Receptor Current Fluctuation Analysis in the Labellar Sugar Receptor of the Fleshfly

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ABSTRACT Fluctuations in the receptor current of the labellar sugar receptor of the fleshfly were analyzed. The receptor current was recorded extracellularly as a drop in potential between the tip and the base of the taste sensillum. After treatment with tetrodotoxin, the taste cells completely lost their impulses but retained their receptor currents, thus facilitating analysis of the receptor current without disturbance by impulses. The current fluctuation increased markedly when the sensillum was stimulated with effective sugars: maltose, sucrose, and fructose. The fluctuation increased in parallel with development of the receptor current, which indicates that it occurs as soon as the sugar reaches the apex of the sensory process. Analysis of fluctuations by computation of autocorrelation functions (ACFs) or power spectra (PS) revealed that: (*a*) the variance (mean square) of fluctuation vs. sugar concentration curve reached a maximum, in contrast to the monotonic increase shown by the receptor current; (*b*) the ACF was approximated by an exponential term, and its time constant differed according to the sugars used and their concentrations. The time constants for fructose and maltose decreased with increases in sugar concentration. At the concentrations of sugars evoking the same magnitude of receptor current, the time constant for fructose was the largest and that for maltose was the smallest. It was strongly suggested that transduction ion channels are present at the tip region of the sensory process of the sugar receptor cell and are operated directly by sugars.

INTRODUCTION

The molecular transduction mechanisms of the taste sensation in higher animals are, for the most part, unresolved. In vertebrates, taste cells contained in a taste bud detect chemical stimuli at the microvilli, which extend out from the epithelium into the saliva or other external solutions containing the stimulants. The ionic concentrations of the stimulant solutions are in some cases very low and thus it is uncertain whether stimulants activate ion channels at the receptive membrane of microvilli (for review, see Beidler, 1971; Sato, 1980; Kurihara et al., 1986).

Among the invertebrates, the fly labellar taste sensillum has been studied most

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intensively (Morita, 1972; Dethier, 1976; Morita and Shiraishi, 1985). In contrast with vertebrate taste cells, the labellar taste cells of the fly are primary sensory cells that extend sensory processes (sensory cilia) into the sensory hair and send axons to the central nervous system (CNS).

The labellar sensory hair (sensillum trichodeum) is surrounded by a cuticle layer and has a small pore (~0.1 μm diam) at its tip for receiving stimuli. It has two compartments, the inner and outer lumina. The inner lumen usually contains four sensory processes for sugar, salt, water, and an unspecified form of stimulus (Morita and Shiraishi, 1985). The outer lumen is filled with receptor lymph, which has an ionic composition similar to that of hemolymph (Kijima H., Y. Okada, S. Oiki, and K. Nagata, manuscript in preparation). It is therefore possible in this case that the opening of a channel by a stimulant may cause a flow of receptor current into the sensory process. Morita and Yamashita (1959) measured the receptor current (generator potential) extracellularly as a voltage drop of ~1–2 mV between the tip and the base of the sensillum. On the basis of this measurement, it was inferred that a receptor current of the order of 10–100 pA flows through the outer lumen toward the tip when the sugar receptor is stimulated by sucrose solution (cf. Fujishiro et al., 1984). Because the outer lumen is electrically coupled to the inner lumen at the distal part of the sensory hair, the receptor current was inferred to flow into the tip of the sensory process, depolarizing the sensory cell to evoke impulses, which are then sent to the CNS via the axon.

It is now well established that conductance of the postsynaptic membrane in the cholinergic vertebrate neuromuscular junction is controlled by ion channels operated directly by their agonist (Katz and Miledi, 1972; Neher and Sakmann, 1976*b*). Morita (1969) developed a theory that quantitatively described the response of the labellar sugar receptor, assuming ion channels opened by binding with sugar molecules. This was assumed partly because the receptor current begins to flow without any appreciable delay after contact of the stimulant solution with the chemosensory hair tip even at low temperatures (Uehara and Morita, 1972). However, it remains to be determined whether such ion channels actually exist for taste reception.

Here we analyze the fluctuation in receptor current recorded extracellularly, and the results strongly suggest the existence of transduction ion channels operated by sugars in the receptor membrane of the fly labellar taste cell. Some properties of these transduction ion channels are also estimated.

MATERIALS AND METHODS

Electrical Measurements

The fleshfly, *Boettcherisca peregrina*, was used 2–8 d after emergence. The head was separated from the body, the brain was destroyed with forceps, and the head was mounted on the top of a glass capillary (tip diameter, ~200 μm) filled with Waterhouse's solution (Buck, 1953) containing 112 mM NaCl, 2 mM KCl, 1.2 mM $NaHCO_3$, 0.7 mM NaH_2PO_4, and 1.1 mM $CaCl_2$. A piece of grounded Ag/AgCl wire was then immersed in this solution within the capillary.

Two methods of sidewall recording (Morita and Yamashita, 1959) were employed to record the receptor current and its fluctuation, the tip-sidewall method and the two-

sidewall method. In both methods, the receptor current was measured as a drop in potential between a distal and a proximal point in the largest type of labellar taste sensillum.

In the tip-sidewall method shown in Fig. 1*A*, a glass microelectrode filled with Waterhouse's solution with a resistance of 50–100 MΩ was inserted into the outer lumen at a point about one-fourth to one-fifth of the total length from the base of the hair, and was connected to one end of a differential amplifier through the Ag/AgCl wire in the glass microelectrode. The other end of the amplifier was connected to a glass capillary, 80–100 μm diam at its apex, filled with a solution containing 0.4 M choline chloride, 0.1 M NaCl (solution A), and a stimulant sugar. The tip of the sensory hair was covered with this glass capillary using an electromagnetically driven apparatus for both stimulation and recording. Solution A scarcely stimulated the salt and the water receptors, and did not inhibit (slightly augmented) the response of the sugar receptor in comparison with the 10 mM NaCl solution.

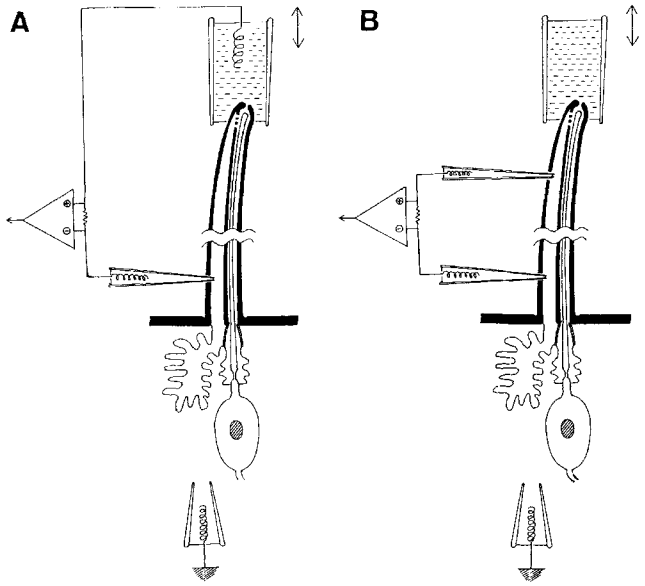

FIGURE 1. Schemes of two extracellular recording methods adopted to record the receptor current of a labellar taste receptor and its fluctuation: the tip-sidewall method (*A*) and the two-sidewall method (*B*).

In the two-sidewall method (Fig. 1*B*), in addition to the use of one sidewall electrode as above, another glass microelectrode was inserted into the outer lumen at a point about 1/10–1/15 of the total length of the hair from the tip. The sugar receptor was stimulated by covering the tip of the sensory hair with a glass capillary containing a sugar in solution A, which was completely isolated from the earth.

With the two-sidewall method, receptor currents were recorded directly coupled (DC). With the tip-sidewall method, fluctuations of the receptor currents were mostly recorded alternately coupled (AC) at high gain, because the total impedance of the measurement system was relatively low (~90–100 MΩ), and the frequency characteristics were better. However, the two-sidewall method was adopted for observation of both the receptor current and its fluctuations simultaneously, even though the total impedance was rather

high (>250 MΩ) and the amount of background thermal noise was greater (cf. legend to Fig. 2). A four-pole Butterworth low-pass filter (24 dB/octave, cutoff frequency, f_c = 2.1 kHz) and a high-pass RC filter (f_c = 1.7 Hz) was applied for AC recordings. DC records were sometimes high-cut by a four-pole Bessel filter at 120 Hz.

All experiments were done at 21–23°C at a relative humidity of 80–90%. Air at 100% relative humidity was blown gently upon the head and the tip of the stimulating capillary during experiments to avoid changes in the concentration of the stimulating solution.

Only sensory hairs that evoked a vigorous sugar impulse train for 50 mM sucrose were used for experiments. After impulse responses to 25–100 mM sugar had been recorded, 100 μM tetrodotoxin (TTX; Sankyo Co., Tokyo, Japan) was applied to the tip of the sensory hair for 4–5 min. Impulses remained blocked for ~2 h after this TTX treatment. After being allowed to rest for ~10 min after TTX treatment, the hair tip was stimulated with various concentrations of a sugar for ~5 s and the electrical signals were fed into an FM data recorder. Intervals between the stimulations were 4–5 min. Usually 24–36 stimulations were performed on one sensillum during a period of 3.5 h, and TTX treatment was repeated every 1.5 h.

Analysis of Fluctuation

Recorded fluctuation signals were A/D converted at a sampling frequency of 5 kHz and both the power spectrum (PS) and the autocorrelation function (or, more precisely, the autocovariance function [ACF]) were calculated for each of the successive 2,048 data points (~0.4 s data length) at the Kyushu University Computer Center. The ACF of the receptor current, $C_I(t)$, is defined as

$$C_I(t) = \langle [I(T) - \langle I \rangle] \cdot [I(T + t) - \langle I \rangle] \rangle, \quad (1)$$

where $I(T)$ and $I(T + t)$ are receptor current at times T and $T + t$ respectively, $\langle I \rangle$ is the average of the receptor current, and the angle brackets indicate the average taken over T. Thus, $C_I(t)$ is the covariance between two values of signal separated by a time interval t (Neher and Stevens, 1977). It was calculated directly or by the modified method of Bendat and Piersol (1971) using an FFT algorithm. The PS was calculated using a cosine FFT subroutine. 10 PS or ACF values were then averaged (total data length: 4.098 s, starting 0.2 s after the onset of stimulation).

Averaged ACF or PS values of control fluctuations obtained for an equal weight per volume of a nonstimulating sugar, α-methyl mannoside (αMM), were subtracted from the ACF or PS of the experiments using stimulating sugars, respectively, and the ACF or PS of the net fluctuation increase was obtained.

The net ACF for a correlation time between 0 and 80 ms was curve-fitted by an exponential term and a background straight line using the nonlinear least-squares method. A background line was used because a small portion of very slowly varying phase often appeared in the ACF (cf. Results and Discussion) and fitting to this by a slow exponential term was not as good as fitting by a line. The fitted exponential term was converted to a Lorentzian term of the PS, and the frequency characteristics of the record and propriety of the curve fittings were ascertained in the PS vs. frequency plot (cf. Fig. 3, *A* and *B*).

RESULTS

Increase in Fluctuation in Parallel with Receptor Current Development

When the labellar sugar receptor was stimulated by 0.1 M maltose dissolved in solution A, both the receptor current and the impulse train from the sugar receptor were observed by the two-sidewall method, as shown in Fig. 2*A* (top

trace). The receptor current was observed as a negative shift at the distal electrode (downward shift in Fig. 2*A*) with reference to the proximal one (Fig. 1*B*), just after the onset of stimulation. On the other hand, no sugar receptor

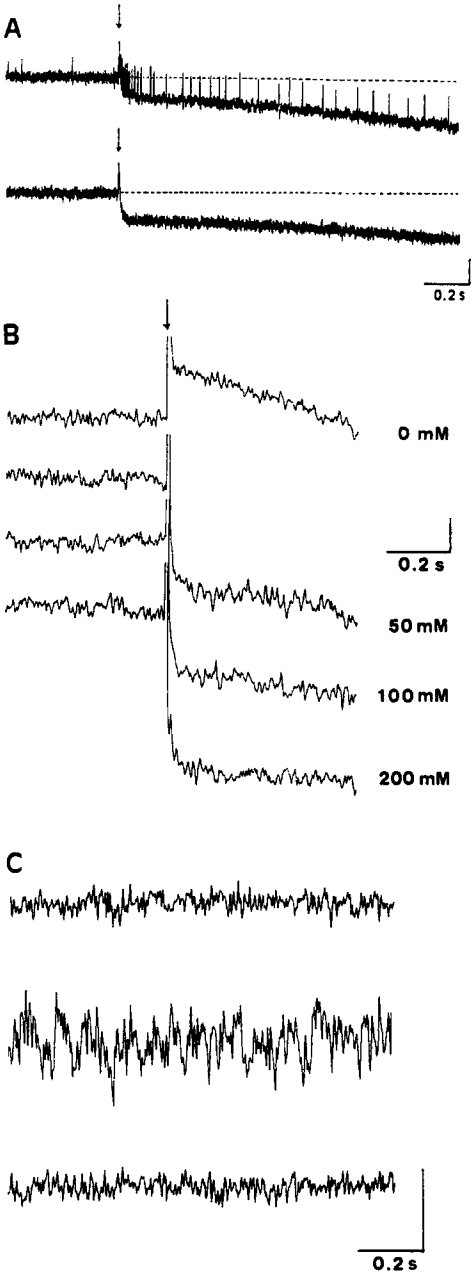

FIGURE 2. Examples of current records obtained by the two extracellular recording methods. (*A*) Low-amplification DC record of the receptor current and impulses obtained by the two-sidewall method, when stimulated with 0.1 M maltose before (upper trace) and after (lower trace) TTX treatment without filters. The upward spike-like change at the onset of stimulation (arrow) is an artifact caused by electrostatic discharge from the stimulating capillary. The increase in fluctuation after the onset of sugar stimulation cannot be distinguished, because of the high level of background thermal noise. The very slow downward change during stimulation might be the diffusion potential caused by chloride ion in solution A diffusing into the outer lumen up to the point of insertion of the distal sidewall electrode (cf. Morita and Shiraishi, 1985). Vertical bar, 1 mV. (*B*) DC records obtained for the same preparation as in *A* but magnified and low-pass filtered with a four-pole Bessel filter (f_c = 120 Hz), upon stimulation with various concentrations of maltose (shown beside the traces). Both the receptor current amplitude and its fluctuation can be recognized. Vertical bar, 0.2 mV. (*C*) Examples of high-amplification AC records of fluctuation after TTX treatment obtained by the tip-sidewall method with a high-pass RC filter (f_c = 1.7 Hz) and a four-pole Butterworth low-pass filter (f_c = 60 Hz). The records were started 0.4 s after the onset of stimulation. Upper trace: control fluctuation with solution A only. Middle trace: fluctuation induced by 25 mM sucrose in solution A. Lower trace: fluctuation induced by 47 mM αMM in solution A. Recorded using a sugar receptor different from that for *A* and *B*. Scale bar, 0.2 mV.

impulses and few water and salt receptor impulses appeared in the control records upon stimulation with solution A containing an equal weight per volume of the nonstimulating sugar αMM (not shown in the figure).

After treatment of the sensory hair with 100 μM TTX for 5 min, the impulses were completely blocked, but the receptor current was not affected at all, as clearly shown in Fig. 2*A* (bottom trace). When DC records after TTX treatment were amplified and passed through a low-pass filter to reduce the background thermal noise, we were able to observe fluctuation increases upon stimulation by various concentrations of maltose (Fig. 2*B*). Control stimulation by solution A only (top record) immediately changed the potential at the distal electrode by 0.3 mV positive with respect to the proximal electrode, and the fluctuation decreased a little, as ascertained by calculation of the variance. This might have been caused by closing of the channels that had opened spontaneously without stimulation. However, this aspect is not further analyzed here.

In the two-sidewall recordings such as those shown in Fig. 2*B*, the receptor current of the sugar receptor cell for a sugar is proportional to the net potential change between the two electrodes, being subtracted from the above control potential change for solution A only.

Upon stimulation by maltose, the fluctuation increased as soon as the receptor current developed. Such a rapid increase of fluctuation in parallel with the receptor current clearly indicates that it occurs as soon as the stimulant sugar reaches the receptor membrane at the tip of the sensory process. It is known that several seconds are required for sugar molecules to reach the base of the chemosensory hair (Ninomiya et al., 1986), so that the fluctuation observed here must have occurred well before the arrival of the sugar at the base. As seen in Fig. 2*B*, the degree of fluctuation in these measurements was greatest at 50 mM and decreased at higher sugar concentrations, while the receptor current increased. This was confirmed by calculation of variances, as described later. An increase in fluctuation was also observed by applying other stimulant sugars, sucrose and fructose. The increase was more clearly visible in the tip-sidewall recording (Fig. 2*C*), in which the degree of background thermal noise was much smaller because of the low total impedance of the system. The fluctuation was markedly increased by 25 mM sucrose. However, nonstimulating sugars, αMM (47 mM [equal, weight per volume, to 25 mM sucrose]; Fig. 2*C*, bottom) or lactose (25 mM) barely increased the fluctuation. Thus, it was concluded that the increase in fluctuation was caused by stimulation of the tip region of the sensory process of the sugar receptor cell.

Analysis of Fluctuation

The nonstimulating sugar αMM (equal weight per volume concentration) was used as a control, since high concentrations of sugar increased the resistance of the stimulating solution and increased the level of thermal noise, as shown later. Examples of the ACF and PS of the control fluctuation obtained for 93 mM αMM and of the net fluctuation (ACF or PS minus the control ACF or PS) for 50 mM maltose are shown in Fig. 3. The control fluctuation consisted mostly of white noise, i.e., the thermal noise originating from the resistance of the whole

system, as judged by the very small values of ACF apparent at any correlation time (t) except the origin, and the nearly flat PS-frequency relationship up to ~1 kHz (Fig. 3*A*). In addition, a very slow decaying phase of ACF or a small increase of PS at lower frequencies was always observed, which may have been caused by a slow drift of the transepithelial potential of ~50–60 mV (Wieczorek, 1982; Kijima et al., manuscript in preparation).

Stimulation by 50 mM maltose markedly increased the fluctuation, and the ACF or PS of the net fluctuation was quite different from the white noise, the ACF (or PS) being approximated by an exponential (or a Lorentzian) term (Fig. 3*B*). This net increase of fluctuation is due to activation of the sugar receptor cell, because other sensory cells in the hair were activated little or activated to the same degree as in the control records obtained for αMM (cf. Fig. 8).

The features of the ACF (or PS) are similar to those of the typical results obtained so far in the analysis of agonist-induced current noise, which reflect the open-shut dynamics of ligand-gated ion channels (Katz and Miledi, 1972; Anderson and Stevens, 1973; Neher and Sakmann, 1976*a*).

The variance of fluctuation gradually decreased with time after the onset of stimulation by 50 mM maltose. The variance between 8 and 10 s after the onset was 62 ± 2% (n = 4) of that for the first 2 s (calculation started at 0.2 s after the onset of stimulation). No significant change in the time constant with time was observed.

Variation of ACF with Sugars and Their Concentrations

Fluctuations were analyzed as above for various concentrations of maltose, sucrose, and fructose. Examples of ACFs of the net increase in fluctuation for 25 mM sucrose and 50 mM fructose are shown in Fig. 4. As seen in Fig. 4*A*, it seemed that ACFs could often be more precisely fitted by two exponential terms, especially in the case of sucrose. However, detailed analysis of this aspect was left for later studies, and ACFs were approximated here by a single-exponential term and a background line (cf. Discussion). As seen in Figs. 3*B* and 4, the variance of fluctuation (the value of ACF at $t = 0$) and the time constant of ACF seemed to differ with different sugars. We therefore examined them systematically at various concentrations. A typical example obtained using one hair preparation for maltose stimulation is shown in Fig. 5. In this sample, three measurements at each concentration agreed fairly well (i.e., they were reproducible). In these experiments, the variance had a peak at 50 mM and decreased at higher concentrations. The time constant of the ACF gradually decreased with the concentration. Similar results were obtained from most of the reproducibly responding hair samples for three kinds of sugars, i.e., maltose (on seven samples), sucrose (on nine samples), and fructose (on seven samples), as shown below (cf. Figs. 6 and 7).

The Attainment of Maximum Variance vs. Sugar Concentration

The variance of the net fluctuation in most cases attained a maximum, as shown in Figs. 5 and 6, when the concentration of sugars was increased, although the peak concentration differed from one preparation to another. Among the nine

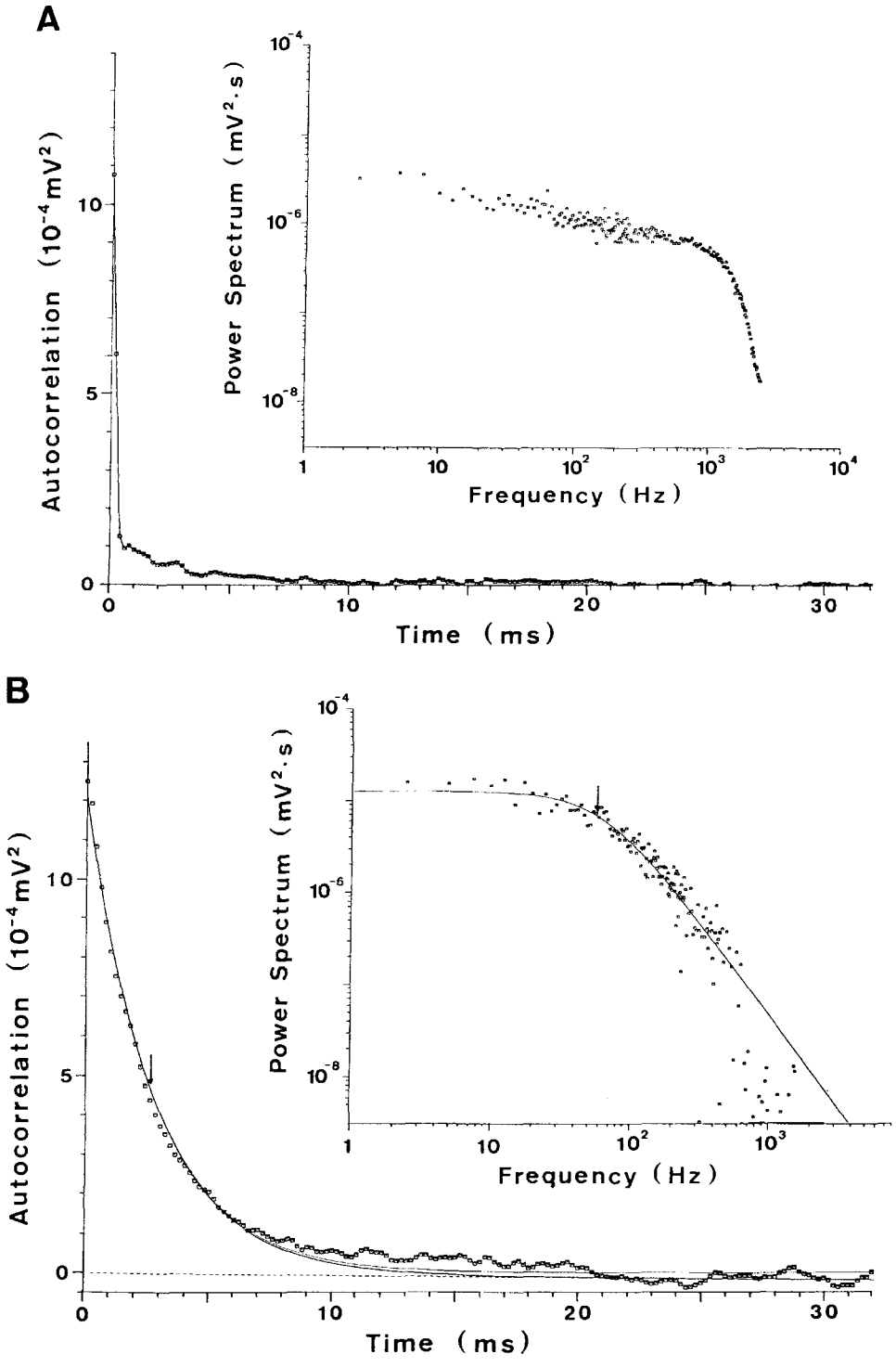
A
Autocorrelation (10^{-4} mV^2)
Power Spectrum (mV^2·s)
Frequency (Hz)
Time (ms)
B
Autocorrelation (10^{-4} mV^2)
Power Spectrum (mV^2·s)
Frequency (Hz)
Time (ms)

FIGURE 3

hair samples for which experiments were reproducible for sucrose, four had a maximum at 25 mM, four at 50 mM, and one at ≤12.5 mM. When maltose was applied, four of seven had a maximum at 50 mM, one at 100 mM, one at ≥200 mM, and one did not show any clear maximum. For fructose, five out of seven had a maximum at 50 mM, one had a maximum at ≤25 mM, and one did not show any clear peak. In total, 19 out of 23 hair samples showed maxima at lower concentrations than the highest.

Among these samples, only those having a maximum at 25 and 50 mM for sucrose, and at 50 mM for maltose and fructose, were averaged and are shown in Fig. 6 to indicate clearly the existence of a maximum. The decrease of variance at 200 mM compared with the peak value was statistically significant for the three kinds of sugars ($p < 0.01$ for sucrose and fructose; $p < 0.05$ for maltose).

The existence of a maximum in the variance of fluctuation is an important feature of the current that flows through ion channels: when the channel open probability is changed from zero to unity, the current fluctuation increases with the increase of current initially, but later it decreases because the fully open channels do not fluctuate between open and shut states (Neher and Stevens, 1977). The above observations are therefore consistent with the open-shut dynamics of ion channels operated by sugars (see also Discussion).

Concentration Dependence of the Time Constant

Fig. 7 shows the dependence on sugar concentration of the time constant of a single-exponential term fitted to the ACF for three sugars, obtained from the hair samples for which experiments were reproducible. The time constant for fructose was larger than that for sucrose and maltose over the whole range of concentration and decreased markedly with concentration. That for maltose was the smallest and also decreased gradually with concentration. It was significantly larger at 12.5 mM than at 50 mM ($p < 0.05$), at 100 mM ($p < 0.01$), and at 200 mM ($p < 0.002$) and also larger at 25 than at 200 mM ($p < 0.005$). However, the time constant for sucrose did not show any significant change except at 0.2 M, where the average time constant was a little smaller than at other concentrations (see Discussion). Different hair preparations showed no remarkable differences in the concentration dependence of the time constants for the three kinds of sugars used, in contrast to the peak concentration of variance.

All the experiments shown in Figs. 6 and 7 were done for one kind of sugar on a hair sample like that shown in Fig. 5. In order to check further the

FIGURE 3. (*opposite*) ACF and PS of the fluctuation obtained from one sensillum. (*A*) ACF of the control fluctuation obtained by applying 93 mM αMM (equal in weight per volume to 50 mM maltose) and PS (inset). High-frequency contribution was cut by a filter (f_c = 2.1 kHz; see Materials and Methods). Average from three experiments on a single sensillum. (*B*) ACF (or PS; inset) of the net fluctuation obtained with 50 mM maltose stimulation. The net ACF (or PS) is the difference between the average of three experiments and the control ACF (or PS) shown in *A*. The thicker solid line is the best-fitted curve, which is the sum of an exponential term (thinner solid curve) and a background line (broken line) (cf. Materials and Methods). The arrow is the time constant (τ = 2.74 ms) of the best-fitted exponential term (or the corner frequency of the Lorentzian term [58.1 Hz]).

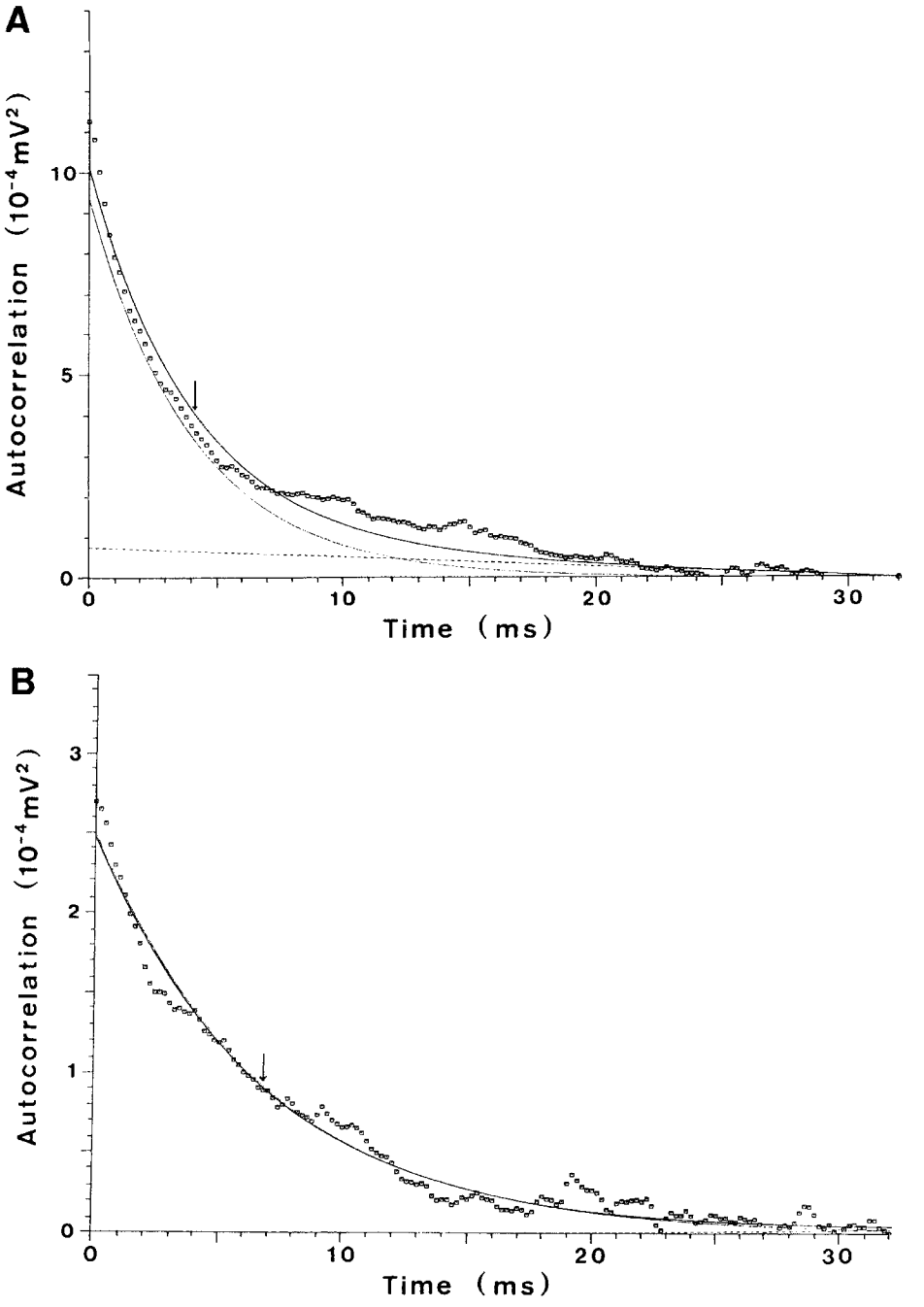

FIGURE 4. Examples of ACFs of net fluctuation increase relative to an equal weight per volume of αMM obtained by stimulation with 25 mM sucrose (*A*) and 50 mM fructose (*B*). Each datum is the average of three experiments obtained on a different sensillum, in the same way as for Fig. 3*B*. The solid lines and broken lines are the same as in Fig. 3*B*. The arrows show the positions of time constants: τ = 4.32 (*A*) and 6.78 (*B*) ms.

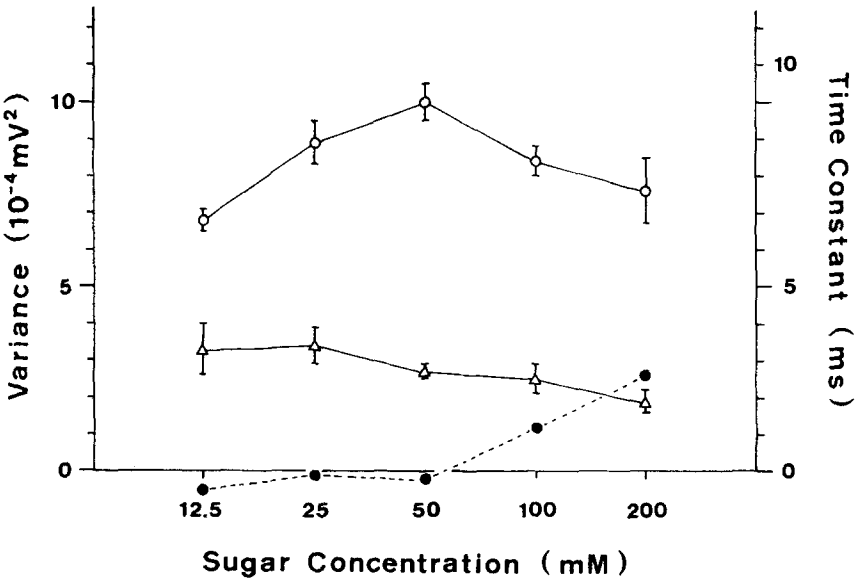

FIGURE 5. Curves of variance and time constant of ACF vs. maltose concentration obtained using one sensillum. The variance of the net fluctuation relative to an equal weight per volume of αMM (open circles) and the time constant (open triangles) of an exponential term fitted to the ACF are the average (± SEM, shown by bars) for three experiments. The filled circles with broken lines are the control variances for αMM (one experiment for each point) relative to solution A only.

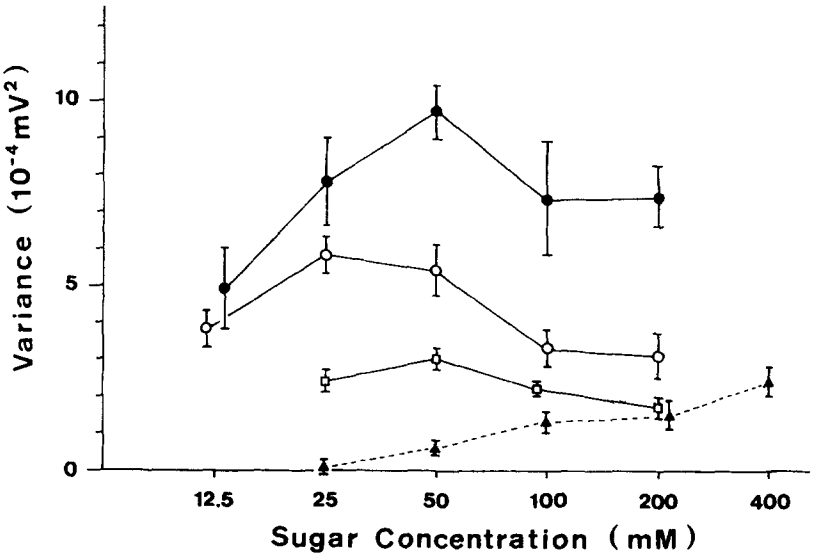

FIGURE 6. The average concentration dependence of the variance of net fluctuation increase relative to an equal weight per volume of αMM and sucrose (open circles, n = 17–19), maltose (filled circles, n = 8–10), and fructose (open squares, n = 15–17) ± SEM. Two or three series of experiments were performed on one receptor. Among the reproducible data, only those having maxima at 25 and 50 mM for sucrose (eight out of nine receptors) and at 50 mM for maltose (four out of seven receptors) and fructose (five out of seven receptors) were averaged (see text). The average control variances obtained for αMM relative to solution A only (filled triangles, n = 24–26) are also shown.

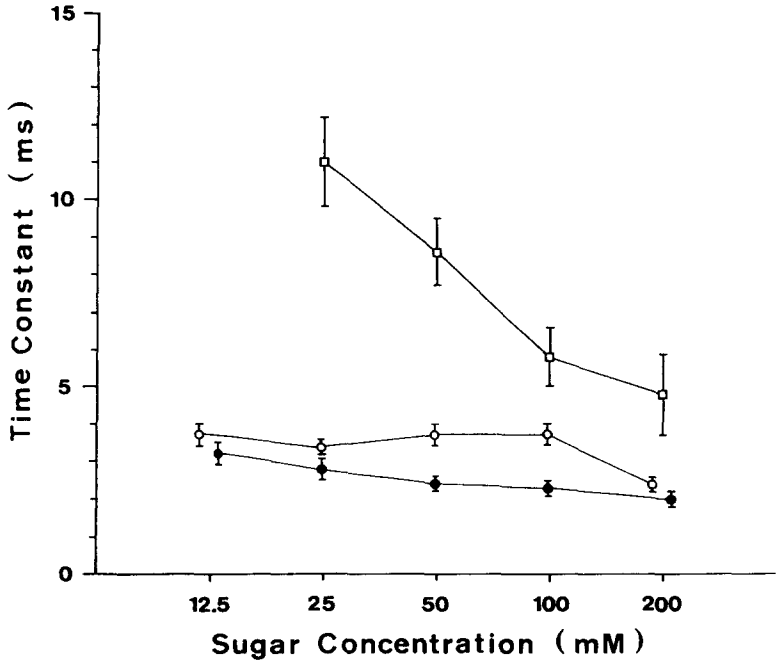

FIGURE 7. Total average (± SEM) for the reproducible data of the time constants of the ACF of the net fluctuation relative to αMM, obtained in the same way as for Fig. 5. Sucrose: open circles, nine samples, n = 25–28; maltose: filled circles, seven samples, n = 20–22; fructose: open squares, seven samples, n = 19–20.

difference in time constants, fluctuations for 0.1 M of three kinds of sugars were examined on a single hair sample. Differences similar to those in Fig. 7 were obtained on each of five hair preparations.

DISCUSSION

The following results were obtained in the present study. (*a*) Upon stimulation with effective sugars, the fluctuation of the potential between the tip and the basal part of the outer lumen of the taste sensillum increased with the development of the receptor current. (*b*) The ACF of the receptor current fluctuation could be approximately described by an exponential term. (*c*) The time constant of this exponential term differed with different sugars. (*d*) The variance of the fluctuation attained a maximum at a certain concentration of stimulating sugar. As discussed below, these results as a whole strongly suggest that the fluctuations observed were those of the receptor current, reflecting the gating of sugar-operated ion channels in the tip region of the sensory process.

Direct Operation of Transduction Ion Channels by Sugars

Result *b* above suggests that the channels obey approximately a simple state-transition scheme containing an open state O and a shut state C:

$$C \underset{\alpha'}{\overset{\beta'}{\rightleftharpoons}} O, \qquad \text{(Scheme I)}$$

where α' and β' are the apparent rate constants of the shut and open transitions, respectively. (See Sakmann and Adams, 1979, and Kijima and Kijima, 1982, 1983, for an explanation of why more complex state-transition schemes reduce to Scheme I under various conditions.) In this case, the ACF of the receptor current, $C_I(t)$, is expressed as (Neher and Stevens, 1977)

$$C_I(t) = i\langle I\rangle(1 - p)\exp(-t/\tau), \tag{2}$$

where i is the single-channel current, p is the channel open probability, and $\langle I\rangle$ is the mean total current, expressed as

$$\langle I\rangle = iNp. \tag{3}$$

N is the number of channels and τ is the time constant, which is given by

$$1/\tau = \alpha' + \beta'. \tag{4}$$

The variance of the current fluctuation, σ_I^2, is given as

$$\sigma_I^2 = C_I(0) = i\langle I\rangle(1 - p). \tag{5}$$

Eq. 4 shows that the reciprocal of the time constant is the sum of the open and shut rates.

Generally, when the channel obeys schemes more complex than Scheme I, the ACF has been shown to be proportional to the time course of a channel in the open state, showing relaxation toward equilibrium, if there is only one open state (Colquhoun and Hawkes, 1977). In any case, the time constant of the exponential term fitted to the ACF reflects the opening and shutting rates of the channel: when the time constant is large, the rate is small, and vice versa. We will compare here the time constants at low sugar receptor responses, since secondary effects may occur at higher responses, as will be discussed below.

The dose-response curves of the sugar and water receptors are shown in Fig. 8. The response is shown by the evoked impulse frequency, which has been shown to be proportional to the receptor current amplitude (Morita, 1972). As seen in the figure, impulses of the sugar receptor responding to 25 mM sucrose, 50 mM maltose, and 50 mM fructose were of similar frequency, i.e., a similar magnitude of receptor current flowed at these concentrations. Nevertheless, the time constant for fructose was much larger than those for sucrose and maltose (Fig. 7).

It is also noticeable in Fig. 8 that a high concentration of fructose (and also sucrose) appreciably reactivated the water receptor (Wieczorek and Koppel, 1978; Wieczorek, 1980), and fluctuation at 50 mM fructose may be the sum of contributions from both the sugar and water receptors. Even taking into account this contribution of the water receptor reactivated by fructose, the above difference in the time constants is valid, since the time constant for fructose was still larger at 25 mM, a concentration at which the water receptor impulses were negligible.

It has been shown (Hanamori et al., 1974; Shimada et al., 1974, 1985; Shimada, 1978; Shimada and Tanimura, 1981) that there are several specific receptor sites (receptor molecules) on the receptor membrane of the sugar receptor cell. Sucrose and maltose react with the same site, the pyranose site, whereas fructose reacts with the furanose site.

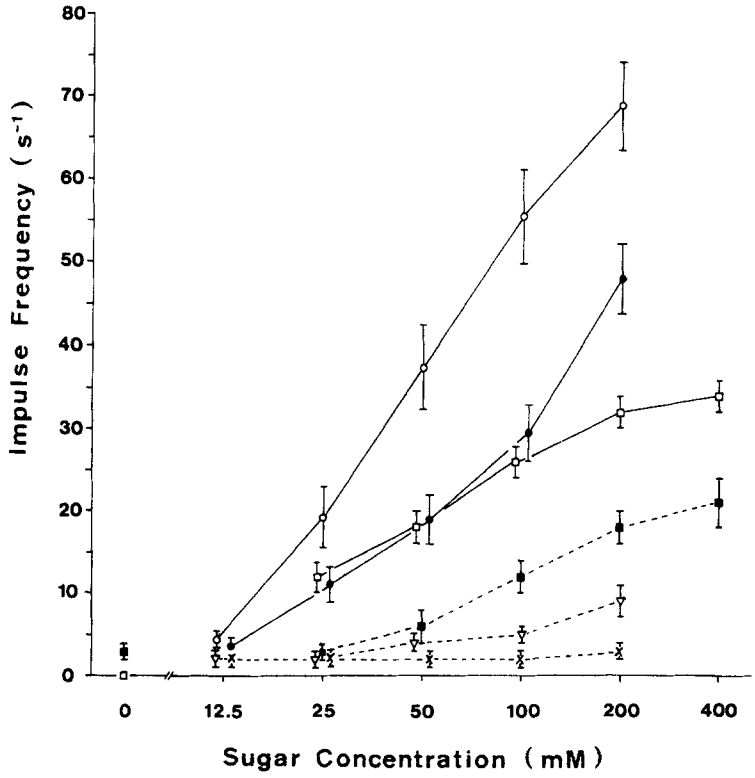

FIGURE 8. Dose-impulse frequency curves of sugars dissolved in solution A, obtained by the tip-sidewall method. Impulses for 1 s starting 0.2 s after the onset of stimulation were counted. Two or three experiments were done on one receptor for each concentration and data for 12 (for sucrose and maltose) and 6 (for fructose) receptors were averaged. Responses (± SEM) of the sugar receptor for sucrose (open circles) and for maltose (filled circles) and of the water receptor for sucrose (open triangles) and for maltose (×'s) were obtained using the same sensilla (n = 22–24). The open and filled squares show the impulses for the sugar and the water receptors, respectively, for fructose (n = 16–18). Solution A only (the open square at 0) and αMM evoked no sugar impulses. The water impulses evoked by αMM were nearly equal to or a little smaller than (at 186 and 372 mM) those obtained with an equal weight per volume of maltose (not shown).

Taking into account the above differences in the receptors, we think that the large difference in time constants for different sugars clearly excludes the following two possibilities: (*a*) most of the current fluctuation is caused by the voltage-dependent channels, which are opened as a secondary effect of the cell depolarization caused by sugars; (*b*) the pyranose site and the furanose site use a

common second messenger for the transfer of information from the receptor to the channel. The reasons for excluding these possibilities are as follows. At 25 mM sucrose, 50 mM maltose, and 50 mM fructose, the receptor cell is depolarized to a similar degree; i.e., the membrane potentials are similar. Therefore, if possibility *a* were true, the voltage-dependent channels would be affected by the similar membrane potentials and would be gated to give a similar gating rate, i.e., a similar time constant of the ACF. Thus, possibility *a* is excluded. If possibility *b* were true, the similar magnitude of receptor currents would mean similar open probabilities of channels gated by the common second messenger; i.e., there would be similar concentrations of the second messenger in the region near the channels within the sensory cell. The similar concentrations of the second messenger would give rise to similar channel gating rates, i.e., similar time constants of the ACF. Thus, possibility *b* can be excluded.

Furthermore, the time constant for 25 mM sucrose (3.41 ± 0.18 ms, $n = 27$) and that for 50 mM maltose (2.37 ± 0.15 ms, $n = 19$) showed a clear statistical difference ($p < 0.001$ by t test) and, at these concentrations, the reactivated water response was negligible compared with that for the control solution containing an equal weight per volume of αMM (Fig. 8). This shows that the two kinds of sugars, sucrose and maltose, which probably react with the same receptor molecule (the pyranose site), caused the receptor current fluctuation with different time constants of the ACF. This indicates that the chemical structure of a sugar that forms a complex with the receptor molecule affects the opening and shutting rates of the ion channel. It is strongly suggested from this fact that the channel corresponding to the pyranose site is directly operated by the sugar. It is conceivable, however, that a receptor may transfer information about the kinds of sugars to the channels by using different kinds of second messengers for different sugars. We think that such a complex transduction mechanism is unlikely. Moreover, the mechanism mediated by the second messenger is unlikely, because the receptor current begins to flow very rapidly within a few milliseconds after the onset of stimulation, without an appreciable latency.

The most likely possibility is that the transduction channel forms a receptor-channel complex, like the well-known nicotinic acetylcholine receptor ion channel at the vertebrate neuromuscular junction (Raftery et al., 1980; Noda et al., 1982).

Dependence of Time Constants and Variances on Sugar Concentration

The time constants of the ACF for fructose and maltose decreased with increasing concentration, as would be expected in simple ligand-gated channel dynamics (Scheme I), but those for sucrose did not show any significant concentration dependence. The reason for this is not clear, but some possible causes can be suggested. (*a*) The channels obey a complex reaction scheme and approximation by a single-exponential term may not be good for the ACFs of sucrose, as seen in the example shown in Fig. 4*A*. (*b*) Fluctuations other than those caused by the transduction ion channels may exist. This possibility cannot be excluded, especially for high concentrations of sucrose, since a very large magnitude of receptor current flows that may largely depolarize the receptor cell and may open the voltage-dependent ion channels.

The observation that variance shows a peak with changes in sugar concentration can be explained as the result of two factors. One is that the fluctuation in conductance is greatest when the channel open probability, p, is 0.5 (see Eq. 2) and the other is the decrease in a single-channel current owing to a decrease in the driving force with an increase in p. The second cause does not exist in voltage-clamp experiments. However, in our experiment, the second cause might have made a significant contribution and shifted the peak position toward $p < 0.5$. The peak concentrations for the three sugars were nearly equal to, or below, the half-maximum concentrations (which should be lower than the concentrations at which $p = 0.5$; Morita and Shiraishi, 1985) in the dose-response curves (Fig. 8).

Number of Channels Open upon Maltose Stimulation

We can estimate the average number of open channels upon sugar stimulation by calculating the ratio, $\langle I \rangle^2/\sigma_I^2$, from Eqs. 2, 3, and 5:

$$\langle I \rangle^2/\sigma_I^2 = \langle I \rangle/[i(1 - p)] = Np/(1 - p). \quad (6)$$

It should be noted that Eq. 6 holds for more general state-transition schemes, if every open state in the scheme has the same single-channel conductance, i.e., gives the same value of i. The variance for 50 mM maltose recorded by the two-sidewall method, which is proportional to σ_I^2, was $7.5 \pm 0.9 \times 10^{-4}$ mV^2, and the average drop in potential that was proportional to the receptor current was 0.69 $\pm$ 0.11 mV ($n = 11$). We do not know the channel open probability, p, at this concentration, but we assume from the dose-response curve that it was 0.3. The average of the open channel number, Np, estimated for each experiment was 530 $\pm$ 90 ($n = 11$). This does not vary more than 30%, even if an extremely small p was assumed. However, this might be an overestimation, since the frequency characteristic of the two-sidewall method was not good, and the high-frequency contribution to the variance might have been decreased.

Comparison with Previous Work

Kaissling and co-workers (Kaissling, 1977; Kaissling and Thorson, 1980), using the olfactory sensilla located on the antennae of male silkworm moths, measured the change in voltage and its fluctuation between the distal cut end of the sensillum and an indifferent electrode located in the hemolymph space within the antenna. In their study, the fluctuations were not analyzed quantitatively and the origin of the fluctuation was not specified. However, qualitatively, their findings would seem to agree well with our present results. When the antennae were stimulated with a pheromone or its analogues, after depression of the nerve impulses by treating the sensillum with (+)trans-permethrin, a negative-going change in the potential difference (called the receptor potential by Kaissling) occurred and was usually accompanied by a fluctuation increase. Moreover, the amplitude and mean cycle of fluctuation appeared to change depending on the structure of the pheromone analogues, and also the fluctuation appeared to pass through a maximum, when the concentration of the pheromone in the air stream was changed.

We were unable to carry out a quantitative analysis of the fluctuation reported here using the method of Kaissling, and instead adopted our own methods. The reason was that in the insect sensilla, a transepithelial potential of several tens of millivolts exists between the space in the sensillum and the hemolymph space (Thurm and Wessel, 1979) and this always shows a considerable drift. In the taste sensillum we studied, this drift (~0.3 mV) made it impossible to analyze the current fluctuation.

Accuracy of Fluctuation Measurements

As indicated by the PS vs. frequency curve of the control (Fig. 3*A*, inset), the overall recording system probably represented the frequency characteristics faithfully up to 1 kHz. However, the high-frequency components of the receptor current might have been short-circuited around the distal part of the sensory process, since the effective length constant of the process becomes shorter with increasing frequency. Thus, the smallest time constant observed (~2.0 ms for 0.2 M maltose in Fig. 7) might not have been correct and the true value might have been smaller. Other time constants significantly larger than this were probably measured accurately.

We have suggested here the existence of sugar-operated transduction ion channels in the fly sugar receptor through analysis of fluctuations in the receptor current flowing through the outer lumen of the sensory hair. The extracellular recording method adopted here is less direct than the patch-clamp method (Neher and Sakmann, 1976*b*) or the whole-cell clamp method. We did not isolate the single-channel current or control the membrane potential and ionic composition inside the receptor cell. However, our method had the advantage of allowing us to analyze quantitatively the dynamics of the transduction channel under the same conditions as those in which the taste cell works physiologically. Using this method, we should be able to determine further the ionic specificity of the transduction channel and examine the transduction mechanisms of the salt and water receptors in the taste sensillum.

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REFERENCES

Anderson, C. R., and C. F. Stevens. 1973. Voltage clamp analysis of acetylcholine produced endplate current fluctuations at frog neuromuscular junction. *Journal of Physiology.* 253:655–691.

Beidler, L. M. 1971. Taste receptor stimulation with salts and acids. *In* Handbook of Sensory Physiology. L. M. Beidler, editor. Springer-Verlag, Berlin. 4(2):200–220.

Bendat, J. S., and A. G. Piersol. 1986. Random Data: Analysis and Measurement Procedures. 2nd ed. John Wiley & Sons, New York. 386–391.

Buck, J. B. 1953. Physiological properties and chemical composition of insect blood. *In* Insect Physiology. K. D. Roeder, editor. John Wiley & Sons, New York. 147–190.

Colquhoun, D., and A. G. Hawkes. 1977. Relaxation and fluctuations of membrane currents that flow through drug-operated channels. *Proceedings of the Royal Society of London, Series B.* 199:231–262.

Dethier, V. G. 1976. The Hungry Fly. Harvard University Press, Cambridge, MA. 489 pp.

Fujishiro, N., H. Kijima, and H. Morita. 1984. Impulse frequency and action potential amplitude in labellar chemosensory neurons of *Drosophila melanogaster*. *Journal of Insect Physiology.* 30:317–325.

Hanamori, T., A. Shiraishi, H. Kijima, and H. Morita. 1974. Structure of monosaccharides effective in stimulation of the sugar receptor of the fly. *Chemical Senses and Flavour.* 1:147–166.

Kaissling, K. E. 1977. Structure of odorant molecules and multiple activities of receptor cell. *In* Olfaction and Taste. J. LeMagnen and P. MacLeod, editors. Information Retrieval, London. 6:9–16.

Kaissling, K. E., and J. Thorson. 1980. Insect olfactory sensilla: structural, chemical and electrical aspects of the functional organization. *In* Receptors for Neurotransmitters, Hormones and Pheromones in Insects. D. B. Sattelle, L. M. Hall, and J. G. Hildebrand, editors. Elsevier/North-Holland Biomedical Press, Amsterdam. 261–282.

Katz, B., and R. Miledi. 1972. The statistical nature of the acetylcholine potential and its molecular components. *Journal of Physiology.* 224:665–699.

Kijima, H., and S. Kijima. 1982. 'Steady/equilibrium approximations' in relaxation and fluctuation. I. Procedure to simplify first-order reaction. *Biophysical Chemistry.* 16:181–192.

Kijima, H., and S. Kijima. 1983. 'Steady/equilibrium approximations' in relaxation and fluctuation. II. Mathematical theory of approximations in first-order reaction. *Biophysical Chemistry.* 17:261–283.

Kurihara, K., K. Yoshii, and M. Kashiwayanagi. 1986. Transduction mechanism in chemoreception. *Comparative Biochemistry and Physiology.* 85A:1–22.

Morita, H. 1969. Electrical signs of taste receptor activity. *In* Olfaction and Taste III. C. Pfaffmann, editor. The Rockefeller University Press, New York. 370–381.

Morita, H. 1972. Primary processes of insect chemoreception. *Advances in Biophysics.* 3:161–198.

Morita, H., and A. Shiraishi. 1985. Chemoreception Physiology. *In* Comprehensive Insect Physiology, Biochemistry and Pharmacology. G. A. Kerkurt and L. I. Gilbert, editors. Pergamon Press, Oxford. 6:133–170.

Morita, H., and S. Yamashita. 1959. Generator potential of insect chemoreceptor. *Science.* 130:922–923.

Neher, E., and B. Sakmann. 1976*a*. Noise analysis of drug induced voltage clamp currents in denervated frog muscle fibres. *Journal of Physiology.* 258:705–729.

Neher, E., and B. Sakmann. 1976*b*. Single-channel currents recorded from membrane of denervated frog muscle fibres. *Nature.* 260:779–802.

Neher, E., and C. F. Stevens. 1977. Conductance fluctuations and ionic pores in membranes. *Annual Review of Biophysics and Bioengineering.* 6:345–381.

Ninomiya, M., M. Ozaki, Y. Kashiwara, and H. Morita. 1986. Destruction and reorganization of the receptor membrane in labellar chemosensory cells of the blowfly. Recovery of responses to sugar after destruction. *Journal of General Physiology.* 87:1003–1016.

Noda, M., H. Takahashi, T. Tanabe, M. Toyosato, Y. Furutani, T. Hirose, M. Asai, S. Inayama,

T. Miyata, and S. Numa. 1982. Primary structure of α-subunit precursor of *Torpedo californica* acetylcholine receptor deduced from cDNA sequence. *Nature.* 299:793–797.

Raftery, M. A., M. W. Hunkapiller, C. D. Strader, and L. E. Hood. 1980. Acetylcholine receptor: complex of homologous subunits. *Science.* 208:1454–1457.

Sakmann, B., and P. R. Adams. 1979. Biophysical aspects of agonist action at frog endplate. *Advances in Pharmacology and Therapeutics.* 1:81–90.

Sato, T. 1980. Recent advances in the physiology of taste cell. *Progress in Neurobiology.* 14:25–67.

Shimada, I. 1978. The stimulating effect of fatty acids and amino acid derivatives on the labellar sugar receptor of the fleshfly. *Journal of General Physiology.* 71:19–36.

Shimada, I., H. Horiki, H. Ohrui, and H. Meguro. 1985. Taste response to 2,5-anhydro-D-hexitols; rigid stereospecificity of the furanose site in the sugar receptor of the fleshfly. *Journal of Comparative Physiology.* 157A:477–482.

Shimada, I., A. Shiraishi, H. Kijima, and H. Morita. 1974. Separation of two receptor sites in a single labellar sugar receptor of the fleshfly by treatment with p-chloromercuribenzoate. *Journal of Insect Physiology.* 20:605–621.

Shimada, I., and T. Tanimura. 1981. Stereospecificity of multiple receptor sites in a labellar sugar receptor of the fleshfly for amino acids and small peptides. *Journal of General Physiology.* 77:23–29.

Thurm, U., and G. Wessel. 1979. Metabolism-dependent transepithelial potential differences at epidermal receptors of arthropods. *Journal of Comparative Physiology.* 134A:119–130.

Uehara, S., and H. Morita. 1972. The effect of temperature on the labellar chemoreceptors of the blowfly. *Journal of General Physiology.* 59:213–226.

Wieczorek, H. 1980. Sugar reception by an insect water receptor. *Journal of Comparative Physiology.* 138A:167–172.

Wieczorek, H. 1982. A biochemical approach to the electrogenic potassium pump of insect sensilla: potassium sensitive ATPases in the labellum of the fly. *Journal of Comparative Physiology.* 148A:303–311.

Wieczorek, H., and R. Koppel. 1978. Effect of sugars on the labellar water receptor of the fly. *Journal of Comparative Physiology.* 126A:131–136.