The Effects of Temperature on the Labellar Chemoreceptors of the Blowfly

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ABSTRACT In the labellar chemosensory hairs of the blowfly, Phormia regina Meigen, stationary amplitudes of the slow potentials induced by salt and sugar stimulations were decreased to 50–80% at 12°C of the values measured at 28°C. The amplitudes induced by water did not show any dependence on temperature change. The maximum rate of rise of the receptor potentials was strongly increased with rising temperature. The value of $K_b$, the apparent Michaelis constant, was less by a factor of six at 28°C compared to the 12°C value for the sugar receptor.

INTRODUCTION

After an early stage, characterized by a slight confusion, in the studies of temperature effects on the insect chemoreceptor, it seemed possible to conclude that the temperature of the stimulating solution had no noticeable effects on the magnitude of response in insect chemoreceptors (Hodgson and Roeder, 1956; Hodgson, 1956; Dethier and Arab, 1958; Mellon, 1961). Gillarly (1966), however, pointed out that the electrophysiological works therefore performed were unreliable, because the temperature of the stimulating solution, which was contained in a capillary of less than 100 μ tip diameter, was actually unknown. He has stated, from the results of his well-controlled experiments, that the response of the salt receptor depends on the temperature of the stimulus. His statement, however, does not seem conclusive, since he did not directly study the temperature effects on the response to the chemical stimulation, but only on the spontaneous discharge of the salt receptor. Considering such a state of our understanding, we attempted to clarify whether or not the temperature of the stimulus has effects on the magnitude of the receptor potential in insect chemoreception.

It has been shown in the labellar sugar receptor that the magnitude of response to sucrose apparently obeys Beidler's theory (Beidler, 1954; Morita and Shiraishi, 1968). That is, the magnitude of response, $r$, is expressed as

$$r = r_m/(1 + K_b/a),$$

where
where $r_m$ denotes the magnitude of maximum response when the concentration of sucrose, $a$, is infinitely high, and $K_b$ is a constant. Morita (1969) studied the implication of the constant, $K_b$, and presented two models for sugar chemoreception in which the dissociation constant between sucrose and the sugar receptor site was considered as one of the component factors of $K_b$. In such a case, as well as in Beidler's theory, a change in the value of $K_b$ with temperature should give us an insight into sugar chemoreception. It was our second objective to disclose this change with temperature.

**MATERIAL AND METHODS**

The receptor potentials and impulses were recorded from the labellar chemosensory hairs of the blowfly, *Phormia regina* Meigen. The range of temperature was limited to about 10°-28°C to obtain reliable data, since we could obtain hardly any reproducible results at high temperatures (>30°C), and since stimulus solutions repeatedly failed to make contact with chemoreceptors and the impulse frequency markedly decreased at low temperatures (<10°C). The arrangement of apparatus for the experiments is schematically shown by Fig. 1. A small room (referred to as the experimental room; 180 X 180 X 180 cm³), in which the experiments were performed, was shielded from the outside both thermally and electromagnetically, and was kept at various temperatures (within ±0.25°C) with an air conditioner and an electric heater. Air was made to pass through water in a bottle kept at 60°C in a water bath and through a thin copper tube of 4 mm outer diameter and 1 m length.

**Figure 1.** Scheme of experimental arrangement, not in scale. The experimental room was thermally and electromagnetically isolated from the outside. a, recording electrode; b, indifferent electrode; $T_1$, temperature at a place near the chemosensory hair tip, to which air saturated with water was blown; $T_2$, temperature at a place which was close to the hair tip but not exposed to the air current.
in the experimental room. The air current obtained in this way was gently blown onto the tips of a chemosensory hair and of a glass capillary (70–100 μ tip diameter) containing a stimulus solution through a glass tube whose tapered end was about 1 mm in diameter. The temperature of the air current was measured at a position just behind the tip of the chemosensory hair with a thermistor thermometer of 2 mm tip diameter calibrated by a standard thermometer. This localized temperature was adopted as the test temperature at which the receptor was stimulated. The thermistor used was Type PT, class 0.5, Shibaura Electric Co. Ltd., Tokyo. An electric current was passed through the thermistor and Joule’s heat was generated. The measurement of temperature, therefore, might be influenced by the rate of flow of the air which was blown onto the chemosensory hair. We examined the effect of airflow as follows. At room temperatures, the thermistor was exposed to an airstream of various flow rates. The temperatures indicated by the thermistor thermometer were completely the same over a range of flow rate from zero to the high value, at which the chemosensory hair vigorously vibrated. The rate of the airstream we used was so low that the chemosensory hair stood quite still. Therefore, we could conclude that the measurement of temperature was not affected at all at the rate of airflow we used. Measurements of temperature showed that the thermal equilibrium was established between the inside and the outside of the air current (Table I A). Measurements of the dew points within the current showed that the air was almost fully saturated with water (98–99 %, Table I B). The lowering of the vapor pressure of the stimulating solution was less than 3.3 % in the concentration range used in the present work (3.3 % in 1 M NaCl and 1.8 % in 1 M sucrose in the temperature range 10°–28°C). Therefore, the temperature change with evaporation and condensation of water near the surface of the stimulating solution could be safely assumed to be negligible. The rate of the test temperature change was at most ±1°C/min. Under such conditions for a blank test, temperatures were measured in water contained in a capillary, which was 3.2 mm in outer diameter and 0.14 mm in wall thickness. The temperature of the water was found to follow that of the air current with a gap of less than 0.5°C. Considering the small dimensions of the stimulating solution and of the chemosensory hair, errors due to thermal nonequilibria could be assumed to be negligibly small.

### Table I

| A. Differences between the Temperatures T₁ and T₂ |
|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| T₂, °C | 15.4 | 17.6 | 24.0 | 25.2 | 29.8 |
| T₁, °C | 16.0 | 18.0 | 23.6 | 26.0 | 30.0 |

| B. Relative Humidity of the Air Saturated with Vapour |
|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| T₁, °C | 17.6 | 18.9 | 19.5 | 24.2 | 26.2 |
| T₁', °C | 17.2 | 18.7 | 19.4 | 23.9 | 26.0 |
| RH, % | 98 | 99 | 99 | 98 | 99 |

For T₁ and T₂, see Fig. 1.
T₁': dew point of the air whose temperature is indicated by T₁.
A recording electrode was a glass microcapillary filled with Waterhouse's solution (Buck, 1953) and had a resistance of 50–150 MΩ. To record the receptor potential, two microelectrodes were inserted into the chemosensory hair as shown in Fig. 1 (a and b), the proximal one serving as an indifferent electrode. The leads from the two electrodes were fed into a dc amplifier. When we needed only to record impulses, an ac amplifier was used, and a piece of platinum wire inserted into the cut end of a proboscis served as an indifferent electrode. Other recording and stimulating methods were the same as described elsewhere (Morita and Shiraishi, 1968).

RESULTS

Receptor Potential

Stationary Amplitude  The slow potential accompanied by impulses is recorded between two electrodes such as a and b in Fig. 1 when a stimulus is applied to the tip of the chemosensory hair. The slow potential attains a stationary level within 0.1 sec after the onset of the stimulus at temperatures above 20°C, and is generally a complex of the receptor potentials originated in two or three chemoreceptors (Morita, 1969; Morita and Yamashita, 1966).

We defined the stationary amplitude of the slow potential as indicated by h in Fig. 2. The value of h was the vertical distance between the two parallel lines drawn on the record, one representing the resting base line and the other the level of the stationary state. As can be seen in Fig. 2, where the record was obtained at 9.8°C, the slow potential change attained the stationary state within 0.1 sec. The rate of rise of the slow potential is higher at higher temperatures, as shown in Fig. 4. Accordingly, a period of 0.25 sec was sufficiently long for the slow potential to arrive at a stationary amplitude at all temperatures in the present work (10°–28°C). Since the stationary state lasts for 1 sec without any marked adaptation, the slow potentials of the same stationary amplitude were expected to result from the same short (0.25 sec) stimulus, even if they were given twice or thrice within a few seconds. Therefore, we obtained two or three records of the slow potential practically at a constant

![Figure 2](image-url)
temperature within a few seconds, while one series of experiments was performed at a temperature which was gradually changed (approximately from 25° down to 10°C, and then from 10° up to 25°C) over the course of about 1 hr. Values of the stationary amplitude at each "constant" temperature were averaged. One of the results is shown in Fig. 3. As shown by this figure, the stationary amplitude of the slow potential induced by water (the filled circles in Fig. 3) did not show any definite dependence on temperature change. This was true in all other chemosensory hairs examined (seven examples). Therefore, it can safely be assumed that the stationary amplitude of the slow potential induced by water is independent of temperature. From such a consideration we can regard the stationary amplitude of the water receptor potential as being independent of temperature, since the slow potential evoked as a result of stimulation by water was almost completely represented by the water receptor potential (for contribution of the salt receptor, see Morita et al., 1966). The temperature dependence of the slow potential induced by a 0.5 M glucose solution (the open circles in Fig. 3) indicates that the sugar receptor potential increased in stationary amplitude with an increase in temperature, since 0.5 M glucose solutions activated almost completely the sugar and the water receptors only. From the same reasoning as above, the
salt receptor potential (the half-filled circles in Fig. 3) is also concluded to have higher stationary amplitudes at higher temperatures.

Such effects of temperature on the stationary amplitudes of the sugar, salt, and water receptor potentials were observed in every labellar chemosensory hair examined, although the degree of temperature dependence varied with the hair. As to the sugar receptor, results showing the same tendency were obtained from stimulations by 0.25 M sucrose: the ratio of the $h$ value at 12°C to that at 28°C was in the range 0.5-0.8.

**Rate of Rise** The maximum rate of rise of the slow potential is the initial slope of the potential, which is represented by the straight line, $a$, in Fig. 2. The temperature dependences of the maximum rates of rise in the slow potentials evoked by water, by 1 M NaCl, and by 0.5 M glucose are represented by the filled, half-filled, and open circles, respectively, in Fig. 4. From this figure, the maximum rate of rise of the water receptor potential can be concluded to be higher at higher temperatures. The logarithm of the maximum rate of rise is plotted against the reciprocal of the absolute temperature in Fig. 4. Accordingly, the activation energy for the rate-limiting process of the generation of the receptor potential by water stimulation, for example, would be obtained from the slope of the line covering the values for the water stimulations, if the maximum rate of rise is proportional to the rate constant in

![Figure 4](image-url)

**Figure 4.** Effects of temperature on the maximum rate of rise, $(R-R)$, whose logarithm is plotted against the reciprocal of the absolute temperature $(1/T)$. The lines are drawn with the method of least squares (the same as in Figs. 7-9).
the rate-limiting process. However, this is quite difficult to prove, because even the proportionality between the amplitude of the recorded receptor potential and the displacement of membrane potential at the receptor locus holds only in a stationary state. Therefore, it should be pointed out only that the temperature dependences of the maximum rates of rise of the slow potentials evoked by glucose and by NaCl were nearly equal in their degree to that of Pacinian corpuscle as described by Ishiko and Loewenstein (1961).

**$K_b$ Value**

As mentioned in the Introduction, the magnitude of response of the sugar receptor to sucrose is expressed as $r = r_m/(1 + K_b/a)$, and this equation is rewritten as

$$a/r = a/r_m + K_b/r_m.$$  

(Beidler's taste equation)

This form of equation shows that the value of $K_b$ is obtained from the $x$-intercept of the straight line representing the $a/r$ vs. $a$ relation.

It has been shown in a temperature range of 18°-25°C that the impulse frequency is proportional to the amplitude of the receptor potential in a stationary state in the labellar sugar receptor in *Calliphora* (Morita and Yamashita, 1966) and in *Phormia* (cited by Morita, 1969). The proportionality was confirmed at low temperatures in the present work as shown by Fig. 5. The amplitude of the receptor potential in this figure cannot be measured directly on the record. However, we can estimate each value of the sugar receptor potential by subtracting that of the water receptor from the height of $h$ as shown in Fig. 2, since the above-mentioned proportionality for the water receptor is sufficiently supported by Morita (1969) and the salt receptor was not activated in this case. Therefore, we used the impulse frequency instead of the receptor potential to obtain the value of $K_b$ for sucrose. At low temperatures (10° and 15°C), as is the case in Fig. 5, the duration of stimulus was 600-700 msec, and the magnitude of response in the stationary state was defined as the end value of an adaptation curve as shown by Fig. 6. The figure shows that the impulse frequency attains a stationary level near the end of the stimulus at low temperatures. Therefore, the proportionality shown in Fig. 5 does not mean that the impulse frequency is proportional to the amplitude of the receptor potential during the whole course of the stationary phase of the latter, but simply justifies our estimation of $K_b$ values from the end value of an adaptation curve. At high temperatures (20° and 25°C), the duration of stimulus was limited to 500 msec, and the magnitude of response was defined as the number of impulses occurring for the last 300 msec, during which the response was actually in a stationary phase.

The preparation shows reproducible responses for about 2 hr after isolation of a proboscis. To complete experiments during this period, $K_b$ values at only
Figure 5. Proportionality between the impulse frequency and the amplitude of the sugar receptor potential in the stationary state, obtained from stimulation by 0.125, 0.25, and 0.5 M glucose solutions at 15°C ± 0.2°C. Impulse frequency was obtained from the end values of adaptation curves as shown by Fig. 6. The amplitude of the sugar receptor potential was obtained by subtracting the water receptor potential from the slow potential evoked by glucose solutions (see text for details). Impulses of the salt receptor were not elicited by water and by glucose solutions in this preparation.

Figure 6. Adaptation curve for 0.6 M sucrose solution at 10°C. Each point is the frequency calculated from the successive interval between impulses.
Figure 7. Beidler's plot for sugar responses at 20° and 25°C. The symbols o and x at 20°C indicate the values obtained in the first and the last run, respectively (the same as in Fig. 8).

Figure 8. Beidler's plot for sugar responses at 10° and 20°C.
two different temperatures were obtained with each sugar receptor. Figs. 7 and 8 show two examples obtained at the highest (25°C) and the lowest (10°C) temperatures in our experiments. Stimulations were given at 20°C as the first run, at 25°C (Fig. 7) or 10°C (Fig. 8) as the second run, and at 20°C again as the last run in each receptor. Since we were interested only in the change of the $K_b$ value with temperature, and since its value varies with receptors, we obtained the value of $K_b/K_b^{20}$ for each preparation, where $K_b^{20}$ is the value of $K_b$ at 20°C. In Fig. 9 are shown the logarithms of the averages of $K_b/K_b^{20}$ values plotted against the reciprocal of the absolute temperature, summarizing the results at four different temperatures. From the slope of the straight line in Fig. 9, the heat of reaction, $\Delta H$, for the complex formation would be estimated as about plus 19 kcal/mole if $K_b$ were the dissociation constant between sucrose and the receptor site.

Figure 9. $K_b$ is the concentration of sucrose at which the sugar receptor shows half-maximum response. $K_b^{20}$ is the value of $K_b$ at 20°C. Bars at each of three points indicate the standard error of the mean (three preparations at each temperature, but six preparations at 20°C).
DISCUSSION

The present results clearly show that the receptor potentials of the labellar chemoreceptors, except for the water receptor, are increased in amplitude in the stationary phase at high temperatures, as suggested by Gillary (1966) for the salt receptor.

Among the labellar chemoreceptors, the proportionality between the impulse frequency and the amplitude of the receptor potential in the stationary phase holds for the sugar and the water receptors (Morita and Hori, unpublished, cited by Morita, 1969). The water receptor does not show responses described by Beidler’s taste equation, and, therefore, the sugar receptor is the only one in which $K_b$ can be considered to be related to events within the receptor membrane. Morita (1969) derived the following equation for the membrane receptor potential, $V$, (depolarization of the receptor membrane):

$$V = V_m/(1 + G/ng),$$

where:

- $n = \text{number of activated receptor sites}$,
- $g = \text{conductance per activated receptor site}$,
- $G = \text{conductance across the receptor membrane when } n = 0$,
- $V_m = \text{the value of } V \text{ when } n = \infty$.

He proposed two models for determination of the value of $n$. One is the “complex” model, in which the receptor site is assumed to be activated simply by complex formation with a stimulant molecule. Then, $n$ is given as

$$n = s/(1 + K/a),$$

where $s$ is the total number of the receptor sites, $K$ the dissociation constant of the complex between the stimulant molecule and the receptor site, and $a$ the concentration of the stimulant substance. Introducing equation 2 into equation 1, we obtain

$$V = V_m/[1 + KG/(sg + G)a],$$

where $V_m$ is the value of $V$ when $a = \infty$. Comparing this equation with that given in the Introduction, $K_b$ can be written as

$$K_b = KG/(sg + G),$$

since $r$ and $r_m$ are proportional to $V$ and $V_m$, respectively. On the other hand, the “regulator” model assumes spontaneous transition of the receptor site from the resting to the activated state. Let the equilibrium constant of this
transition be $1/L$, the dissociation constant of the complex between the stimulant molecule and activated receptor site be $K^*$, the same for the resting receptor site be $K$, and $K^*/K$ be $c$, then

$$V = V_0/[1 + GLK^*/(sg + G + GLc)a].$$  \(5\)

In this case, $K_b$ is given as

$$K_b = GLK^*/(sg + G + GLc).$$  \(6\)

In the complex model, $K_b$ is composed only of dissociation constant and membrane conductances. The conductances might be temperature dependent. However, they make a component factor of $K_b$ as a ratio, so that the temperature dependence of this factor may be diminished as a whole. Accordingly, the temperature dependence of $K_b$ has to be attributed to that of $K$. If such were the case, the complex formation between the stimulant molecule and the receptor site would be an endothermic reaction whose heat of reaction is as high as 19 kcal/mole. It is well known that almost all binding reactions as well as physical adsorption are exothermic, and the only exception we know is the formation of hydrophobic bonds. Insofar as we assume that polar groups (–OH) of sugar molecules are essential for binding with the receptor site (Evans, 1963), the complex model should be excluded (see the Appendix).

The situation is quite different in the regulator model. As equation 6 shows, $K_b$ contains explicitly the equilibrium constant $L$ for transition of state from the active to the resting. Assuming that the temperature dependence of $K_b$ is completely attributed to that of $L$, absorption of heat (19 kcal/mole) is needed for transition of the receptor site from the resting to the active state. Morita (1969) estimated $1/L$ to be about $10^{-4}$, corresponding to +5.45 kcal/mole for free energy change. From those values, entropy is calculated as being increased in the active state by as much as 50 cal/mole·degree. If the value of $K^*$ is increased with high temperatures, as ordinarily occurs in binding reactions, the entropy increase for the transition to the active state must be still more. Thus, the present results concerning the temperature effects on the labellar sugar receptor enable us to deduce a more reasonable explanation for the sugar reception with the regulator model than with the complex model.

Compared with the results for $K_b$, those for the stationary amplitude of the receptor potential were not so accurate. However, combining both results, we may discuss another important constant, $V_\infty$, to some extent. The value of $h$, when stimulated by 0.25 M sucrose, decreased by 20–50% with lowering of the temperature from 28°C to 12°C. The value of $K_b$ increased by a factor of about six with the same range of temperature drop. Since the $K_b$ value was 0.02–0.06 M at 28°C, the stationary amplitude of the receptor potential at 12°C is calculated as 50–80% of that at 28°C with a constant value of $V_\infty$. The value of $h$ contains the amplitude of the water receptor potential, so
that the net effect of temperature on the amplitude of the sugar receptor potential should have been stronger than was seen in the results for the $h$ value. Nonetheless, the value of $V_\infty$ can never be assumed to increase by more than 100% with a rise in temperature from 12° to 28°C. Thus, Paton's rate theory (1961) and all other theories which relate the amplitude of the receptor potential to a certain reaction rate are least plausible for the labellar sugar receptor.

As long as we assume that a response of the salt receptor is determined only by association of ions with the receptor site, its positive temperature coefficient shown in Fig. 3 is difficult to explain: the assumption could be wrong. However, since any constants valuable for understanding of the salt reception mechanisms could not be examined, this problem as well as that in the water receptor is left open for further studies.

In conclusion, a rise in temperature stabilizes the excited state in the sugar receptor membrane (probably also in the salt receptor). It follows that the entropy is increased in the excitation (including all the processes such as binding of the sugar molecules, conformational changes within the membrane, conductance changes, etc.), which is associated with an order-disorder transition. This is true whatever the mechanism is, if the receptor potential is governed by a certain state of (not by a rate of a process occurring in) the membrane.

APPENDIX

The "complex" model also assumes implicitly a transition of state of the receptor site (activation of the receptor site by a complex formation). If we take it in the most strict sense, i.e. if all and only the receptor sites binding the sugar molecules change their state from the resting ($S$) to the activated ($S^*$) state, the processes may be described, explicitly expressing the transition of state, as

\[ S \rightarrow S^* \]

and

\[ A + S^* \rightarrow AS^* \]

where $A$ represents a stimulant molecule. Accordingly, the net result is

\[ A + S \rightarrow AS^*. \]

Obviously, this is a special case for the "regulator" model, where $c$ in equation 6 is zero.

Another scheme explicitly expressing the transition may be given, in a somewhat less strict sense of the complex model, as

\[ A + S \rightarrow AS \rightarrow AS^*. \]
Denoting that $K' = [A][S]/[AS]$ and $L = [AS]/[AS^*]$, equations 2, 3, and 4 are rewritten, respectively, as

$$n = s/(1 + L + K'L/a),$$
$$V = V_0/[1 + GK'L/(sg + G + GL)a],$$

and

$$K_b = GK'L/(sg + G + GL).$$

(1\ a)

When $L \ll 1$, equation 1\ a is reduced to

$$K_b = GK'L/(sg + G).$$

(2\ a)

Comparing equation 2\ a with equation 4, we obtain

$$K = K'L.$$

Thus, the heat absorption in the reaction $A + S \rightarrow AS^*$ may be attributed to the step of the transition of $AS^*$. 

Received for publication 13 July 1971.

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