Stimulation of the Salt Receptor of the Blowfly

II. *Temperature*

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ABSTRACT The response of the salt receptor of the blowfly increased with increasing ambient temperature. At constant ambient temperature, the response increased as the relative humidity was raised. At low relative humidity, the temperature of the stimulating solution near its air interface was markedly below ambient temperature, due to evaporation. Warming and cooling the tip of the sensillum while recording from its side respectively raised and lowered the frequency of "spontaneous" action potentials of the receptor. The results indicate that the response of the salt receptor is dependent on the temperature of the stimulus. The Q_{10} is probably several times greater than one.

The effect of temperature on the electrophysiologically recorded response of the salt receptor of the blowfly has been the subject of several reports. Hodgson and collaborators (1955) merely state that "responses are modified by temperature changes." Hodgson and Roeder (1956) heated and cooled the whole stimulated preparation and found a direct correlation between the temperature recorded from the labellum and the spike frequency of the salt receptor (termed the "L fiber" by them). However, the temperature of the stimulating solution was not determined and, consequently, neither was the precise site of the effect. Hodgson (1956) states that an increase in the temperature of the preparation has an inverse effect on the spike frequency of a receptor during salt stimulation but that changes in the temperature of the stimulating solution have no effect on this. (It is not clear whether or not it is the salt receptor.) However, the absence of quantitative data and of adequate description of methods makes these statements inconclusive. Dethier and Arab (1958) state that "it must be concluded that the action of chemical stimuli at the tip of labellar hairs is temperature-independent," but their published electrophysiological evidence supporting this is also inconclusive. Mellon (1961) who attempted a quantitative study of the effect of temperature on the salt receptor, claims to have varied the temperature of his stimulating solution between 0 and 24°C with no noticeable effect on the response. However, considering the dimensions of the stimulating electrode and the procedure used, the temperature of the stimulating solution in contact with the tip of the sensillum was actually unknown, and could be determined only by very detailed and complicated analysis of the thermal events there or by actual measurement. Such determinations have not been carried out by previous investigators. A more careful examination of the dependence of the response of the salt receptor on the temperature of the stimulating solution was attempted here.

METHODS

Action potentials (spikes) were recorded from the type I labellar sensillum of the blowfly, *Phormia regina* Meigen (Evans and Mellon, 1962 *a*). The preparation and recording technique were essentially those used by Evans and Mellon (1962 *b*). The recording electrode was a glass capillary of about 100 μ diameter, filled with the stimulating solution and placed over the tip of the sensillum; the indifferent electrode was a similar capillary filled with saline solution and placed at a more proximal part of the proboscis. Stimulation evoked a train of impulses from the salt receptor. Spikes of other fibers were ignored. Response is defined as the number of salt receptor spikes occurring in the 0.5 sec interval beginning 0.1 sec after the onset of stimulation. The techniques of measuring and controlling temperature and relative humidity are described in the appropriate sections with the results. The methods have been described elsewhere in greater detail (Gillary, 1966 *a*, *b*).

RESULTS

Ambient Temperature and Relative Humidity

The effects of varying independently the temperature and relative humidity surrounding the whole preparation were determined, using an apparatus which will briefly be described here. Flowing air from an institutional air line was dried by passing it through $CaCl_2$ and $CaSO_4$, or humidified by bubbling through water or LiCl solutions of various concentrations, and independently heated or cooled by passage through a coil immersed in an ice bath or heated by means of an electric heating coil. The air then passed into a 130 cm^3 chamber housing the preparation where temperature was monitored with a thermometer calibrated to 0.1°C. Following this, it entered a flask where the relative humidity was measured using an electronic sensing element. The flow rate, which was controllable and monitored with a gas flow meter, was maintained at 200 liters/hr. Variations between 50 and more than 500 liters/hr showed no detectable effect on the response.

The results were clear and are illustrated by Figs. 1, 2, and 3. In the first figure, the response to 1 M NaCl stimulation increases at a rate of about 10%/°C within the range of 23 to 28°C. Correction due to the effect of temperature on relative humidity raises this to a value between 10 and 15%/°C at constant relative humidity, which corresponds to a Q_{10} of about 3 or 4. At constant ambient temperature, the response to 1 M NaCl increased with increasing relative humidity at a rate of between 0.5 and 1% per per cent change in relative humidity. Furthermore, relative humidity affected the whole curve of response vs. concentration, including the threshold value. All responses were normal as described in a preceding section. The effect of relative humidity was also demonstrated by recording from an intact fly, in addition to the standard preparation.

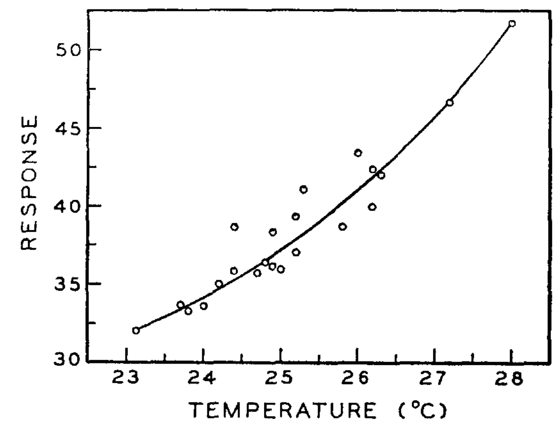

FIGURE 1. Response as a function of ambient temperature. The ambient temperature was raised and lowered through five cycles while concurrently recording temperature and response to 1 M NaCl. Each point is the average response of seven receptors on a single preparation. The total water content of the air was constant during the experiment, the relative humidity at 23°C being about 80% and less at higher temperatures (about 60% at 28°C). (Response is defined in Methods.)

Localized Temperature Measurements Using Thermocouples

Localized temperatures were measured using calibrated thermocouples of 25 μ diameter wire with a junction diameter of less than 75 μ. The temperature of the stimulated preparation, measured with a thermocouple thrust into the mouth or pressed against the labellum, was found to be identical to the ambient temperature and independent of the relative humidity. However, for low relative humidity (30% or less), the temperature recorded when the thermocouple was positioned near the interface of the stimulating solution, where the tip of the sensillum was located during stimulation, was approximately 10°C lower than ambient temperature, and approached ambient temperature up the shank of the electrode. With fresh solution flowing out of the electrode tip, the temperature near the interface approached ambient temperature but upon cessation of flow, fell back down to its steady low value within a fraction of a second. The effect of salt concentration on the thermal

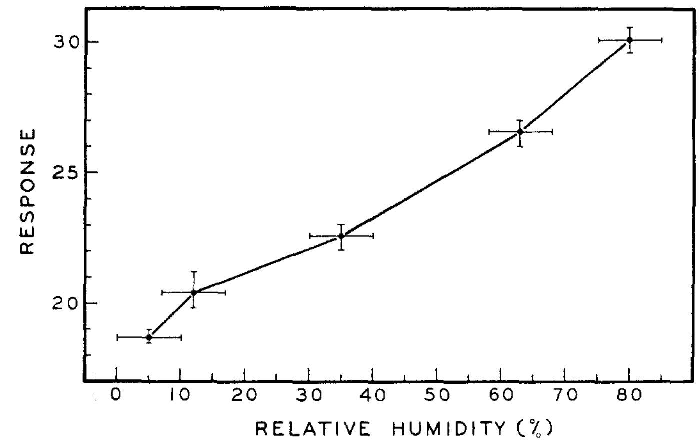

FIGURE 2. Response as a function of relative humidity. Points are averages of the responses of five receptors on one preparation tested 20 times each with 1 M NaCl while the relative humidity was increased and decreased through two cycles. Temperature was held constant well within 1 °C.

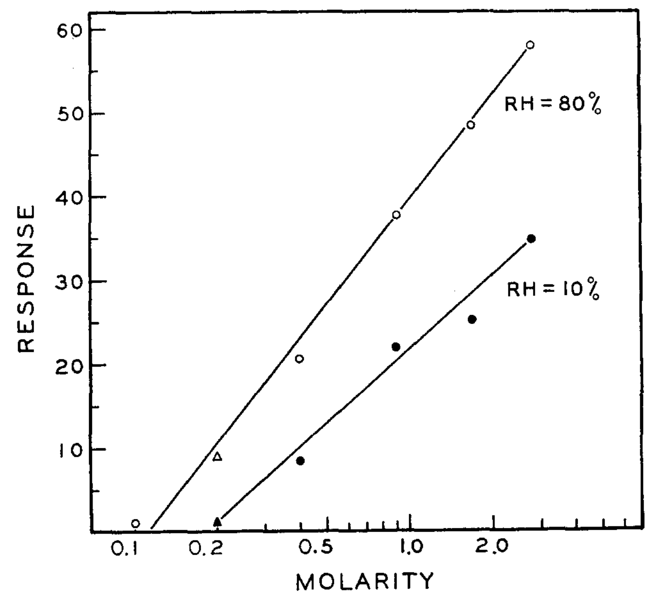

FIGURE 3. Effect of humid and dry air on the NaCl response at several concentrations. Each point is the average of the responses of four and two receptors on preparations 1 (O) and 2 (△) respectively. Relative humidity was varied back and forth between 10 and 80% while testing a given concentration several times, the sequence of preparation 1 being 0.9, 0.4, 2.8, 1.7, and 0.1 M. Twenty-six tests were made per receptor on preparation 1, 4 on preparation 2. The 10% relative humidity response at 0.1 M was below threshold. Since the results were obtained over a long period of time and from different preparations, one would not expect plots at a given relative humidity to be linear. However, their apparent near linearity made normalization unnecessary.

behavior of the stimulating electrode was found to be insignificant. Care was taken to prevent cooling of the thermocouple due to the Peltier effect (Lewis and Randall, 1961) and since thermal conduction along the wires would have tended to raise the recorded temperature towards ambient temperature, these results are probably not artifacts due to the presence of the thermocouple.

The temperature near the air interface of water in vessels of different sizes ranging from 100 μ to 10 cm in diameter and filled to varying degrees under different conditions of relative humidity was measured using the above mentioned technique. Lower relative humidity, smaller vessel diameter, greater degree of filling, or convexity of the interface all resulted in a greater lowering of the temperature from ambient temperature.

Evaporation

The foregoing data strongly indicate that the temperature of the solution in the tip of the stimulating electrode is lowered considerably by evaporation under conditions of low relative humidity. The rate of evaporation from a capillary identical to those used for stimulation was measured during conditions of approximately 25% relative humidity and the rate of heat dissipation due to evaporation calculated to be on the order of 10^{-4} watts. This is more than sufficient to account for a steep fall in temperature of a volume of solution equal to that contained in the tip of the electrode if it were thermally isolated. The measurements with thermocouples indicate that heat flowing into the tip does not reduce the thermal gradient to an undetectable level.

Sidewall Experiment

Recording from the side of the sensillum with an electrode filled with concentrated KCl using the "sidewall" technique described by Evans and Mellon (1962 *a*) often results in a fairly steady frequency of salt spikes. Bringing a sealed capillary containing ice near the tip of the sensillum of such a preparation resulted in marked inhibition of the salt fiber frequency. Bringing a heated capillary which was warm to touch near the tip resulted in a marked increase. It is very unlikely that the temperature of any part of the preparation other than the tip of the sensillum was significantly affected by this procedure.

DISCUSSION

The data warrant certain minimal conclusions. The response of the salt receptor is sensitive to temperature changes at the tip of the sensillum. The Q_{10} is certainly significantly greater than one and probably several times as great. Under conditions of low relative humidity, evaporation occurs from the stimulating solution. If one uses a stimulating electrode of dimensions similar to those employed in these studies, evaporation can rapidly and significantly alter the temperature of the solution near its air interface.

In addition to affecting the temperature of the stimulating solution, evaporation can affect its concentration. Other investigators (Wolbarsht and Dethier, 1958; Evans and Mellon, 1962 *a*; Stürckow, 1963) were aware of possible effects on the concentration of the stimulating solution due to evaporation. However, these effects were not assessed quantitatively. Effects of evaporation on temperature were not considered at all. Stürckow sought to maintain a constant and known concentration of solution in the stimulating electrode by continual replacement by means of a flowing system. The others simply replaced the contents of the stimulating electrode with fresh solution a second or more prior to stimulation with the stationary solution. The rapid rate of evaporation under conditions of low relative humidity suggests that the latter precautions alone are probably inadequate to eliminate variation in concentration due to evaporation.

It is possible that relative humidity can exert an effect on the response of the preparation independent of its effect on the temperature and concentration of the stimulating solution but this would have to be demonstrated by holding the temperature and concentration of the solution constant while varying the relative humidity, which was not done. The current view that the effects of relative humidity on the response were mediated solely via thermal changes in the stimulus seems the simplest hypothesis and will be adopted for the present.

The results suggest experimental precautions to be taken when attempting quantitative study, namely, the careful control of temperature and relative humidity. If, as in the current studies, stimulation is effected with a nonflowing solution, it is suggested that a fresh solution in an environment of constant temperature and high relative humidity be used, if the solution is to be at room temperature. If lower relative humidities are used, care must be taken to insure that the tip of the sensillum is thrust well beyond the interface where evaporation can significantly increase the concentration of the stimulating solution, and that the same or identically constructed electrodes be used, since apparently subtle differences can markedly affect their thermal properties. The validity of quantitative relationships between stimulus and response presented in many publications on insect chemoreception is in doubt because such precautions were not observed.

The results of the sidewall experiment confirm the dependence of the response on the temperature of the tip of the sensillum. However, in this experiment the site of stimulation of the receptor was unknown. If the spikes were elicited by salt near the recording electrode at the side of the receptor, then temperature could have some effect independent of the action of salt at the tip. However, salt from the stimulating electrode could have been transported to the tip of the sensillum. If so, an observable temperature effect may require salt at the tip of the sensillum. This would suggest that temperature and salt act at the same site. Data bearing on the problem are currently lacking.

The size of the generator potential mechanically elicited from the receptor membrane of the Pacinian corpuscle recorded by Ishiko and Loewenstein (1961) is strongly temperature-dependent, although temperature alone does not stimulate. Their conclusions may apply to the salt receptor.

The temperature dependence of the stimulation process should prove useful in evaluating the importance of things such as different types of binding reactions, enzymatic processes, or ion pumps in the receptor mechanism. However, pending additional data, speculation on this seems premature.

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