BEHAVIORAL AND ELECTRICAL RESPONSES TO SUGARS IN *DROSOPHILA MELANOGASTER*

KUNIO ISONO AND TOSHIHIDE KIKUCHI

Department of Biological Science, Tohoku University,
Sendai 980

Received February 8, 1973

Identification of sugar receptor sites both in insects and vertebrates has been a challenging problem to sensory physiologists as they research for mechanisms involved in the initial process of sugar reception. Some proteins which bind sugar molecules have been isolated from tongues of certain mammalian species, but their relation to sugar reception is not clear. The studies of the chemosensory hairs of dipterous insects such as the blowfly and the flesh fly have a certain advantage, because they have much simpler neural systems: sensory surfaces are localized at the tips of the hairs and each chemosensory cell involved in a single hair is highly specialized to given stimulants (Morita and Shiraishi 1968). A hypothesis that the enzyme α -glucosidase is one of sugar receptor sites in insects has been proposed from the comparisons of various aspects of the enzyme with those of sugar reception in the blowfly (Hansen 1968; Amakawa *et al.* 1972; Kawabata *et al.* 1973; Koizumi *et al.* 1973; Kijima *et al.* 1973), but the existence of several isozymes in the fly has complicated the attempts to prove the hypothesis.

In the past few years some important development has been seen in behavioral genetics, in which behavioral mutants of *Drosophila* are induced to define neural functions underlying complex behavioral pattern; visual senses (Benzer 1967; Hotta and Benzer 1967, 1970; Pak *et al.* 1970), olfaction (Kikuchi 1973a, b), circadian rhythm (Konopka and Benzer 1971) and leg-shaking under ether anaesthesia (Ikeda and Kaplan 1970). For genetic dissection to be successfully made on the systems involved in sugar reception of *Drosophila*, it is necessary to establish a physiological method to assay sugar responses. At present there is no such method available and hence little is known about the sugar response of the fly. This paper describes the nerve response patterns of a labellar chemosensory hair of *D. melanogaster* to stimulation by various sugars and then compares the results with those of the behavioral response to these stimulants.

MATERIAL AND METHODS

D. melanogaster strain: An isogenic line AA75-3 was isolated from the strain AA75-2 (Kikuchi 1973b), which was originally established from the *Oregon R* wild strain, by the following mating: a virgin female of the strain AA75-2, to which an

attached X chromosome was inserted, was mated with a male of the marker strain having a genotype *Cy/Pm*; *Ubx/Sb*. A female hybrid, *Cy/+*; *Ubx/+* with an attached X chromosome, was then backcrossed to a single male of the marker strain. The homozygote (strain AA75-3), the autosomes of which came from one side of the chromosomal pair of the strain AA75-2, was isolated in descendents.

Electron microscopy: External features of the mouth parts and labellar hairs of the fly were examined by scanning electron microscopy. The whole fly was carefully air dried to eliminate surface distortions and then mounted with cement onto a metal stub and gold coated according to the method of Beilder (1969).

Behavioral assay: Flies aged 0 to 3 days, reared on an usual corn-yeast medium, were allowed to take for 2 hrs 0.1 M sucrose soaked in filter paper. They were then starved for 20 hr at 20°C. About a hundred flies thus treated were transferred to a petri dish (85 mm inside diameter and 20 mm depth), in which four glass rings (22 mm inside diameter and 5 mm depth) were fixed with cement onto the bottom of the dish. The rings were filled with appropriate stimulants: a pair of rings placed opposite each one were filled with freshly prepared 2 per cent agar solution (1 ml each), whereas the others were filled with 1 ml of sugar in 2 per cent agar (Figure 1). Each of the dishes with flies was arranged on a plate glass set in an apparatus for a flash light photograph and then set in a dark room maintained at 25°C. The apparatus employed, shown in Figure 2, consisted of a box without a cover (*B*, 50×50×50 cm), a flash lamp (*FL*) with a lens (*L*), a plate glass (*PG*, 50×50 cm width and 3 mm thickness) and

Fig. 1. Photograph of petri dish with four rings. W, water-ring with 1 ml of 2% agar solution alone; S, sugar-ring with 1 ml of sucrose in 2% agar. Each tiny spot represented is an individual fly printed by flash light photography.

Fig. 2. Apparatus for flash light photography. *B*, a box without cover; *PG*, a plate glass; *PB*, a black plastic board; *PP*, a printing paper; *PD*, petri dishes with glass rings; *FL*, a flash light lamp with a lens (*L*).

a black plastic board (*PB*, the same make-up as the plate glass *PG*), one edge of which was fixed with hinges. This was a device for routine exchange of the printing papers (*PP*) put between the plate glass (*PG*) and the plastic board (*PB*). The apparatus could provide simultaneously the prints of 20 dishes. The flash light, with a duration of approximately 0.3 second, was operated by a button switch set apart from the apparatus. The prints showed spots of the flies licking given containers in the four rings and those loitering at the bottom of the dishes, but not the flies hanging beneath the covers of the dishes approximately apart 25 mm from printing paper. The distribution of flies among the four rings were monitored every 15 minutes through a 2 hr-test period. The printing paper between the glass and the board was replaced immediately after exposure with a flash light (the behavior of flies was not disturbed through the above operation).

The response of the flies to a given concentration of sugar and at a given time was evaluated as the ratio of the individual numbers printed on the paper in the two sugar-rings to the total number printed in the four rings. Response value for sugar (*S*) vs. water (*W*) at a given time (*t*) was represented as $K_{S/W}^t$. Eight values obtained from every-15-minute-measurements were usually independent of the time elapsed, but, when high concentrations of the sugar were presented, they exhibit a remarkable diminution as a function of the time. The values obtained during the first 15 minutes showed a considerable fluctuation, presumably due to some excitation of the flies. For those reasons the response value for a given sugar concentration was defined as the value at the 15 minutes ($K_{S/W}^{15}$), which was estimated by the "y-intercept" at 15 minutes

Fig. 3. Time-course in response to 1, 0.1 and 0.01 M sucrose solutions. Magnitude of response is represented as the response value $K_{S/W}$: the ratio of number of flies found on sugar-rings containing sucrose in 2% agar to the total number of flies found both on sugar- and water-rings containing 2% agar alone. P, R and Q represent the estimated values at 15 min. ($K_{S/W}^{15}$) for 1, 0.1 and 0.01 M sucrose, respectively.

of the regression line obtained from the eight values (see Q and R in Figure 3). For the high sugar concentrations, to which response values showed a remarkable decrease with time, the initial four to six plots were employed (see also P in this figure).

Recording of nerve response: The stimulation and the recording of the nerve response were performed at the tip of the labellar sensory hair. A fly aged 0 to 2 days was cooled and its legs and wings were removed. The fly was then entered with an indifferent electrode (about $100\ \mu$ tip diameter and filled with Ephrussi and Beadle's solution) through the thorax into the back of the head. The technique for stimulation and recording for nerve response was the "tip recording method" which was employed on the blowfly, *Phormia regina* (Hodgson *et al.* 1955); A glass capillary with a tip diameter of 50 to $70\ \mu$, which served as both a stimulator and a recording electrode, was filled with sugar dissolved in 10^{-4} M NaCl solution. The recording electrode was maneuvered by a micromanipulator until the tip of a single hair just penetrated the surface film of the electrode solution. The stimulation led to evoke nerve response originating both in the "sugar receptor" and in the "salt receptor". Application of higher concentrations of NaCl solution resulted in a greater conductivity, but also resulted in the inhibition of the spikes initiated in the sugar receptor and made it difficult to discriminate the spikes originating in the sugar receptor from those originating in the

salt receptor. Therefore, 10^{-4} M NaCl was employed in the present experiments. The recording electrode was coupled with a capacitor to filter a.c. potential changes. The capacitor was connected to the input of a preamplifier to record potential changes between electrode pairs. Potential changes thus amplified were recorded by a cathode-ray oscilloscope and then photographed. The duration of the stimulation was within 1 second. Stimulations to the single hair were repeated at intervals of at least 5 minutes which seemed enough to recover sugar receptor function. The magnitude of the response was evaluated by the number of spikes originating in the sugar receptor during the period of 0.2 to 0.4 second after the initiation of each stimulation. All experiments were performed at 20°C in a relative humidity range from 60 to 70.

Chemicals: D-Glucose and D-xylose were of reagent grade purchased from Tokyo Kasei Co.; maltose, sucrose, D-fructose, D-galactose, lactose and D-mannose were of the same grade from Kanto Chemical Co. Agar was obtained from Kanto Chemical Co.

RESULTS AND DISCUSSION

Behavioral responses to sugars

The response time-course of the flies transferred to the petri dishes, in which they were allowed to choose freely either of the four rings filled with water or sugar solution, was examined with eight sugars: sucrose, maltose, fructose, glucose, xylose, mannose, galactose and lactose. Figure 3 shows typical examples of the behavioral responses to 1, 0.1 and 0.01 M sucrose vs. water presented in the four rings through a 2 hr-period. The response of the flies to 0.1 M sucrose vs. water is definitely independent of the time elapsed, showing response values ($K_{S/W}$) as high as 0.92 to 0.99. The time-course in response to 0.01 M sucrose indicates that the response is approximately independent of the time, giving an average value of about 0.78. When 1 M sucrose is presented, the response of the flies to this sugar (as compared with that of water) decreases as a function of time and falls to a low level of 0.56 at the end of the period, while the flies show the highest degree of preference: close to the value of 0.98 at the beginning of the period. Response curves similar to the one for 1 M sucrose were obtained with all of the other seven sugars: 1 M fructose, 1 M lactose, 1 M maltose, 1 M mannose, 1 M galactose, 3×10^{-1} M glucose and 3×10^{-1} M to 1 M xylose.

One possible explanation for the above-mentioned effect is that the sugar contamination on the surface of the water-rings caused by flies freely choosing among the four rings leads to increase the number of the flies appearing on the water-rings, whereupon the response value for a given sugar decreases. Figure 4 shows the number of flies appearing on the water-rings through a 2 hr-period, as compared with the number on the rings containing 1 M sucrose. The number of flies appearing on the water-rings, shown in this figure, increases as time elapsed, which indicates the possibility that the flies tend to choose the water-rings in order to lick some contaminated sugar. The flies appearing on the sugar-rings give remarkable changes in response: the number of flies on the sugar-rings decreased with time and fell to a level of 12 per cent of the number observed on the sugar-rings after the first 15 minutes. It appears there-

Fig. 4. Changes in the number of flies found on sugar-rings containing 1M sucrose in 2% agar, and the number on water-rings containing 2% agar alone.

fore that the change in the value for 1M sucrose is due to a decrease in the number of the flies on the sugar-rings rather than an increase in the number of flies on the "contaminated" water-rings. On the other hand, it has been demonstrated that the feeding of high concentrations of the appropriate sugars to the blowfly (*Phormia*) elevated its tarsal threshold to sucrose, fructose and glucose (Evans and Dethier 1957). The changes in the responses of *Drosophila* might be brought by the ingestion of given sugars.

Figure 5 shows the relation of the concentration of the eight sugars (sucrose, maltose, fructose, glucose, lactose, mannose, galactose and xylose) to the response of the flies represented as the response value for the given sugar at 15 minutes ($K_{S/W}^{15}$) (see Method). The magnitudes of the behavioral responses to given sugars expressed as the value $K_{S/W}^{15}$ increase approximately as a sigmoid function for the logarithm of the stimulus concentrations. The responses for sucrose, maltose, glucose, fructose and xylose, thus tend to level off at the high concentrations, whereas for lactose, galactose, and mannose, the responses are still not saturated at the highest concentration examined. For comparison of the responses among the eight sugars, the sensitivity of the flies for given sugars was defined conveniently as the concentration value $C_{0.75}$, which gives the response value ($K_{S/W}^{15}$) of 0.75. The eight sugars thus divided themselves into two groups according to their degree of sensitivity defined as $C_{0.75}$ (Table 1): (1) maltose, sucrose, glucose and fructose to which the flies are substantially sensi-

Fig. 5. Relation between logarithm of sugar concentration and response value at 15 min. ($K_{S/W}^{15}$). The response value was estimated by the method shown in Figure 3.

Fig. 6. A—Ventral view of the mouth parts (proboscis) of *Drosophila* with scanning electron microscopy. Magnification: 300 $\times$. There appear two types of labelar hair: long hair (type L) and short hair (type S). B—Side view of type L hair; C—Side view of type S hair. Notice the tips of both hairs forking into two branches. Magnification for B and C: 3,000 $\times$.

tive, giving the $C_{0.75}$ values of 1.7×10^{-3} , 3.9×10^{-3} , 7.0×10^{-3} and 2.8×10^{-3} M, respectively; (2) lactose, xylose, galactose and mannose to which the flies are less sensitive than to the former, showing at least tenfold $C_{0.75}$ values of the former: 6.9×10^{-2} , 1.3×10^{-1} , 2.0×10^{-1} and 3.2×10^{-1} M, respectively.

External structure of labellar hair

To investigate the chemosensory hairs sensitive to those sugars, the external structures of the labellar hairs were examined by scanning electron microscopy. As shown in Figure 6, there appear two types of labellar hairs: long hairs (*L*) 2–3 μ in diameter at the base and 45–55 μ long, and short hairs (*S*) 2–3 μ in diameter at the base and 20–30 μ long. The noteworthy points of the labellar hair of *Drosophila* demonstrated in this figure are as follows:

- (1) the tip of the hair shows a characteristic structure, forking into two branches, which enables us to discriminate the chemosensory hair from others;
- (2) the hair length is larger than in expected from its body size; and
- (3) the number of labellar hairs shown in *A* of this figure is small as compared with that of the blowfly, which offers an advantage for the stimulation and recording of nerve response from a single hair. The type *L* hair was then employed in the present electrophysiological studies.

Nerve responses of labellar chemosensory hairs

Figure 7 shows the photographs of typical records obtained from a stimulation of 2×10^{-1} M sucrose with 10^{-4} M NaCl as an electrolyte to the tip of the type *L* hair (*c* in Figure 7), comparing them with those obtained from the stimulations of the elec-

Fig. 7. Photographs of spike potentials appearing on oscillograph screen in response to stimulation by 10^{-4} M NaCl (upper record (a)), 10^{-1} M NaCl (middle (b)) and 2×10^{-1} M sucrose plus 10^{-4} M NaCl (lower (c)). *N*, *S* and *W* express the spikes originating in salt, sugar and water receptor, respectively.

trolytes, 10^{-4} and 10^{-1} M NaCl (*a* and *c*). The electrical responses from a single hair consisted either of one or two of the three series of spikes potentials (*N*, *W* and *S* in Figure 7), each series apparently originating in a single neuron. The larger spike (*N* in *a* and *b*) predominated when electrode contained NaCl alone; the middle spike (*S* in *c*) dominated when electrode contained 2×10^{-1} M sucrose solution with 10^{-4} M NaCl; and the smallest one (*W* in *a*) was frequently evoked with the *N* spike by a trace of electrolyte such as 10^{-4} M NaCl, indicating that the spike *W* originated in the water receptor. The spike *S* was also initiated by maltose, glucose and fructose (not shown), which indicates the spike *S* originates in the sugar receptor, whereas the spike *N* evoked by stimulations of KCl, LiCl and NaCl originates in the salt receptor. Two series of the spikes, *N* and *S*, represented above presumably correspond to the *L* and *S* fiber responses described and based on the preliminary experiments on certain labelar hairs of the *Drosophila* (Hodgson and Reoder 1956). Initiation of the spike *W*, originating in the water receptor, was however inhibited to some degree both by 10^{-1} M NaCl and 2×10^{-1} sucrose solutions (*b* and *c*). Application of 10^{-4} M NaCl with 2×10^{-1} M sucrose resulted in the diminution of the frequencies of the *N* spike, as shown in the record *c*. NaCl initiates a low frequency of spontaneous spikes under conditions approaching zero stimulation (not shown). This might be comparable to the fact that water initiates impulses in the salt receptor of the fleshfly, *Boettchrisca* (Morita 1967).

Figure 8 shows the response curves of maltose, sucrose, glucose and fructose. The

Fig. 8. Nerve response curve for maltose, sucrose, glucose and fructose plotted against logarithm of sugar concentration. Magnitude of response is represented as the number of impulses from type *L* hair during the period of 0.2–0.4 second after onset of stimulation.

Table 1. Comparison of sugar concentrations giving a response value of $0.75(C_{0.75})$ in preference-aversion tests, with their maximal numbers of impulses per 0.2 seconds (R_m) from a labellar hair and with their concentrations giving half values of $R_m(C_{Rm/2})$.

Sugars	Concentrations (M)		Maximal numbers of impulses/0.2 sec
	$C_{0.75}$	$C_{Rm/2}$	
Maltose	1.7×10^{-3}	4.8×10^{-3}	29.4
Sucrose	3.9×10^{-3}	5.0×10^{-3}	26.6
Glucose	7.0×10^{-3}	5.6×10^{-3}	21.6
Fructose	2.8×10^{-3}	3.0×10^{-3}	14.6
Lactose	6.9×10^{-2}	3.0×10^{-1}	—
Xylose	1.3×10^{-1}	$1.0 <$	—
Mannose	3.2×10^{-1}	$1.0 <$	—
Galactose	2.0×10^{-1}	$1.0 <$	—

magnitude of the nerve response is represented as the number of impulses evoked during the period from 0.2 to 0.4 second after initiation of the stimulation to the tip of the labellar hair. The magnitude of the response increases approximately as a sigmoid function of the logarithm of the sugar concentration. The responses thus saturate or level off at the concentration of 10^{-2} to 10^{-1} M, but the maximal responses differ remarkably from sugar to sugar. Maltose and sucrose yield substantial impulse frequencies of 29.4 and 26.7 per 0.2 second, respectively, whereas glucose yields relatively small responses (21.6 per 0.2 second). Fructose yields the smallest response among the four sugars examined, giving approximately half the value of maltose (Table 1). However, the concentrations of the four sugars ($C_{Rm/2}$) which give the half value of the maximal response ($Rm/2$) are almost the same, showing the $C_{Rm/2}$ values of 4.8×10^{-3} , 5.0×10^{-3} , 5.6×10^{-3} and 3.0×10^{-3} M for maltose, sucrose, glucose and fructose, respectively (Table 1). These values ($C_{Rm/2}$) obtained from the nerve responses correspond roughly to the concentration values $C_{0.75}$ estimated from the behavioral responses, the $C_{0.75}$ values being 1.7×10^{-3} M for maltose, 2.8×10^{-3} M for fructose, 3.9×10^{-3} M for sucrose and 7.0×10^{-3} M for glucose.

For the other four sugars, xylose, lactose, mannose and galactose, the attempts to record nerve response from the labellar chemosensory hairs (type *L*) have not been successful, even when the highest concentration of the sugars such as 1 M xylose, 1 M galactose, 1 M mannose and 3×10^{-1} M lactose were applied. Since the *Drosophila* could discriminate, as shown in Figure 5, each of those sugars from water in the preference-aversion tests, the fact that the type *L* hair examined is insensitive to those sugar implies that there remain unknown sensory hairs sensitive to these sugars.

The results obtained from the present experiments indicate that the *Drosophila* is substantially sensitive to a variety of sugars. The fact that the labellar hairs examined were not sensitive to sugars such as lactose and xylose, to which the fly showed a behavioral response, demands a further investigation for other types of chemosensory

hairs sensitive to those sugars. Since it is possible to isolate the behavioral mutants of *Drosophila* (Benzer 1971; Kikuchi 1973b); the success, both in single hair recording and in the development of a simple method for behavioral assay, may pave the way to the analysis of a complex system of contact chemoreception.

SUMMARY

A simple method for the measurement of the behavioral responses of *Drosophila melanogaster* to the various sugars and a method for recording nerve responses from a single chemosensory hair were described and the results were analyzed. The fly showed preference for maltose, sucrose, glucose, fructose, xylose, lactose, mannose and galactose, especially for the former four sugars, in the preference-aversion test between sugar and water. Electrophysiological responses from a labellar hair by stimulation of those sugars showed that the labellar hair examined was sensitive to maltose, sucrose, glucose and fructose, giving a series of spikes different from those initiated by NaCl and water. The magnitude of nerve responses to these four sugars was arranged in the order of the maximal number of impulses from high to low as follows: maltose, sucrose, glucose and fructose. The labellar hair examined was insensitive to xylose, lactose, mannose and galactose.

ACKNOWLEDGMENTS

We wish to thank Drs. H. Morita and A. Shiraishi for helpful suggestions and Miss Y. Shinohara for excellent technical assistance. This work was aided in part by Scientific Research Fund of the Ministry of Education of Japan (Nos. 4050 and 738040).

LITERATURE CITED

- Amakawa, T., K. Kawabata, H. Kijima, and H. Morita, 1972 Isozymes of α -glucosidase in the proboscis and legs of flies. *J. Insect Physiol.* **18**: 541-553.
- Beidler, L. M., 1969 The use of the SEM in sensory biology. *Proc. Engis Stereoscan Colloquim* 109-122.
- Benzer, S. 1967 Behavioral mutants of *Drosophila* isolated by countercurrent distribution. *Proc. Nat. Acad. Sci.* **58**: 1112-1119.
- Benzer, S., 1971 From the gene to behavior. *J. Amer. Med. Assoc.* **218**: 1015-1022.
- Dethier, V. G., D. R. Evans, and M. V. Rhoades, 1956 Some factors controlling the ingestion of carbohydrates by the blowfly. *Biol. Bull.* **111**: 204-222.
- Evans, D. R., and V. G. Dethier, 1957 The regulation of taste thresholds for sugars in the blowfly. *J. Insect Physiol.* **1**: 3-17.
- Hansen, K., 1969 The mechanism of insect sugar reception, a biochemical investigation. In "Olfaction and Taste" 3 (Paffman ed.) pp.382-391. Rockefeller University Press, New York.
- Hodgson, E. S., J. Y. Lettvin, and K. D. Roeder, 1955 Physiology of primary chemoreceptor unit. *Science* **122**: 417-418.
- Hodgson, E. S., and K. D. Roeder, 1956 Electrophysiological studies of arthropod chemoreception. I. General properties of the labellar chemoreceptors. *J. Cell. Comp. Physiol.* **48**: 51-76.

- Hotta, Y., and S. Benzer, 1969 Abnormal electroretinograms in visual mutants of *Drosophila*. *Nature* **222**: 354-356.
- Hotta, Y., and S. Benzer, 1970 Genetic dissection of the *Drosophila* nervous system by means of mosaics. *Proc. Nat. Acad. Sci.* **67**: 1156-1163.
- Ikeda, K., and W.D. Kaplan, 1970 Unilaterally patterned neural activity of gynandromorphs: mosaic for neurological mutant of *Drosophila melanogaster*. *Proc. Nat. Acad. Sci. U.S.A.* **67**: 1480-1487.
- Kawabata, K., H. Kijima, A. Shiraishi, and H. Morita, 1973 α -Glucosidase isozymes and labellar sugar receptor of the blowfly. *J. Insect Physiol.* **19**: 337-348.
- Kijima, H., O. Koizumi, and H. Morita, 1973 α -Glucosidase at the tip of the contact chemosensory seta of the blowfly, *Phormia regina*. *J. Insect Physiol.* **19**: 1351-1362.
- Kikuchi, T., 1973a Specificity and molecular features of an insect attractant in a *Drosophila* mutant. *Nature* **243**: 36-38.
- Kikuchi, T., 1973b Genetic alteration of olfactory functions in *Drosophila melanogaster*. *Japan. J. Genetics.* **48**: 105-118.
- Koizumi, O., H. Kijima, K. Kawabata, and H. Morita, 1973 α -Glucosidase activity on the outside of the labella and legs of the fly. *Comp. Biochem. Physiol.* **44B**: 347-356.
- Konopka, R.J., and S. Benzer, 1971 Clock mutants of *Drosophila melanogaster*. *Proc. Nat. Acad. Sci. U.S.A.* **68**: 2112-2116.
- Morita, H., 1967 Effects of salts on the sugar receptor of the fleshfly. In "Olfaction and Taste" 2 (T. Hayashi ed.) pp.787-798 Pergamon, New York.
- Morita, H., and A. Shiraishi, 1968 Stimulation of the labellar sugar receptor of the fleshfly by mono- and disaccharides. *J. Gen. Physiol.* **52**: 559-583.
- Pak, W.L., J. Grossfield, and K.S. Arnold, 1970 Mutants of the visual pathway of *Drosophila melanogaster*. *Nature* **227**: 518-520.