Further Studies on the Roles of Sodium and Potassium in the Generation of the Electro-Olfactogram

Effects of mono- , di- , and trivalent cations

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ABSTRACT   In the negative EOG-generating process a cation which can substitute for Na^+ was sought among the monovalent ions, Li^+, Rb^+, Cs^+, NH_4^+, and TEA^+, the divalent ions, Mg^{++}, Ca^{++}, Sr^{++}, Ba^{++}, Zn^{++}, Cd^{++}, Mn^{++}, Co^{++}, and Ni^{++}, and the trivalent ions, Al^{+++} and Fe^{+++}. In Ringer solutions in which Na^+ was replaced by one of these cations the negative EOG's decreased in amplitude and could not maintain the original amplitudes. In K^+-Ringer solution in which Na^+ was replaced by K^+, the negative EOG's reversed their polarity. Recovery of these reversed potentials was examined in modified Ringer solutions in which Na^+ was replaced by one of the above cations. Complete recovery was found only in the normal Ringer solution. Thus, it was clarified that Na^+ plays an irreplaceable role in the generation of the negative EOG's. The sieve hypothesis which was valid for the positive EOG-generating membrane or IPSP was not found applicable in any form to the negative EOG-generating membrane. The reversal of the negative EOG's found in K^+- , Rb^+- , and Ba^{++}-Ringer solutions was attributed to the exit of the internal K^+. It is, however, not known whether or not Cl^- permeability increases in these Na^+-free solutions and contributes to the generation of the reversed EOG's.

The ionic mechanisms underlying the generation of the electro-negative and positive "electro-olfactograms" (EOG's) have been studied in previous papers (Takagi and Wyse, 1965; Takagi, Wyse, and Yajima, 1966; Takagi, 1968; Takagi, Wyse, Kitamura, and Ito, 1968). Briefly, the results showed that the negative EOG's primarily depend upon the entry of Na^+ and the simultaneous exit of K^+, and that neither Li^+, tetraethyl ammonium (TEA^+), $choline^+$, hydrazine, nor sucrose can substitute for Na^+; the positive EOG's primarily depend upon the entry of Cl^- and the simultaneous exit of K^+, and three anions, Br^-, F^-, and HCO_2^- can substitute for Cl^-.

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The present experiments were intended to supplement the above work. A substitute cation was sought among many other mono-, di-, and trivalent ions, and the effects of these cations upon the negative EOG's were examined. It is concluded that none of the cations tested can substitute for Na^+ in the generation of the negative EOG's. Finally, ionic mechanisms of the EOG's in other animals are considered.

METHODS

The experimental procedures are entirely the same as in the previous papers (Takagi et al., 1966, 1968).

Preparation

The ceiling part of the olfactory epithelium which covers the olfactory cavity of the bullfrog, *Rana catesbiana*, was excised and used for experiment.

It is well-known that the excised olfactory epithelium produces EOG's for about 3–4 hr just as the excised eye yields the ERG's (Hosoya and Yoshida, 1937; Yoshida, 1950; Ottoson, 1956; Higashino and Takagi, 1964; Shibuya and Takagi, 1963 *a, b*; Takagi and collaborators, 1959, 1960 *a, b, c*, 1964, 1965, 1966, 1968, 1969; Gesteland, Lettvin, and Pitts, 1965). Thus, these scientists used the olfactory epithelium devoid of blood circulation for the study of the EOG's. However, only recently considerable differences between the shapes of the EOG's elicited in the excised olfactory epithelium and the EOG's shapes in vivo (in immobilized bullfrog with *d*-tubocurarine) were pointed out by Takagi, Aoki, Iino, and Yajima (1969). In excised epithelia 122 odorants were examined at three different concentrations (1/36, 1/6, and 1/1). Of these 87 % (106 odorants) produced only the negative EOG's which simply increased in amplitude with increasing concentration (group I odorants). Among the remaining 16 odorants 6 produced only the positive EOG's which simply increased in amplitude with increasing concentration (group II); 7 generated the negative EOG's followed by positive afterpotentials (group III); and the last 3 odorants produced the negative EOG's at low concentration (1/36) and the positive EOG's at high concentration (1/1) (group IV). However, when these odors were examined in vivo, most of them produced only the negative EOG's, and only a few odors among groups II, III, and IV produced the positive on- and -after potentials.

Solutions

Normal Ringer solution had the following composition (mM): Na^+, 117.6; K^+, 2.5; Ca^{++}, 2.0; Cl^-, 121.5; HPO_4^-, 1.1; $H_2PO_4^-$, 0.4. Na^+ in Ringer solution was replaced by one equimolar (117.6 mM) monovalent ion or by one equimolar (84.4 mM) divalent cation, and thus various Na^+-free Ringer solutions were formed. For the sake of simplicity, these isotonic solutions are called Li^+-Ringer solution, or Sr^{++}-Ringer solution and so forth in the present experiment.

Stimulants

Saturated vapors of amyl acetate and ethyl ether were used to elicit the electronegative EOG's of the "on"-type and the "on-off" type, respectively. In earlier experi-

ments saturated vapor of chloroform was applied to elicit the electropositive EOG's. In this way the negative and positive EOG's were elicited (cf. Takagi et al., 1968). Although the positive EOG's were dominant in excised epithelium when chloroform vapor was applied, no considerable difference was found between the EOG's elicited in excised epithelium and in vivo by amyl acetate and ethyl ether. The saturated vapors of these odorants were used in this and previous experiments (Takagi et al., 1966, 1968), because the EOG's elicited by them at lower concentrations were very small in amplitude when recorded with the Ringer-gelatin-ZnSO$_4$-Zn electrodes, and except for their amplitudes, no essential difference was found between the EOG's elicited by the saturated vapors and those elicited by diluted vapors of amyl acetate, ethyl ether, and chloroform (Takagi et al., 1969).

Recording Apparatus

Olfactory receptor potentials were recorded by means of a pair of nonpolarizable (Ringer-gelatin-zinc sulfate–zinc stick) electrodes. The potential changes between these electrodes were amplified with a DC amplifier and recorded with a Nihon-Kohden ink-writing recorder (Tokyo).

Experimental Procedure

At the beginning of each series of experiments the olfactory epithelium was spread flat in a container and immersed in Ringer solution for 5 min. After the fluid level was lowered to the level of the filter paper, the EOG's were recorded and they were used as controls for subsequent trials. Next, Ringer solution was replaced with one of the Na$^+$-free Ringer solutions. The test solution was changed two or three times during the 5 min immersion. After the fluid level was lowered, EOG's were recorded. By repeating these procedures in fresh solutions, temporal changes of the EOG's were examined. The recovery processes of the potentials were studied after the test solution was replaced by normal Ringer solution. Each time three recordings were made for each of the three kinds of potentials. The replacement of the test solution or Ringer solution and the recording of nine EOG's took about 20 min, including 5 min for immersion. The average amplitude of the three EOG's elicited by the same odors in each of the solutions was plotted at intervals of 20 min. Since the similarity between the negative-on and -off EOG's has been shown (Higashino and Takagi, 1964), and since the changes in the negative-off EOG's in test solutions generally resemble those in the negative-on EOG's, only the behavior of the latter is shown in Figs. 2, 4, and 6.

K$^+$-Ringer Test

In a previous paper (Takagi et al., 1968), it was shown that the negative and positive EOG's reversed their polarities when the olfactory epithelium was immersed in K$^+$-Ringer solution in which the concentrations of sodium and potassium ions were simply reversed; but both recovered completely or nearly completely when the K$^+$-Ringer solution was replaced by normal Ringer solution after the epithelium had been immersed in the K$^+$ solution for only 5 min; moreover, the recoveries progressed or continued steadily during the several subsequent immersions in normal Ringer solutions only. But such striking recoveries never occurred in Li$^+$- and other Ringer

solutions. This phenomenon was used to determine whether cations or chemicals can substitute for Na^+. In the present experiment, the same experimental procedure was repeated to seek a substitute for Na^+ among many cations. For convenience this experimental procedure is referred to as the "K^+-Ringer test" in the present paper. Since the mere finding that the negative EOG's decrease in amplitude in several Na^+-free Ringer solutions is not considered sufficient evidence, this test was performed to confirm and to clarify the role of Na^+ in the generation of the negative EOG's.
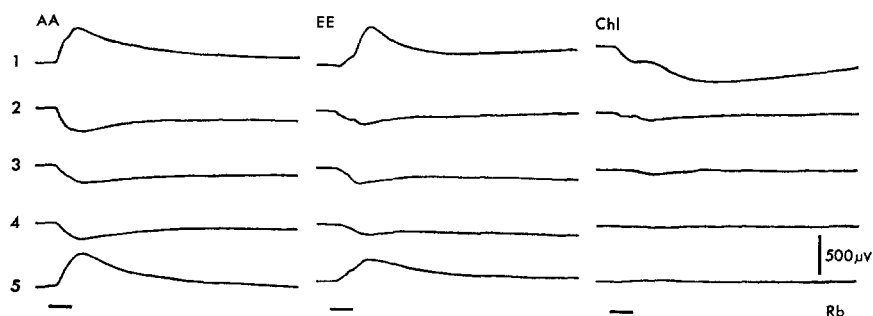

FIGURE 1. Effect of Rb^+. Negative EOG's elicited by amyl acetate (AA), and ethyl ether (EE) vapors (shown as controls in 1) reversed their polarity in Rb^+-Ringer solution (2, 3, 4), but recovered their negativity again in normal Ringer solution (5). Positive EOG's elicited by chloroform vapor (Chl) (shown as control in 1) decreased their electropositivity in Rb^+-Ringer solution (2, 3, 4) and did not recover when Rb^+ was replaced by Na^+ (5).

RESULTS

1. Li^+ and TEA^+

In a previous paper (Takagi et al., 1968), it was shown that these cations cannot substitute for Na^+ in the generation of the negative EOG's. The positive EOG's increased initially and decreased later in the TEA^+-Ringer solution, while they simply decreased in the Li^+-Ringer solution.

2. Rb^+

In Rb^+-Ringer solution the negative EOG's promptly reversed their polarity and became positive just as in K^+-Ringer solution (Fig. 1). After the reversal of the EOG's was confirmed in K^+-Ringer solution, K^+ was replaced by Rb^+. The reversed (originally negative) EOG's did not recover but remained positive (Fig. 2). Thus, Rb^+ resembles K^+ to a large degree in this respect. On the other hand, the positive EOG's decreased in amplitude and disappeared, but they never reversed their polarity in Rb^+-Ringer solution, although the positive EOG's reversed their polarity in K^+-Ringer solution. After being treated in the Rb^+ solution, the negative EOG's recovered fairly

well in Ringer solution, but the positive EOG's recovered only slightly. Thus, it is clear that the action of Rb^+ is similar to that of K^+ with the negative EOG's, but not with the positive EOG's.
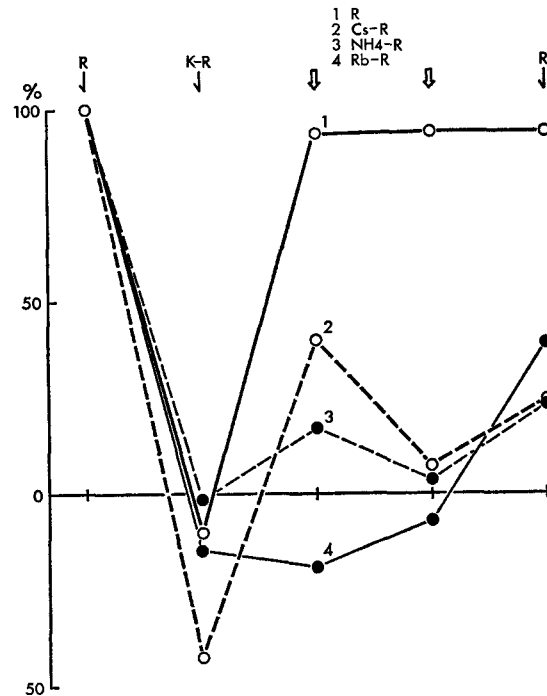

FIGURE 2. K^+-Ringer test with monovalent cations. The negative EOG's (indicated by R) reversed their polarity in K^+-Ringer solution (K-R) but recovered to various extents in Cs^+- and NH_4^+-Ringer solutions, or did not recover in Rb^+-Ringer solution (indicated by hollow arrows and by 2, 3, and 4, respectively). This is in contrast with the complete or nearly complete recovery in normal Ringer solution, as indicated by 1. After having been treated in Cs^+-, NH_4^+-, or Rb^+-Ringer solution the EOG's tend to recover. The magnitudes of the EOG's are shown in percentages as related to those of the control EOG's (indicated by R).

3. Cs^+ and NH_4^+

When Na^+ was replaced by Cs^+ or NH_4^+ in Ringer solution, the negative EOG's decreased in amplitude. In some cases, they even reversed their polarity after two or more immersions (Fig. 3). It is worthy of note that the positive EOG's immediately or rapidly reversed their polarity in these solutions (Figs. 3 and 4). NH_4^+ completely resembles Cs^+ in its effect upon the positive EOG's. After being treated with Cs^+, the negative EOG's recovered slightly in Ringer solution, while the positive EOG's did not recover. After being treated with NH_4^+, the negative EOG's recovered remarkably, while the positive EOG's recovered to a lesser degree.

When K^+-Ringer solution was replaced by Cs^+- or NH_4^+-Ringer solution, the reversed (originally negative) EOG's regained electronegativity and recovered to some extent. However, the EOG's that had recovered now decreased in amplitude during subsequent immersions (Fig. 2). In many cases, they even became electropositive again, especially in NH_4^+ solutions. After these procedures, the negative EOG's recovered only slightly in normal Ringer solution, but the positive EOG's did not. From these experiments, it is clear that neither Cs^+ nor NH_4^+ can replace Na^+ in the generative mechanism of the negative EOG's.
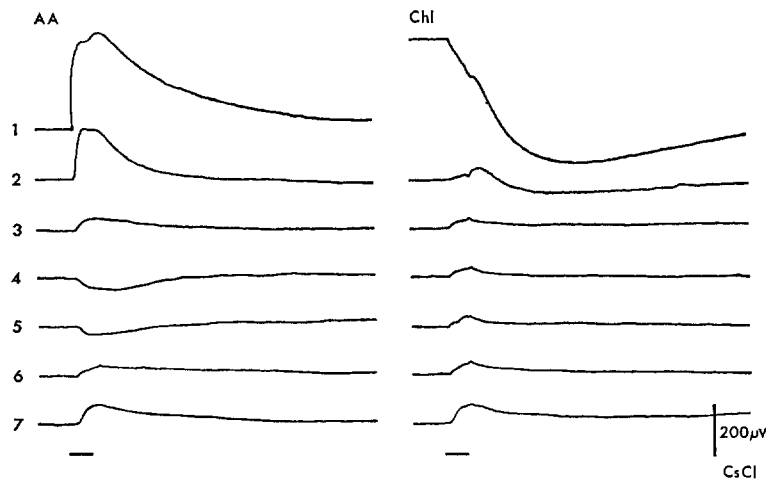

FIGURE 3. Effect of Cs^+. Negative EOG's (1) decreased in amplitude and reversed their polarity during several immersions in Cs^+-Ringer solution (2 to 5). Positive EOG's rapidly decreased in amplitude and reversed their polarity in this solution (2 to 5). Recoveries of the negative EOG's were not remarkable, while the positive EOG's did not recover at all (6 and 7).

4. Ba^{++}

It has been shown previously that 1 mM Ba^{++} selectively suppresses the positive EOG's while the cation hardly affects the negative ones (Takagi et al., 1968). When Na^+ in Ringer solution was replaced by equimolar Ba^{++}, the negative EOG's suddenly reversed their polarity, and large positive EOG's appeared. This phenomenon was rarely observed in the winter, but it was common in the other seasons. The amplitudes of the positive EOG's were very large and were in many cases equivalent to the amplitudes of the negative EOG's before Ba^{++} treatment. However, the positive EOG's were not mirror images of the negative EOG's, because the declining phases of the positive EOG's were much steeper than those of the negative EOG's (Fig. 5). The large positive EOG's soon decreased in amplitude during repetitive

stimulations. The mechanism of this phenomenon will be discussed later. These effects were not different when $Ba(NO_3)_2$ was used instead of $BaCl_2$. The (originally) positive EOG's were immediately suppressed in Ba^{++}-Ringer solution, as was anticipated from the effect of 1 mM Ba^{++}. When Ba^{++}-
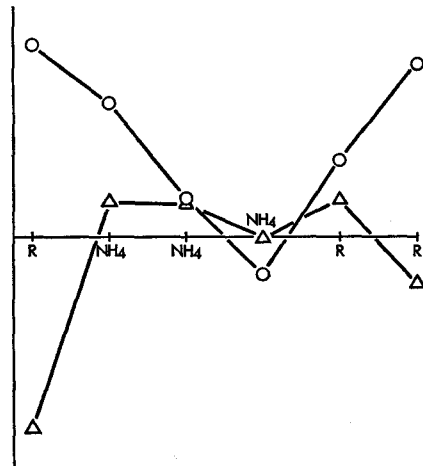

Figure 4. Effect of NH_4^+. Negative EOG's decreased in amplitude and reversed their polarity during several immersions in NH_4^+-Ringer solution (indicated by NH_4). Positive EOG's immediately reversed their polarity in this solution. The negative EOG's recovered remarkably in Ringer solution (indicated by R), while the positive ones recovered less remarkably.
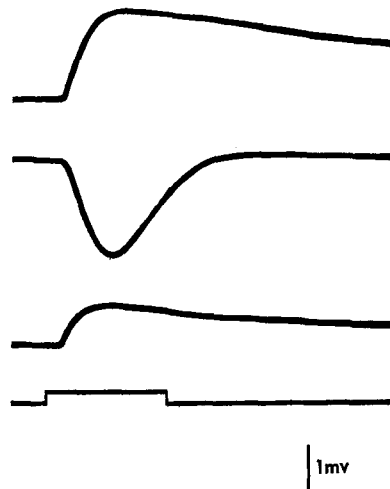

Figure 5. Effect of Ba^{++}. Negative EOG's immediately reversed their polarity in Ba^{++}-Ringer solution. Further explanation is found in the text. From the top to the bottom are (1) a control negative EOG, (2) a reversed EOG in Ba^{++} solution, (3) a half-recovered EOG in Ringer solution, and (4) indication of stimulation (6 sec).

1mv

Ringer solution was replaced by normal Ringer solution, the reversed (originally negative) EOG's recovered nearly completely or considerably, but the positive EOG's recovered only partially. In the K^+-Ringer test, the reversed (originally negative) EOG's recovered once in Ba^{++}-Ringer solution, but the recovery stopped and the negative EOG's became positive. Thus, it is clear that Ba^{++} cannot substitute for Na^+ in the generation of the negative EOG's (Fig. 6).

In order to study the ionic mechanism of this potential reversal, K^+ was increased or decreased in Ba^{++}-Ringer solution. When K^+ was increased to 10 mM and then to 20 mM, the reversed EOG's decreased only slightly in amplitude and nearly disappeared or regained electronegativity. Then, when the concentration of K^+ was returned to normal, the reversed EOG's increased in amplitude again. Conversely, when K^+ was removed from Ba^{++}-Ringer solution, the reversed (positive) EOG's increased in amplitude. All these
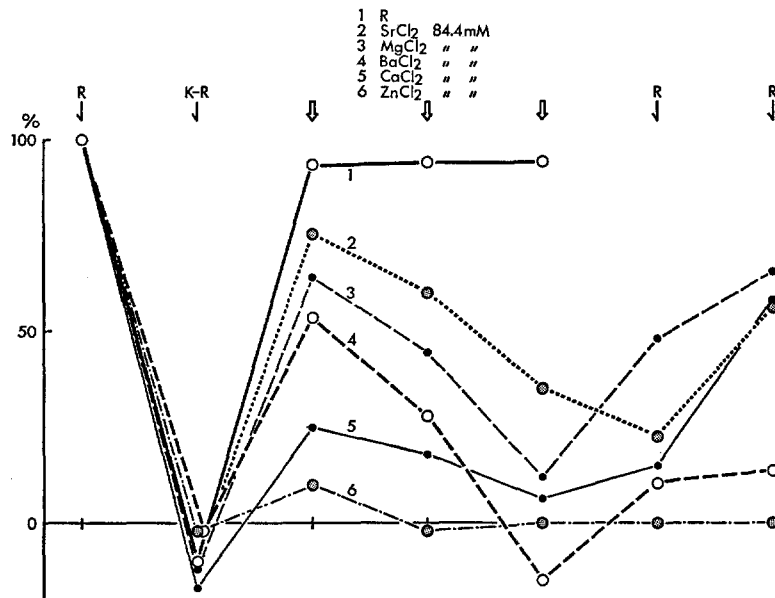

FIGURE 6. K^+-Ringer test with divalent cations. Negative EOG's (controls indicated by R) reversed their polarity in K^+-Ringer solution (K-R), but regained electro-negativity and recovered to various extents in Ringer solutions in which Na^+ was replaced by one of the divalent cations. However, such recovered EOG's were not maintained but decreased in amplitude again (indicated by three hollow arrows). Recoveries in normal Ringer solution were not complete (indicated by two R's on the right).

results suggest that the reversal of the negative EOG's mainly depends upon the action of K^+, which may very probably be the exit of the internal K^+. They also suggest that even isotonic Ba^{++} does not have an influence upon the K^+ permeability across the receptive membrane which generates the negative EOG's.

5. Other Divalent Cations

When Na^+ in Ringer solution was replaced by the equivalent amount (84.8 mM) of one of the divalent cations, Ca^{++}, Sr^{++}, Zn^{++}, Cd^{++}, Mg^{++}, Mn^{++}, Co^{++}, and Ni^{++}, the negative and positive EOG's decreased in amplitude,

and in some cases nearly disappeared. Recoveries of the EOG's in normal Ringer solutions were poor with the first four cations, but slightly better with the last four. In the K^+-Ringer test, the reversed (originally negative) EOG's recovered their negativity quite well in Sr^{++}- or Mg^{++}-Ringer solution, but such a recovery did not last, and the EOG's decreased in amplitude during the subsequent immersions. In the case of the other cations, recoveries were even slighter. Thus, it became clear that none of these cations is able to substitute for Na^+ (Fig. 6).

5. Trivalent Cations

In a Ringer solution in which Na^+ was replaced by Al^{+++} or Fe^{+++}, the EOG's quickly disappeared, and they never recovered in normal Ringer solution. It is clear that these cations are destructive with regard to the EOG-generating membranes.

DISCUSSION

Role of Na^+

It has been shown that the electrical activities of some excitable cells, such as frog spinal ganglion cells (Tasaki, 1959), frog nerve fibers (Greengard and Straub, 1959), crustacean muscle fibers (Fatt and Katz, 1953; Fatt and Ginsborg, 1958; Werman, McCann, and Grundfest, 1961), and insect muscle fibers (Werman and Grundfest, 1961) are maintained in Na^+-free solutions containing alkali-earth cations. It is believed that the action potential is produced in these solutions by increasing the membrane permeability to the alkali-earth cations (Fatt et al., 1953, 1958; Werman et al., 1961; Nishi, Soeda, and Koketsu, 1965). In fact, the movement of Ca^{++} (Ba^{++} or Sr^{++}) as a charge carrier of the inward positive current was proven in barnacle muscle fibers (Hagiwara and Naka, 1964). Thus, the alkali-earth and other ions can substitute for Na^+ in the generation of the action potential (Koketsu, 1961).

In a previous experiment (Takagi et al., 1968) a substitute was not found for Na^+ among Li^+, TEA^+, choline$^+$, hydrazine$^+$, and sucrose. The same experimental procedures were repeated in the present experiment to search for such a substitute among the other monovalent cations, K^+, Rb^+, Cs^+, NH_4^+ and Ag^+; divalent cations, Mg^{++}, Ca^{++}, Sr^{++}, Ba^{++}, Zn^{++}, Cd^{++}, Mn^{++}, Co^{++}, and Ni^{++}; and trivalent cations, Al^{+++} and Fe^{+++}. None of these cations including the alkali-earth ions could show an ability to substitute for Na^+ in the generation of the negative EOG's. It was concluded, therefore, that the sodium ion is the only cation that can generate and maintain the negative EOG's. Thus, the result of this experiment was found to be very different from that of similar experiments on other nervous tissues and muscles.

Generative Mechanism of Negative EOG's

In the present research, the ionic mechanism of the negative EOG's was further studied without considering the origin of the EOG's. In fact, it is not possible to clarify the origin in this type of research. The origin of the EOG's has been discussed elsewhere (Takagi, 1969), after reviewing all the research which is concerned with this problem. In the present stage of the research, however, it has not yet been clarified whether or not the receptive membrane of each olfactory cell responds to odors in a unitary manner to produce the EOG; or whether the slow potentials elicited by organic solvents such as ether and chloroform are produced in the same membrane as that in which odorous molecules are received and differentiated or as that in which the negative slow potentials in response to amyl acetate and other general odors are produced. Although these problems are yet to be solved, our previous experiment (Takagi et al., 1968) disclosed that Na^+ and K^+ contribute mainly to the generation of the negative EOG's. This finding closely resembles the ionic mechanism of the end plate potential (Takeuchi and Takeuchi, 1960). In many nervous tissues the ionic mechanisms of the receptor potentials have been studied and the permeability of these receptive membranes to Na^+ and other ions has been discussed (Diamond, Gray, and Inman, 1957, 1958; Furukawa and Hanawa, 1955; Hamasaki, 1963; Stieve, 1965; Kikuchi, Naito, and Tanaka, 1962; Eguchi, 1965; Ottoson, 1963, 1964; Calma, 1965; Edwards, Terzuolo, and Washizu, 1963; Morita, Hidaka, and Shiraishi, 1966; Morita, 1967). Consequently, it is believed to be possible to discuss the results of the present experiment from the viewpoint of ion permeability. In the following two paragraphs, applicability of the sieve hypothesis and reversal of the negative EOG's in modified Ringer solutions in which Na^+ is replaced by K^+, Rb^+, or Ba^{++} are discussed in relation to the negative EOG-generating membrane.

Sieve Hypothesis

A sieve hypothesis was proposed to explain the anion permeability of the inhibitory postsynaptic membrane of the spinal motoneuron. It was proven that anions with hydrated ion sizes below a certain limit can pass the membrane, but those with hydrated ion sizes above that limit cannot (Araki, Ito, and Oscarsson, 1961; Ito, Kostyuk, and Oshima, 1962). In the positive EOG-generating membrane, anion permeability was studied and the sieve hypothesis was found to be valid on the basis of naked ion sizes but not on the basis of hydrated ion sizes (Takagi et al., 1966).

Now, when the naked sizes of the monovalent cations are compared, only Li^+ is smaller than Na^+ (Table I). If a sieve hypothesis were applicable to the olfactory receptive membrane on the basis of naked cation size, as was found

in the case of anions, Li$^+$ should be a substitute for Na$^+$. In a previous paper, however, it was shown that Li$^+$ cannot substitute for Na$^+$ (Takagi et al., 1968). Next, if the hypothesis were applicable on the basis of hydrated cation sizes just as in IPSP, cations smaller than Na$^+$ should pass through it, when the olfactory receptive membrane is excited by odors. Cations which have hydrated ion sizes smaller than Na$^+$ are K$^+$, Rb$^+$, Cs$^+$, and NH$_4^+$. When Na$^+$ in Ringer solution was replaced by one of these ions, the negative EOG's decreased in amplitude or reversed their polarity and were never maintained. Moreover, in the K$^+$-Ringer test, the negative EOG's did not recover in

TABLE I

SIZES OF (A) THE MONOVALENT CATIONS,
AND (B) THE DIVALENT CATIONS IN NAKED AND
HYDRATED STATES ARE INDICATED

On the right side, the relative hydrated ion sizes are shown as multiples of the size of K$^+$.

		Naked ion size	Relative hydrated size
	Li	0.60	1.948
	Na	0.95	1.493
A	K	1.33	1.0
	Rb	1.48	0.961
	Cs	1.69	0.954
	NH$_4$	1.48	1.0

		Naked ion size	Relative hydrated size
	Mg	0.65	2.85
	Ni	0.70	2.61
	Co	0.72	2.91
B	Zn	0.74	2.78
	Cd	0.97	2.85
	Ca	0.99	2.52
	Sr	1.13	2.34
	Ba	1.35	2.34

Ringer solutions in which Na$^+$ was replaced by one of these cations. Thus, a substitute ion for Na$^+$ was not found among these monovalent cations. In the same way, a substitute ion for Na$^+$ was sought among the divalent cations but in vain (Table I). Now, it is clear that the sieve hypothesis is applicable to the negative EOG-generating membrane neither on the basis of naked ion size nor on the basis of hydrated ion size, and that the mechanism of Na$^+$ permeability in the negative EOG-generating membrane is unique and entirely different from that of anion permeability in the positive EOG-generating membrane. Thus, it can be concluded that there are two basically different generative mechanisms of the EOG's in the olfactory epithelium. This coin-

cides well with the conclusion reached in the study on the effects of polarizing currents upon the EOG's (Higashino and Takagi, 1964), and with the conclusion reached in the impedance measurement of the olfactory epithelium during the EOG's (Gesteland, Lettvin, and Pitts, 1965).

K^+ and Reversal of Negative EOG

When Na^+ in Ringer solution was replaced by K^+, the negative EOG's reversed their polarity and became positive (Takagi et al., 1968). In this state, K^+ is still richer in concentration in the interior of the cell than on the exterior (Asano and Hurlbut, 1958), and hence, K^+ is supposed to exit across the receptive membrane when the membrane is excited. From the conclusion that the negative EOG's depend upon the entry of Na^+ and the exit of K^+ (Takagi et al., 1968), the reversal of the negative EOG's in Na^+-free, K^+-rich media may be explained solely by the exit of the internal K^+. It is not yet known, however, whether the permeability of the negative EOG-generating membrane to Cl^- increases in Na^+-free media or not. If it does, Cl^- also plays a role in the generation of the reversed EOG's.

Reversal of the negative EOG's also occurred in Rb^+-Ringer solution. In its effect upon the negative EOG's, Rb^+ completely resembles K^+. Rb^+ is akin to K^+ in ion size both in the naked and hydrated states. If Rb^+ is assumed to be equivalent to K^+ in its permeability, the potential reversal can be explained by a similar mechanism, namely the exit of the internal K^+. Although the reversal occurred gradually and after two or more immersions, it was shown that Cs^+ and NH_4^+ have reversal actions on the negative EOG's similar to those of K^+ and Rb^+. The effects of Cs^+ and NH_4^+ were far more conspicuous in that both cations reverse the polarity of the positive EOG's. When these four cations are compared (Table I), it is found that K^+ and Rb^+ have very similar naked and relative hydrated ion sizes. Nearly the same relation is found between Cs^+ and NH_4^+. It is interesting, however, that K^+ and NH_4^+ have the same relative hydrated size (1.0), and very similar naked ion sizes (1.33 A and 1.48 A). On the other hand, Rb^+ and Cs^+ have very close relative hydrated ion sizes (0.961 and 0.954), and also close naked ion sizes (1.48 and 1.69). Cs^+ (0.954) has a hydrated ion size very close to that of K^+ (1.0), while Rb^+ has the same naked ion size as NH_4^+ (1.48). Thus, these four cations in general have very similar ion sizes both in the naked and hydrated states. Since their actions upon the negative and positive EOG's are different, it may be said that the properties of these cations, other than the ion sizes, mainly affect the EOG-generating mechanism.

The most pronounced reversal of the negative EOG's was found when Na^+ was replaced by Ba^{++}. If the permeability of the receptive membrane to Ba^{++} is increased by olfactory stimulation, negative EOG's should occur. On the contrary, the positive EOG's occurred. Consequently, it is presumed that Ba^{++}

cannot pass the membrane, or can barely pass through. It has been shown that the contribution of Cl^- to the generation of the negative EOG's can very probably be ruled out (Takagi et al., 1968). If so, the potential reversal can be explained by the exit of the internal K^+ only. It is very likely that Cl^- does not play a role in the generation of the reversed EOG's in Ba^{++}-Ringer solution because Cl^- entry is blocked by only 1 mM of Ba^{++} (Takagi et al., 1968). The action of K^+ upon the EOG's was examined by changing the K^+ concentration in the Ba^{++}-Ringer solution. When the external K^+ concentration was increased or decreased, the reversed EOG's decreased or increased in amplitude, although only slightly. In this way, it was suggested that K^+ can pass through the receptive membrane even in Ba^{++}-Ringer solution and the above explanation was strengthened. This, however, does not exclude a possibility that the potential reversal is produced by some other mechanism.

In Ba^{++}-Ringer solution, the negative EOG's immediately reversed their polarity. However, in the K^+-Ringer test it was found that the once reversed EOG's in K^+-Ringer solution recovered and temporarily regained electronegativity when the K^+-Ringer solution was replaced by the Ba^{++}-Ringer solution (Fig. 6). Such a temporary recovery was observed in all other cases except in Rb^+-Ringer solution (Figs. 2 and 6). It may be presumed that these recoveries are produced by increases in membrane permeability to these mono- and divalent cations, and that the subsequent decreases in amplitude of these once recovered EOG's are due to the accumulation of these cations inside the receptive membrane, because mechanisms may not exist which can pump them out. However, in a previous paper, the similarly temporary but far more striking recovery was proven in sucrose-Ringer solution in which Na^+ was replaced by sucrose (Takagi et al., 1968). Sucrose has a large molecular size and does not have an electric charge. Consequently, it is assumed that the temporary recovery in the sucrose-Ringer solution is due to the recovery of the resting membrane potential. In instances of recovery in Ba^{++}- and other Na^+-free Ringer solutions, the same explanation may be applied. It is believed that this latter explanation is more probable than the former explanation of an increase in membrane permeability to these cations.

Ionic Mechanism of EOG in Other Animals

Tucker (1963) recorded the EOG's in the terrestrial tortoise, *Gopherus polyphemus*, by flowing aqueous solutions of odorants over the olfactory epithelium. The solution contained 1.4 mM $CaCl_2$ and 170 mM $NaCl$ or its osmotic equivalent of sucrose. Systematic investigation of the solution parameters showed in this case that the olfactory receptors are insensitive to variation in ionic strength, and to variation in pH over several units.

In these respects, our results in the present and previous experiments on the bullfrog EOG's (Takagi et al., 1966, 1968) differ from Tucker's results on

the tortoise EOG's. However, our findings do resemble his results in that the EOG's are insensitive to variation of osmotic pressure over a range of about 20 to 25%, and in that the EOG's disappear and the receptors are killed in a short time when a solution free of Ca^{++} flows over the olfactory epithelium. Tucker with Shibuya (1965) further studied the effects of several cations upon the same negative EOG's (they are now referred to as "underwater" EOG's). When K^+ was reduced in the bathing solution, no demonstrable effects appeared in the EOG's. On the other hand, when K^+ concentration was increased in exchange for Na^+, the EOG's decreased in amplitude and eventually disappeared when 90% of the Na^+ was exchanged for K^+. In this high K^+ solution, the negative EOG's reverse their polarity in the bullfrog, while Tucker and Shibuya did not observe such a reversal in the tortoise. When Na^+ was replaced by Ca^{++}, Sr^{++}, or Mg^{++}, the negative EOG's in the tortoise became smaller than the control EOG's recorded with the normally used solution containing 170 mM NaCl and 1.4 mM $CaCl_2$. Similar results were obtained in the bullfrog EOG's in our experiments. In a previous paper (Takagi et al., 1968), it was discussed from our viewpoint that the underwater EOG which is usually less than 5 mv becomes extraordinarily large (over 60 mv) when Na^+ is replaced by equimolar sucrose.

When these results on the tortoise are compared with the results obtained in the present experiments, the EOG's recorded in the two kinds of animals are similar in some respects, but very different in others. Especially the effects of low and high K^+ are entirely different in that this ion plays an essential role in the generation of the EOG's in the bullfrog. It seems that the EOG's in the bullfrog are more sensitive to variations in concentrations of various ions. These differences may be attributed mostly to the differences in their species.

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