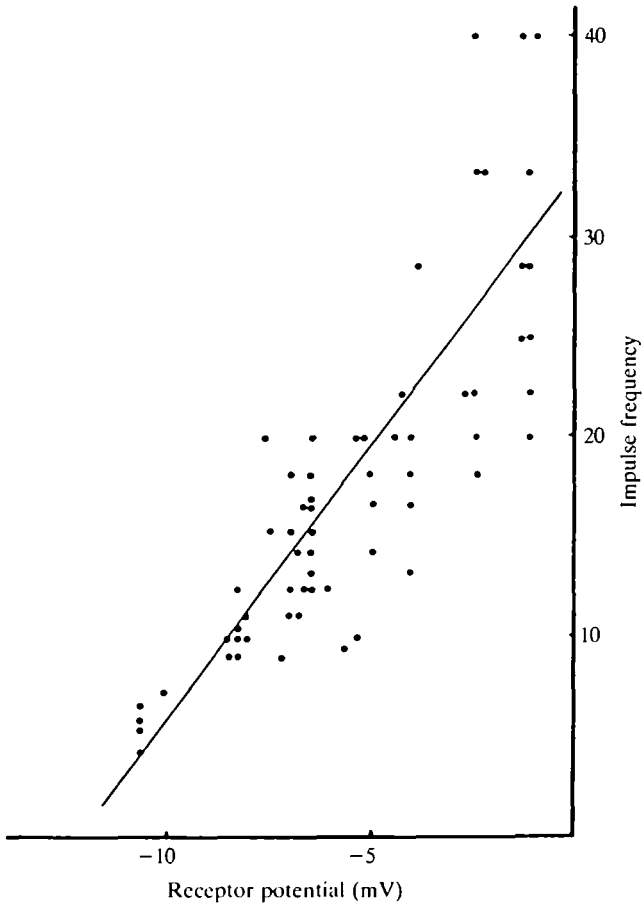


Fig. 4. Correlation between amplitude of receptor potentials and impulse frequency. The regression line is $y = 33.8 - 2.9x$ (where x is receptor potential and y is impulse frequency).

relationship between the amplitude of receptor potential and impulse frequency (Fig. 4).

Amplitudes of impulses were uniform and intervals between spontaneous impulses were regular in some preparations (Fig. 2), whereas in others two different-sized spikes occurred and spike intervals were less regular (Figs 6, 7).

Conductance changes during carbon dioxide stimulation

Changes of membrane potentials induced by injection of current were recorded to measure the membrane conductance. The current-voltage relationship in response to hyperpolarizing currents was almost linear (Fig. 5). Input resistance of the receptor cell was determined from the slope. For depolarizing current, the current-voltage relationship was rectified and became non-linear because of the production of bursts of impulses. A similar current-voltage relationship has been

reported in the patch-clamp preparation of lobster olfactory cells (Anderson & Ache, 1985).

During carbon dioxide stimulation, mean input resistance was $23 \pm 11 \text{ M}\Omega$ (mean \pm s.d., $N = 16$). In the receptor cell shown in Fig. 6A,B, the input resistance was about $38 \text{ M}\Omega$ before carbon dioxide stimulation, and increased to $50 \text{ M}\Omega$ during 3% carbon dioxide stimulation. In another receptor cell (Fig. 6C), the input resistance during carbon dioxide stimulation became about 1.6 times as large as the pre-stimulus level: the input resistance was changed from 33.5 to $56.3 \text{ M}\Omega$ in this preparation. Increase in membrane resistance during carbon dioxide stimulation was observed in all seven receptor cells examined.

During current injection, responses to carbon dioxide were changed. Fig. 7 shows that depolarizing currents decreased the amplitude of the receptor potential in response to 3% carbon dioxide, whereas hyperpolarizing currents increased it. As shown in Fig. 7B, the reversal point of the receptor potential was at a more positive potential than the membrane potential adapted to 0% carbon dioxide. Similar effects of current injections upon receptor potentials were observed in five preparations.

DISCUSSION

In the present study, the receptor cells of the temporal organ of the house centipede *T. hilgendorfi* exhibited a hyperpolarizing receptor potential, which was accompanied by a reduction in impulse frequency, in response to carbon dioxide stimulation. This appears to be the first measurement using conventional microelectrode techniques of receptor potentials in arthropod chemoreceptor cells. Membrane

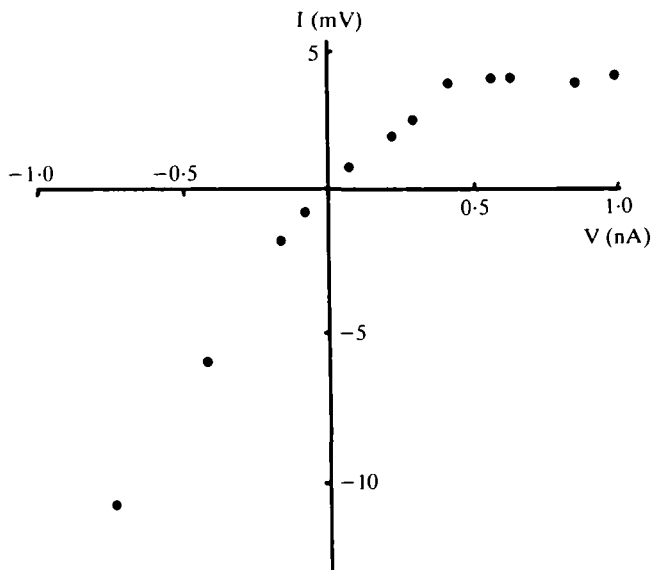


Fig. 5. A current-voltage curve of the receptor cell. The membrane potential changed linearly in response to hyperpolarizing currents, but not to depolarizing currents.

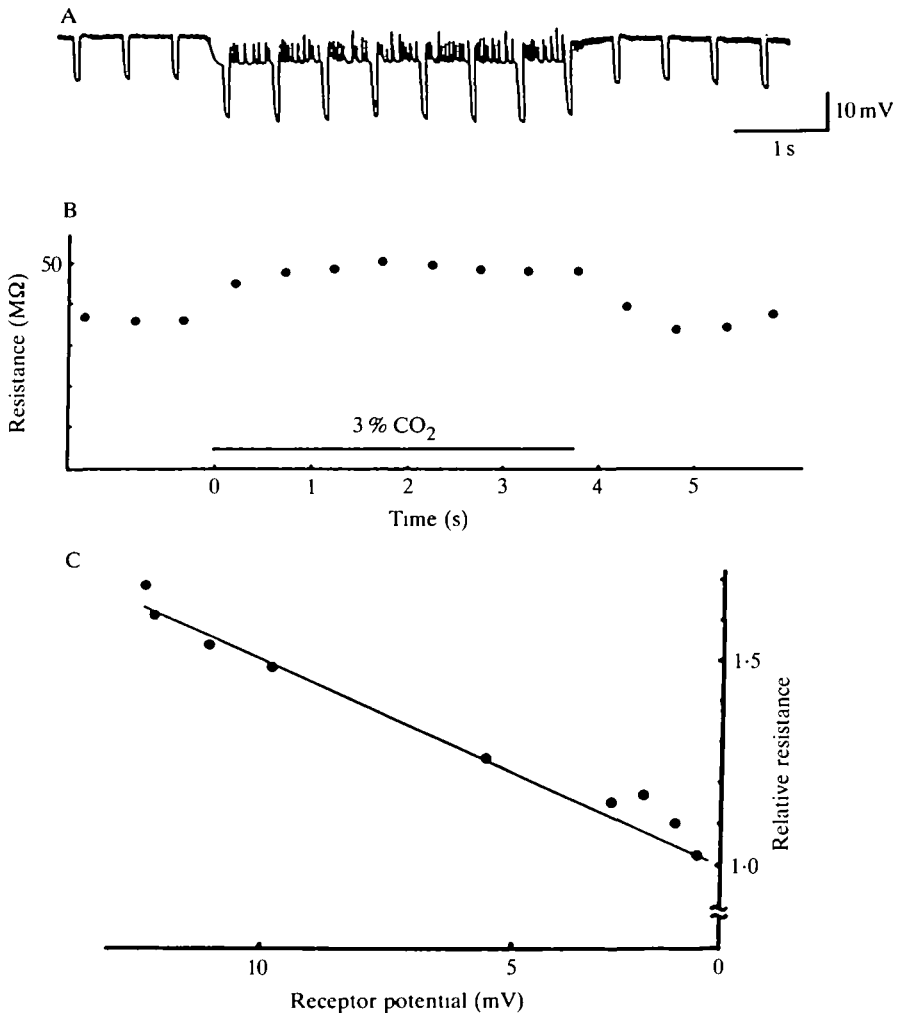


Fig. 6. Membrane resistance during the resting state and during 3% carbon dioxide stimulation measured by brief extrinsic hyperpolarizing currents. (A) Recording; (B) a time course of resistance change obtained from the record in A. (C) Relationship between relative resistance and receptor potentials. The membrane resistance in the resting state is taken as 1.0. The resting potential was -38 mV.

properties of lobster olfactory cells have been investigated by patch-clamp techniques (Anderson & Ache, 1985).

As reported in lobster olfactory cells (Anderson & Ache, 1985) and in vertebrate chemoreceptors as well as in other sensory cells (see references given in the Introduction), the receptor potentials were accompanied by changes in membrane conductance. The reduction in membrane conductance during the receptor potential suggests that the receptor membrane is less permeable to some ions during carbon dioxide stimulation than it is in CO₂-free air. Thus, an equilibrium or reversal potential for ions related to carbon dioxide reception should be near the resting level

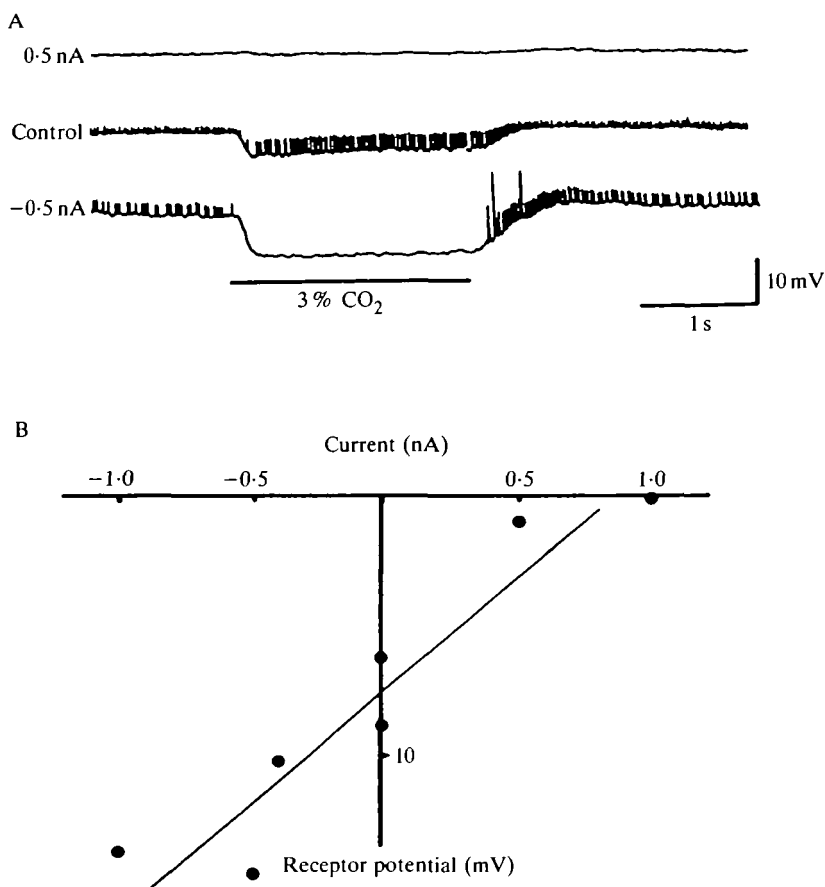


Fig. 7. Effect of current injection upon amplitude of receptor potential during 3% carbon dioxide. (A) Responses to injection of 0.5 nA and -0.5 nA and 0 nA (control). (B) Relationship between applied current and amplitude of receptor potential. The resting potential was -38 mV.

or at a more positive level. This assumption agrees with the effects of current injection upon the amplitude of the receptor potential: a hyperpolarizing current enhanced the receptor potential, whereas a depolarizing one attenuated it.

A hyperpolarizing receptor potential accompanied by a decrease in membrane conductance is also found in the photoreceptor cells of the vertebrate retina upon stimulation (Tomita, 1965; Toyoda, Nosaki & Tomita, 1969).

The receptor potential and impulse frequency were linearly related to the logarithm of carbon dioxide concentration. Similar data for impulse frequency were obtained by Yamana *et al.* (1986). The receptor potential and impulse frequency are thus roughly linearly related, conforming to the suggestion of Morita (1972) based upon extracellular recordings.

The spike intervals in Figs 6 and 7 are less regular than the extracellularly recorded spike intervals shown in fig. 2 of Yamana *et al.* (1986). This irregularity may reflect some modification of the firing pattern of the receptor cell by penetration with

microelectrodes in the present study. But any such damage does not seem to impair the reception process, because both transient conductance changes and potential changes are elicited by carbon dioxide.

The different amplitudes of the spikes shown in Fig. 7 have two alternative interpretations. First, the different amplitudes may result from damage to the receptor cell. Second, the different amplitudes may originate in different spike initiation sites. Small spikes in Fig. 7 are assumed to originate from electrotonically spread axonal spikes. Large spikes may be somatodendritic spikes triggered by summation of receptor current and an action current of an axonal spike, because they occur at the end of stimulation when the cell returns to the depolarized state. Large spikes have a notch on their rising phase, which is common for somatic spikes of nerve cells (Takeda & Kennedy, 1964).

In summary, it is proposed for the primary process of carbon dioxide reception in the temporal organ of the house centipede *T. hilgendorfi* that carbon dioxide reduces the membrane conductance of the receptor cell, which results in the generation of a hyperpolarizing receptor potential. The ionic basis of the receptor potential is currently under investigation.

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