Stimulation of the Salt Receptor of the Blowfly

III. *The alkali halides*

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ABSTRACT Application of solutions of each of the alkali halides to the tip of a labellar sensillum of the blowfly elicited a repetitive neural discharge from the salt receptor. The records were qualitatively similar to those for NaCl. For each of the alkali chlorides and sodium halides, the shapes of the curves of the response of the salt receptor as a function of concentration were similar to that for NaCl. The alkali halides exhibited a regular pattern of relative stimulating effectiveness for the salt receptor. The effectiveness of the anions increased monotonically with atomic number. The effectiveness of the cations was greatest for potassium and declined as the atomic number was increased or decreased. This hierarchy for stimulating effectiveness was maintained at all tested molarities. The response to a mixture of two salts appeared to be an average of those to the single salts at concentrations equal to the total concentration of the mixture. Cross-adaptation was observed between the alkali halides. The results indicate that an explanation of stimulation of the salt receptor must apply to all the salts tested and that both the anion and the cation affect a salt's stimulating effectiveness.

Associated with the labellar gustatory sensilla of the blowfly are several primary receptor cells (Wolbarsht and Dethier, 1958; Evans and Mellon, 1962 *a*). One of these responds to certain salt solutions. The effects of stimulation of this cell (termed the salt receptor) with solutions of NaCl have been considered elsewhere (Gillary, 1966 *a*, *b*). In these studies, an attempt was made to broaden the spectrum of stimulation by testing solutions of the other alkali halides and comparing the effects with those of NaCl. Specific questions concerned whether or not all the alkali halides stimulate in the same qualitative manner, the relative importance of the anion and cation in stimulation, and the dependence of stimulating effectiveness on ionic atomic number.

METHODS

Action potentials (spikes) were recorded from the type 1 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. The contents of the stimulating electrode were replaced with fresh solution less than 0.1 sec before each test, using a technique described by Evans and Mellon (1962 *a*). Tests were done every 3 min and each lasted less than a second. Stimulation evoked a train of impulses from the salt receptor. Spikes of other fibers were not treated quantitatively. Response is defined as the number of salt receptor spikes which occurred in the 0.5 sec interval beginning 0.1 sec after the onset of stimulation. The methods have been described elsewhere in greater detail (Gillary, 1966 *a*, *b*).

RESULTS

Individual Salts

QUALITATIVE ASPECTS OF RECORDINGS Recordings during stimulation by each alkali halide were similar in exhibiting (*a*) no more than two spike types, which were always clearly distinguishable and of which one was always the salt receptor if the concentration was above threshold, and (*b*) a regular frequency of salt receptor spikes whose time course exhibited the high initial phase and subsequent steady phase seen during NaCl stimulation, except when very concentrated LiCl solutions were used. Furthermore, responses to all alkali halides exhibited the reproducibility characteristic of the NaCl response, and so could be dealt with quantitatively. Typical recordings during stimulation with 1 M alkali chloride and sodium halide solutions may be seen in Fig. 1.

RESPONSE VS. CONCENTRATION The shape of the response vs. concentration curve for each of the alkali chlorides and sodium halides was determined by testing a sequence of ascending concentrations. The responses to each salt fell on smooth curves which were similar to that of NaCl in exhibiting a threshold above which the response rose at a declining rate as the concentration was raised. The plots of response vs. the logarithm of the molarity through 3 M were linear for NaCl, NaBr, NaI, KCl, and RbCl, within experimental precision. The plot for NaF was also typical but could be extended over only a small range since its solution saturates at about 1 M. That for CsCl was linear through 2 M. Above this, the responses to tests at about 3.5 M (4 molal), 8 molal, and the saturated solution leveled off to a constant value. The LiCl curve was also approximately linear through a concentration of 2 M after which it leveled off to a plateau value through about 5 M (5.5 molal). Responses to 8 molal LiCl were about half those of the plateau, and those to 20 molal still less. However, while all other tests yielded the typical time course of spike frequency, stimulation at the last two concentrations yielded a continually de-

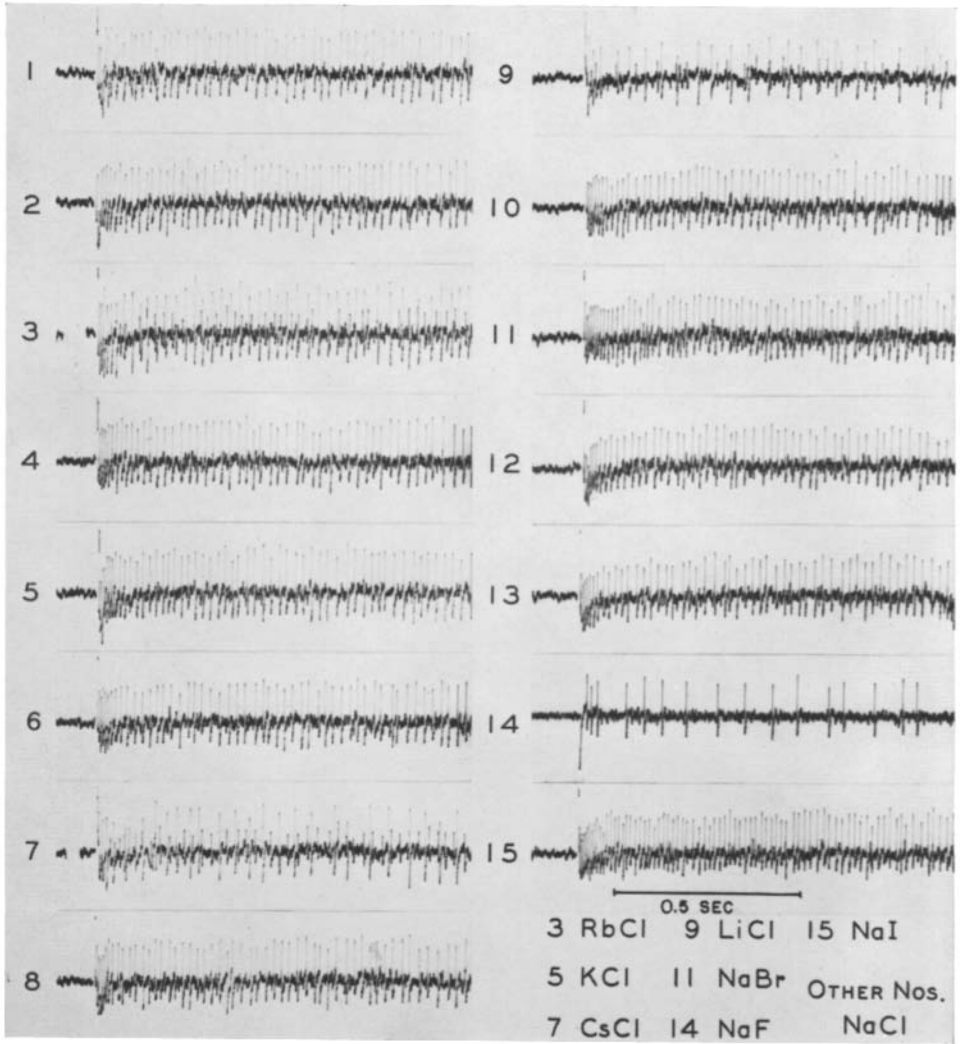

Figure 1. Records during stimulation with 1 m alkali halide solutions. The first 13 records are all those obtained from a single sensillum in their tested order. The last two are from different sensilla of similar sensitivity under similar test conditions. Salt spikes are 0.5 mv in height and initially positive.

clining frequency with no apparent steady phase. This apparent anomaly, which is also described by Evans and Mellon (1962 *b*) and Mellon (1961), may have been the result of some secondary effect due to these extremely concentrated solutions, since their thermodynamic activities are 41 and 1,250, respectively (Robinson and Stokes, 1959).

comparison of responses The relationship of the response vs. concentration curves of the alkali chlorides and sodium halides can be seen in Figs. 2

and 3, respectively. In these experiments, a small part of the response curve of one salt was compared with that of another, usually NaCl. Each curve for a given salt is the result of tests on many different preparations at widely different times. In addition, adaptation could have differentially influenced the precise relationships between the curves at different concentrations. Hence, it is not surprising that in the figures, experimental points for a given salt do not always fall on the smooth curve obtained when an ascending series of concentrations was tested on a single receptor during a relatively short time.

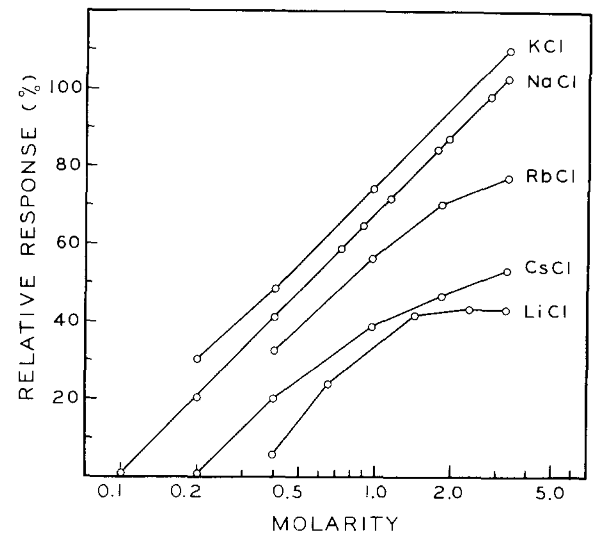

FIGURE 2. Comparison of alkali chloride responses at different concentrations. Experimentally, comparisons were made by repeated alternating tests with NaCl. NaCl responses were defined to be on a linear plot of response vs. log M with a 0.1 M threshold and 100% response at 3 M. Results are averaged from more than 850 tests on more than 80 receptors.

What is clearly demonstrated is that the different salts were differentially effective as stimuli. At no point do the individual curves cross each other but instead maintain a constant hierarchy for stimulating effectiveness at all concentrations.

Although the data in Figs. 2, 3, and 5 are plotted using a molarity scale, experimental comparisons were made with solutions of equal thermodynamic activity. The relative deviation between the two concentration scales for NaBr and NaI at high concentrations is considerable, as can be seen from Fig. 3. Plotted on an activity scale, the response curves intersect at these high concentrations. This was not encountered for data from tests using any other concentration or any other salt, whether the scale was molarity, molality, or activity. Subsequent experiments comparing NaBr and NaI at 2 M concentrations, not included in the figure, showed the NaI response to be significantly greater than that to NaBr, as would be extrapolated from the data at lower concentrations where the relative deviations between scales are smaller. The absence of this anomalous intersection for equal molar comparisons supports the view that molarity may be a more useful scale to use than thermodynamic activity.

The relative responses of the salt receptor to stimulation by 1 M alkali halide solutions are presented in Fig. 4. LiF is excluded because its solution saturates at about 0.1 M, at which concentration it was nonstimulating. The regular pattern of relative stimulating effectiveness is evident.

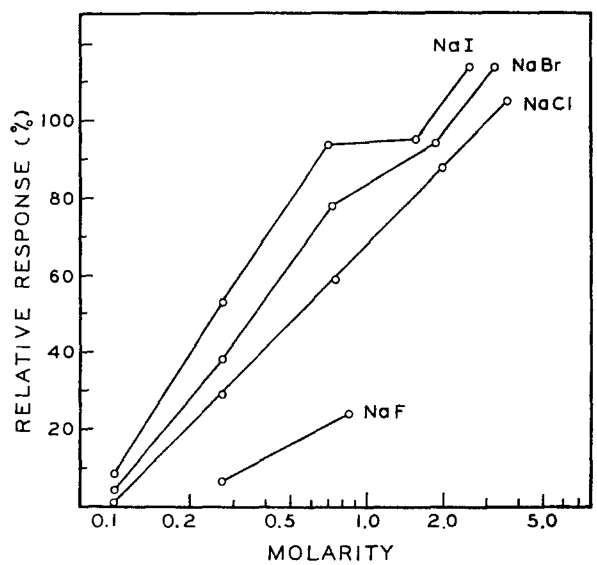

FIGURE 3. Comparison of sodium halide responses at different concentrations. Data were obtained and plotted as described for Fig. 2. Results are averaged from more than 450 tests on more than 60 receptors.

Mixtures

The effects of stimulation with mixtures of two salts were examined, one always being NaCl and the other, another alkali chloride or sodium halide. A mixture of equal concentrations with respect to the two salts was compared with the responses of the same receptor to tests by the individual salts made up to the same total concentration as the mixture.

Early experiments employed mixtures including several concentrations each of KCl and CsCl and one of LiCl. In these, the concentration scale was thermodynamic activity; Harned's rule (Robinson and Stokes, 1959) was employed in calculations for making up the mixtures. In later experiments, 1 M mixtures including RbCl, NaBr, and NaI, and 0.5 M mixtures containing NaF were tested and compared in the above manner. The results of the above experiments were clear and independent of whether activity or molarity was used.

Qualitatively, the recordings during stimulation by mixtures appeared to be related to both of the recordings during stimulation with solutions of the individual salts. All NaCl records had practically only salt spikes. The mixture records had salt spikes and sometimes the smaller type, depending on whether or not it was also present during stimulation by the other pure salt.

Within the experimental precision, the response to the mixture was equal to the average of the responses to the two solutions containing single salts at

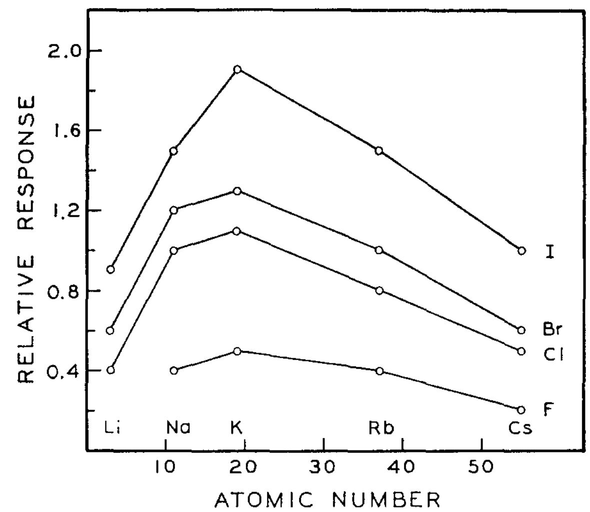

FIGURE 4. Relative stimulating effectiveness of 1 M alkali halides. Salts were compared by repeated alternating tests between two stimuli on a number of sensilla yielding regular and reproducible responses, averaging the responses to their respective stimuli, and calculating the ratio. Experimentally, comparisons were made as follows: (*a*) All alkali chlorides were compared with NaCl. (*b*) All sodium halides were compared with NaCl and in addition, NaI was compared with NaBr. (*c*) All other alkali halides were compared with at least two other salts, the Na salt of common anion and the chloride of common cation if a fluoride or bromide, and the bromide of common cation if an iodide. All ratios were consistent with those plotted within ± 0.1. There were no exceptions from any receptor to the order of ratios plotted in the figure.

concentrations equal to the total concentration of the mixture. The data definitely excluded the possibility that the response to the mixture was equal to the sum of the responses to solutions of the individual salts at their respective individual concentrations in the mixture. Data for mixtures of CsCl and NaCl are presented in Fig. 5.

Cross-Adaptation

Experiments were performed to see whether stimulation with one salt affects the response to tests by another. Each alkali chloride and sodium halide was paired with an equal molar (1 or 2 M) NaCl solution. First, one salt of a pair was tested at fixed intervals. Between tests, either nothing, the same salt, other salt, or pure water was applied to the sensillum for a considerably longer time than the usual test duration of less than 1 sec. The relative effects of these on the response to the tested salt were examined. Then the second salt of the pair was tested and the procedure repeated.

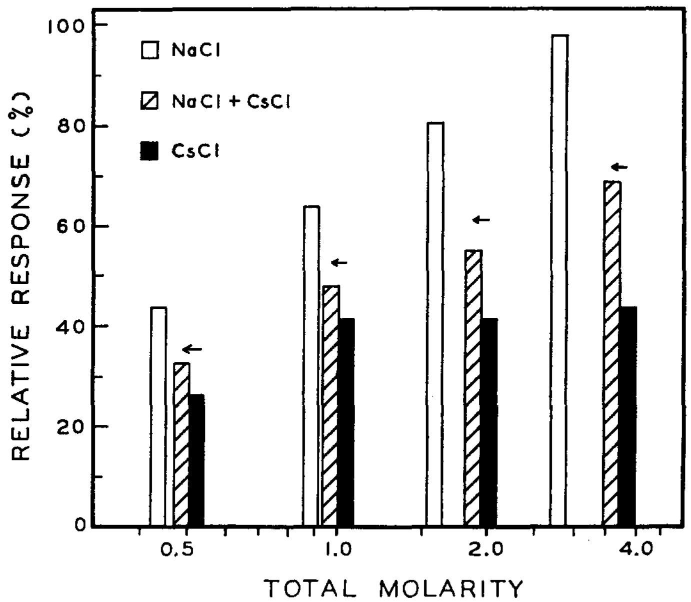

FIGURE 5. Comparison of the responses to mixtures of CsCl and NaCl with those to the individual salts. On a given preparation, three solutions of NaCl, NaCl + CsCl, and CsCl respectively, all of which had equal total thermodynamic activities, were repeatedly tested on several receptors in a varied sequence. This was done at each of four activities. Responses were averaged from more than 420 tests on 46 receptors. NaCl responses were defined to be on a linear plot of response vs. log M with a 0.1 M threshold and 100% response at 3 M. The dispersion along the abscissa within each group of responses to tests at a single activity is a result of conversion from the activity scale to the molarity scale. Arrows indicate the average of the responses to the two solutions of the single salts whose respective molarities equal the total molarity of the mixture.

Adaptation is used to mean (*a*) that the response to the tested salt was decreased by prior stimulation with salt more than it was by application of water or nothing at all to the sensillum, (*b*) that this decreased response exhibited the normal frequency time course, and (*c*) that this decrease was reversible; i.e., the response returned towards its former unadapted value with time when unstimulated. Adaptation was seen to some degree in all cases. NaCl stimulation resulted in adaptation of responses to tests by each of the other alkali chlorides and sodium halides, and application of each of these other salt solutions resulted in adaptation of the NaCl response. In addition, prior stimulation with each salt resulted in adaptation of its own response.

Salts which yielded higher responses appeared to be more effective in adapting the salt receptor. This suggests that the ability of a solution to adapt is a function of the neural response it elicits and not some simple function of its bulk concentration. However, this conclusion can be arrived at with confidence only after more careful, quantitative experiments corroborate this.

DISCUSSION

Several important conclusions are suggested by the data. First of all, the similarity of (*a*) the quality of the recordings during stimulation and (*b*) the relationships of response vs. concentration for all single alkali halide solutions tested as well as for mixtures strongly suggest that any theory of stimulation should apply to all these salts. There seems to be no current reason for the exclusion of any salt tested on the basis that it stimulates atypically, as was asserted by Evans and Mellon (1962 *b*). Second, the alkali halides differ in their relative ability to stimulate the salt receptor. Furthermore, in addition to the significant role of the cation in stimulating effectiveness, the data clearly indicate that the anion is also very important. This is contrary to the beliefs of Mellon (1961) and Evans and Mellon (1962 *b*) that only the cations play a significant role in stimulation.

The data of Fig. 4 are consistent with the idea that the effects of the cation and anion are additive. However, while there is little doubt that the order of ratios presented in Fig. 4 is correct, the absolute values may not be. This is because they were obtained experimentally from responses from repeated tests between different salts of equal concentration, which admits the possibility of error due to adaptation. More accurate values may be obtainable by comparing the concentrations of different stimuli yielding the same response.

The effect of the anion on the relative stimulating effectiveness of alkali halides of a given cation is a monotonically increasing function of its atomic number and so could be simply correlated with any of many ionic properties which are direct functions of atomic number. However, this is clearly not so for the cations. Many parameters of the stimulating solutions were examined in an attempt to find one which correlates with the observed pattern of stimulating effectiveness. This attempt proved unsuccessful. Two different ionic properties which are monotonically correlated with atomic number could oppositely influence an ion's effectiveness in stimulation, resulting in the observed optimum. Furthermore, this optimum could be affected by events in the sensillum other than those at the primary receptor site (see Gillary, 1966 *a*, *b*). However, at present it seems best simply to state that such an optimum exists.

If the cationic optimum is due to events at the receptor membrane, several mechanisms described by others could be applicable in explaining the data described here. Mullins (1960) has proposed a theory relating the rate of passage of ions through pores in a membrane to ionic size, an ion of optimum size having the greatest rate and smaller or larger ones, lesser rates. Results of his experiments on cation movement through amphibian muscle membranes indicate that potassium passes through most easily, rubidium less, and cesium still less. Sodium moves through less readily than does potassium. These data are strikingly similar to those for the relative stimulating effectiveness of cations presented here. If stimulation involves ionic association with a receptor surface, the cationic pattern could be determined simply by some differential affinity or a differential effect after adsorption, both of which might have some determining optimum function of atomic number. However, current evidence is insufficient to support one of these mechanisms preferentially.

The existence of cross-adaptation tends to indicate that adaptation is due to some process common to stimulation by all salts. The observation that a mixture stimulates with an effectiveness of that of the average of the responses to the two individual salts whose respective concentrations equal the total concentration of the mixture indicates that the shape of the response vs. concentration curve probably has a common origin for all salts. If the origin of adaptation and the relationship between response and concentration is at the primary receptor site, an implication is that the different salts could affect the same site. However, if these effects originate subsequently, such as where spikes are initiated, then different salts could act at different primary sites. In the light of the current deficiency of evidence which bears on this problem, it seems premature to attempt to draw specific conclusions regarding the primary receptor site from these results.

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