The pH Dependence of the Receptor Potential of the Hermit Crab Photoreceptor *

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The pH dependence of the receptor potential (ReP) of isolated layers of photoreceptor cells from the hermit crab compound eye (*Eupagurus bernhardus* L.) was determined. Measurements were performed, using extracellular electrodes, in the range pH 3.5–9.5 in three different buffer systems: Tris, glycine, and phosphate.

The amplitude of the ReP was highest at pH 7.5 and decreased in more acidic and more alkaline salines (Fig. 4). Relative to the changes in ReP amplitude, the changes in time course and shape of ReP were small.

Salines of pH other than 7.5 caused an increase of the latent period but decreased the peak amplitude time and the repolarizing phase (t_2).

Alkaline salines caused about the same changes in the ReP as acidic salines. The only observed difference was that repolarisation was more strongly influenced by alkaline solutions and that the plateau magnitude was depressed relatively more than the peak magnitude in acidic environments.

Of the three buffers used, Tris had the weakest influence on the ReP and phosphate buffer the strongest. In contrast to the good reproducibility of the experimental results, the reversibility of the pH effects was generally poor; the effects with glycine-buffer were more reversible than those with Tris.

Introduction

The light initiated response of photosensitive cells – the receptor potential – is known to be influenced by light-induced reactions in visual pigments and by changes in conductivity of the photoreceptor membrane.

Measurements of vertebrate rhodopsin-reaction kinetics and investigation of rhodopsin conformational changes initiated by light indicate that the conversion of metarhodopsin I to metarhodopsin II is most probably coupled with the conductivity change of the photoreceptor cell membrane responsible for the production of the receptor potential [1–3].

There exist a number of papers which deal with a pH dependence of the photopigment reactions in vertebrates [2–11]. Ostroy and coworkers [2, 3] were interested in the correlation of the chemical changes of rhodopsin and the late ReP of the retina. They found that illuminated cattle rhodopsin takes up a proton during the meta I → meta II conversion and releases protons during later stages of the photochemical conversion of rhodopsin. Similar findings have been obtained by others on cattle rhodopsin solutions [4–6]. Hydrogen ion changes after illumination were also measured in cattle and frog rod outer segments (ROS) suspensions [7–10]. In our laboratory Stieve, Wilms, and Nöll [11] found that, for cattle rhodopsin, the transitory existence of meta-rhodopsin I is longest at pH 7. This could mean that the coupling between photochemical and conductivity change is most efficient at this pH.

Only few papers dealing with the influence of pH on invertebrate rhodopsin have been published [12–14]. Hubbard and St. George [12] demonstrated that squid metarhodopsin contains a single acidic-binding site with a pK of 7.7. Wald [14] demonstrated that the crayfish 562 nm pigment (in digitonin extract) is selectively destroyed at pH 9.0.

The pH also influences those properties of the cell membranes which control ionic transport.

Hille's experiments on the nerve membrane have shown that hydrogen ion changes cause changes of the sodium permeability [15]. Deuticke found that pH influences the red blood cell membrane and causes shape changes. He refers those changes to altered membrane properties [16].

The effect of pH on the ReP was investigated and discussed by Abrahamson and Ostroy [1] and Wong

* Dedicated to Prof. Dr. K. von Haffner (Hamburg).

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and Ostroy [3]. The suggest that the meta I → meta II reaction most likely causes or leads to the generation of the receptor potential of the retina. Ward and Ostroy [2] investigated the relation between rhodopsin photolysis, accompanied by hydrogen ion changes and the late receptor potential in the bull frog retina. They also investigated the late receptor potential as a function of different buffers, different buffer-concentrations and pH and concluded that increasing the pH of the environment or increasing the buffer concentration increased the ReP amplitude.

Sickel described that acidic environments can mimic light- and alkaline environments can mimic dark-adaptation in frog retinas. The mechanisms of these phenomena are not understood; it may be important that calcium, in this respect, acts similar to hydrogen ions [17].

All the referred studies are confined to the light response of vertebrate photoreceptors. Little is known about the influence of pH on invertebrate photoreceptors. Stieve (1964) reported that salines of pH from 6.5 to 9.5 had little effect on the receptor potential of the hermit crab eye [18]. This paper presents a continuation of our study on the ionic mechanism of the light initiated receptor potential of the invertebrate photoreceptor [18–22].

In the experiments, the results of which are presented below, the effects of prolonged application of salines of different pH, adjusted and stabilised by different buffer systems, were examined.

Material and Methods

The experiments described in this paper were performed in three groups based upon the buffer substance used to adjust the pH of the saline: Tris, glycine, and phosphate. Excised retinas of *Eupagurus bernhardus* L. were stimulated by light and the response, the receptor potential (ReP), was recorded using extracellular electrodes. In each group of experiments the RePs obtained in test saline were compared with the RePs obtained in saline of pH 7.5 adjusted with the same buffer. Preparation of the retina, the amplifying and recording apparatus have been described previously (Stieve *et al.* [19, 20]).

Solutions

a. Physiological solution [18]

The saline (prior to buffering) contained:

	Na^+	K^+	Ca^{++}	Mg^{++}	Cl^-	SO_4^{--}
m eq/l	523.3	10.0	10.2	10.5	553.7	10.5

The pH was adjusted to the desired value using the following buffers:

b. Buffer solutions

1. Tris

10 mM Tris-HCl (hydroxymethyl-aminomethane-hydrochloride) was added to the saline to obtain solutions buffered at pH 3.5, 5.5, 7.5, and 9.5. (Buffering capacity at pH 3.5 was very much reduced.) The osmotic pressure was held constant by an equivalent reduction in the amount of sodium chloride.

2. Glycine

Salines containing 5.0 mM glycine (final concentration were titrated with HCl or NaOH to yield pH values of 5.5, 7.5, and 9.5.

3. Phosphate

Salines of pH 5.5, 7.5, and 9.5 were prepared using minimal volumes of 12 mM stock phosphate buffer prepared after Britton and Robinson [23].

The temperature during the experiments was approximately 10 °C. The hydrogen ion concentrations were measured with a pH-meter (Fa. Knick, type pH 22).

Procedure

The retina was illuminated by white light at regular time intervals, and the electrical response to light, the ReP of the visual cells, was recorded using extracellular electrodes. In most of the experiments the retina was stimulated every 10 min with a one second light flash. Every 30 min a shorter light flash of about 20 ms duration was applied.

Apart from these light stimuli the preparation was kept in the dark during the experiment which lasted 3 hours or more. The duration of the experiment, *t*, was measured from the beginning of the pre-period.

After a pre-period of 60 min, during which the retina was superfused with saline of pH 7.5, the preparation was exposed to the test saline of different pH for 60 min (the test period). The experiments ended after another 60 min of superfusion with saline of pH 7.5 (the after-period). In the evaluation, the ReP registered after the 60 min sojourn in the test saline (b-value) and the ReP after 60 min superfusion, again with normal saline, (c-value) were compared with the last ReP of the pre-period (a-value).

In all figures negative voltage is plotted in the upward direction.

The following measurements were made to characterise the RePs and served as a basis for the evaluation:

a) For short light flashes (τ about 20 ms):

h_{max} – the amplitude of the maximum of the ReP [mV],

t_1 – the latency – the time from light flash onset until the first visible increase of the ReP [ms],

t_{max} – the peak-amplitude time – the time from light flash onset until the maximum is reached [ms],

t_2 – the time in which the ReP decreases from h_{max} to $h_{max}/2$ [ms] (repolarization time).

b. For long light flashes (τ about 1000 ms):

h_{max} – the maximal amplitude of the ReP (transient) [mV],

h_e – the plateau value – the amplitude at stimulus end [mV],

h_a – the amplitude 500 ms after the end of the stimulus [mV],

t_{max} – the peak-amplitude time [ms].

The shape-quotient, h_{max}/h_e, was also determined. In the following the stimulus duration τ will appear, if necessary, as a subscript to the respective measured quantity (*e.g.* $h_{max_{20}} = h_{max}$ after light flash of 20 ms duration).

For evaluation of a group of experiments the measured parameters of the RePs are normalized (%) with respect to the values obtained at the end of the pre-period.

Results

1. Tris-buffer

Fourteen experiments were performed with Tris buffered saline at four different pH values; 3.5, 5.5, 7.5, and 9.5.

1.1 pH 3.5

The measured values of ReP parameters elicited both by short and long light flashes in four experiments at pH 3.5 are presented in Table I.

Fig. 1 shows RePs recorded by short and long light flashes in one experiment of this series and Fig. 2 shows the changes of some parameters with time during this experiment.

The RePs caused by short light flashes in this acidic saline were markedly reduced in size (maximal amplitude, h_{max}, about 25 per cent) and decreased yet more during the after-period, reaching values as low as 19 per cent. The latency, t_1, was irreversibly prolonged (to about 120 per cent). The peak-amplitude time, t_{max}, was reduced to about

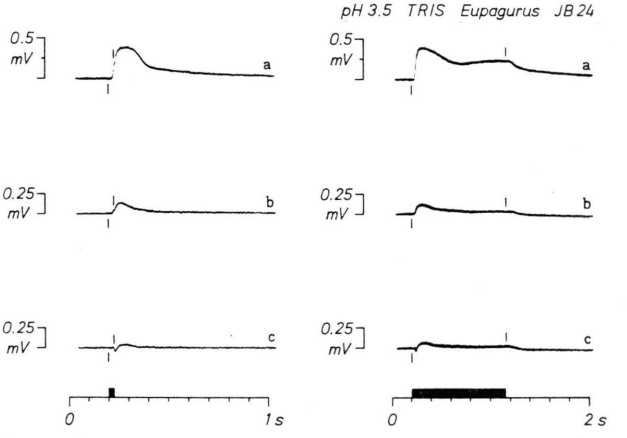

Fig. 1. Receptor potentials of an isolated *Eupagurus* retina in Tris-buffered-saline, pH 3.5, recorded after short (τ about 20 ms) and long (τ about 1000 ms) light flashes (JB 24). Times of recordings: a-values: Pre-period, pH 7.5; b-values: Superfusion with pH 3.5-saline; c-values: After-period, pH 7.5. Temperature 10 °C.

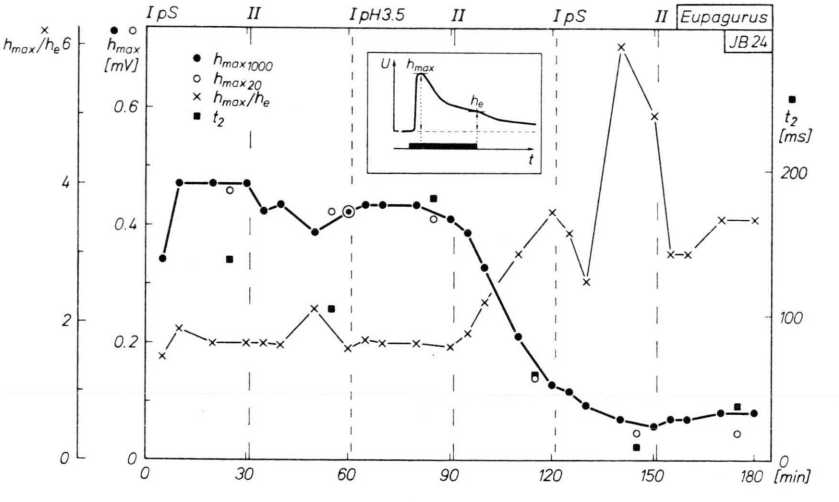

Fig. 2. A graph showing changes of peak-amplitude $h_{max_{20}}$, repolarization time t_2, peak-amplitude $h_{max_{1000}}$ and shape quotient h_{max}/h_e, of the RePs recorded during superfusion with Tris-buffered-saline of pH 7.5 (pS) and pH 3.5 (JB 24).

Table I. Measured parameters of the RePs in Tris-buffered-saline, pH 3.5 (mean ± S.E. of mean). Peak amplitude (h_{max}), plateau-value (h_e), shape-quotient (h_{max}/h_e), peak-amplitude-time (t_{max}) and amplitude 500 ms after end of stimulus (h_a) for long light flashes (τ about 1000 ms), and peak-amplitude (h_{max}), latency (t_l), peak-amplitude-time (t_{max}) and repolarization-time (t_2) for short light flashes (τ about 20 ms). a-Values: Pre-period, pH 7.5-saline; b-values: Superfusion with Tris-buffered saline, pH 3.5; c-values: After-period, pH 7.5-saline. Temperature 10 °C. $n = 4$ (JB 24–27).

Stimulus duration τ ca. 20 ms

	time [min]	h_{max}	t_l	t_{max}	t_2
a	55	0.23 ± 0.05 mV	24.8 ± 0.9 ms	85.7 ± 10.8 ms	110.5 ± 17.2 ms
b	115	25 ± 6%	120 ± 9%	87 ± 6%	34 ± 12%
c	175	19 ± 2%	125 ± 15%	90 ± 11%	27 ± 11%

Stimulus duration τ ca. 1000 ms

	time [min]	h_{max}	h_e	h_{max}/h_e	h_a	t_{max}
a	60	0.26 ± 0.06 mV	0.17 ± 0.03 mV	1.53 ± 0.21	0.07 ± 0.2 mV	131.0 ± 32.6 ms
b	120	33 ± 13%	25 ± 12%	151 ± 27%	—	94 ± 11%
c	180	22 ± 13%	21 ± 14%	125 ± 31%	—	101 ± 17%

90 per cent. The repolarization-time, t_2 was irreversibly and markedly decreased to about 30 per cent.

The amplitudes, h_{max} and h_e, of RePs produced by long light flashes were both reduced but h_e was reduced somewhat more than h_{max}, yielding a shape quotient of about 150 per cent. During the after period, h_{max} decreased more than h_e, yielding a shape quotient of 125 per cent. The t_{max}-period was not significantly changed.

1.2 pH 5.5

The results of three experiments in which retinas were superfused with saline of pH 5.5 are summarized in Table II.

The only significant change observed in this series of experiments is in the reduction of $t_{max_{20}}$. All other changes are not significant but are, generally, in the same direction as in the preceding experiments.

1.3 pH 9.5

The results of seven experiments in which retinas were superfused with saline of pH 9.5 are summarized in Table III. Oscillograms of RePs elicited by short and long light flashes in one experiment are presented in Fig. 3.

The effects of pH 9.5 saline on the measured characteristics of RePs generally resembled those of acid salines but were less marked. Peak amplitudes

Table II. Measured parameters of the RePs in Tris-buffered-saline, pH 5.5. Conditions as in Table I; $n=3$ (JB 21–23).

Stimulus duration τ ca. 20 ms

	time [min]	h_{max}	t_l	t_{max}	t_2
a	55	0.40 ± 0.14 mV	25.43 ± 1.62 ms	92.86 ± 7.66 ms	187.34 ± 2.86 ms
b	115	91 ± 19%	111 ± 14%	89 ± 7%	108 ± 16%
c	175	83 ± 15%	110 ± 6%	87 ± 3%	96 ± 6%

Stimulus duration τ ca. 1000 ms

	time [min]	h_{max}	h_e	h_{max}/h_e	h_a	t_{max}
a	60	0.47 ± 0.13 mV	0.33 ± 0.07 mV	1.44 ± 1.57	0.15 ± 0.03 mV	219.05 ± 65.72 ms
b	120	90 ± 19%	94 ± 8%	94 ± 13%	81 ± 14%	101 ± 6%
c	180	77 ± 12%	86 ± 7%	88 ± 7%	85 ± 22%	101 ± 11%

Table III. Measured parameters of the RePs in Tris-buffered-saline, pH 9.5. Conditions as in Table I; $n=7$ (J; JB 16–20).

Stimulus duration τ ca. 20 ms

	time [min]	h_{max}	t_1	t_{max}	t_2
a	55	0.33 ± 0.08 mV	41.9 ± 3.8 ms	193.3 ± 36.2 ms	258.1 ± 41.9
b	115	92 ± 13%	111 ± 3%	78 ± 10%	77 ± 16
c	175	81 ± 8%	118 ± 4%	82 ± 10%	139 ± 24

Stimulus duration τ ca. 1000 ms

	time [min]	h_{max}	h_e	h_{max}/h_e	h_a	t_{max}
a	60	0.39 ± 0.09 mV	0.25 ± 0.05 mV	1.51 ± 0.15	0.12 ± 0.04 mV	203.8 ± 54.3 ms
b	120	85 ± 10%	85 ± 13	107 ± 10	85 ± 16%	80 ± 10%
c	180	81 ± 6%	95 ± 11	88 ± 6	125 ± 19%	114 ± 6%

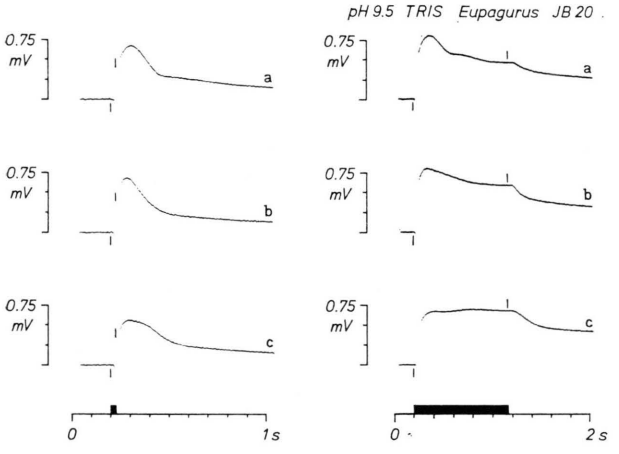

Fig. 3. Receptor potentials of an isolated *Eupagurus* retina in Tris-buffered-saline, pH 9.5, recorded after short (τ about 20 ms) and long (τ about 1000 ms) light flashes (JB 20). Times of recordings: a-values: Pre-period pH 7.5; b-values: Superfusion with pH 9.5-saline; c-values: After-period, pH 7.5. Temperature 10 °C.

were irreversibly reduced to about 80 per cent, t_2 was decreased and then irreversibly increased, amplitudes of h_e and h_a were reduced but exhibited reversibility (h_a even exceeded control values after wash out of the test saline) as did t_2 and $t_{max_{1000}}$.

2. Glycine-buffer

Four experiments were performed with glycine-buffered saline; two at pH 5.5 and two at 9.5.

2.1 pH 5.5

Table IV summarizes the results of experiments with saline buffered with glycine at pH 5.5.

The results obtained with glycine buffered saline, pH 5.5, resemble the effects obtained with Tris-buffered saline, pH 5.5, but were more drastic and more reversible. The only noteworthy difference is that the repolarisation time, t_2, was irreversibly reduced and that glycine-buffered saline did not change the peak-time, t_{max}, until the end of the recovery period. The latency, t_1, was reversibly prolonged to 135 per cent.

2.2 pH 9.5

Table V summarizes the results obtained from retinas superfused with glycine-buffered saline at pH 9.5.

The results obtained with glycine-buffered saline, pH 9.5, were at variance with those obtained in Tris-buffered saline of the same pH. Peak amplitudes of the RePs increased during the after-period, t_{max} was little affected and the plateau magnitude, h_e was irreversibly enhanced, what reduced the shape-quotient to about 70 per cent. The only similarity in the effect of glycine and Tris buffered saline was that both lengthened the latent period of the RePs. The peak-amplitude times $t_{max_{20}}$ and $t_{max_{1000}}$ were not significantly changed.

3. Phosphate-buffer (Britton-Robinson solution)

The results of eleven experiments in which retinas were superfused with phosphate buffer are summarized in Table VI (5 expts. – pH 5.5) and Table VII (6 expts. – pH 9.5). Only 500 ms light flashes were used in these experiments.

Table IV. Measured parameters of the RePs in glycine-buffered-saline, pH 5.5. Conditions as in Table I; $n=2$ (HH 190–191).

Stimulus duration τ ca. 20 ms

	time [min]	h_{max}	t_1	t_{max}	t_2
a	55	0.25 ± 0.05 mV	13.2 ± 1.2 ms	56.4 ± 1.2 ms	73.2 ± 1.2 ms
b	115	56 ± 20%	135 ± 15%	102 ± 7%	84 ± 1%
c	175	72 ± 4%	110 ± 10%	93 ± 5%	71 ± 2%

Stimulus duration τ ca. 1000 ms

	time [min]	h_{max}	h_e	h_{max}/h_e	t_{max}
a	60	0.28 ± 0.06	0.14 ± 0.003 mV	2.04 ± 0.43	74.1 ± 2.9 ms
b	120	58 ± 20%	61 ± 11%	92 ± 16%	110 ± 4%
c	180	78 ± 6%	78 ± 15%	102 ± 11%	87 ± 1%

Table V. Measured parameter of the RePs in glycine-buffered-saline. pH 9.5. Conditions as in Table I; $n=2$ (HH 192–193).

Stimulus duration τ ca. 20 ms

	time [min]	h_{max}	t_1	t_{max}	t_2
a	55	0.34 ± 0.03 mV	27.6 ± 3.6 ms	60.0 ± 8.4 ms	57.6 ± 3.6 ms
b	115	96 ± 31%	137 ± 17%	103 ± 6%	81 ± 3%
c	175	118 ± 8%	113 ± 3%	99 ± 6%	93 ± 2%

Stimulus duration τ ca. 1000 ms

	time [min]	h_{max}	h_e	h_{max}/h_e	t_{max}
a	60	0.44 ± 0.02 mV	0.20 ± 0.04 mV	2.26 ± 0.29	147.3 ± 52.3 ms
b	120	92 ± 32%	132 ± 13%	68 ± 17%	110 ± 10%
c	180	121 ± 19%	192 ± 10%	64 ± 13%	103 ± 8%

Table VI. Measured parameters of RePs in phosphate-buffered-saline, pH 5.5. Conditions as in Table I; $n=5$ (HH 211–215).

Stimulus duration τ ca. 500 ms

	time [min]	h_{max}	h_e	h_{max}/h_e	t_{max}
a	60	0.10 ± 0.02 mV	0.09 ± 0.02 mV	1.10 ± 0.04	117.3 ± 36.0 ms
b	120	30 ± 12%	24 ± 6%	146 ± 28%	91 ± 18%
c	180	60 ± 3%	42 ± 9%	178 ± 62%	73 ± 14%

Table VII. Measured parameters of the RePs in phosphate-buffered-saline, pH 9.5. Conditions as in Table I; $n=6$ (HH 205–210).

Stimulus duration τ ca. 500 ms

	time [min]	h_{max}	h_e	h_{max}/h_e	t_{max}
a	60	0.08 ± 0.01 mV	0.06 ± 0.01 mV	1.35 ± 0.18	70.3 ± 12.4 ms
b	120	35 *	$75 \pm 7\%$	64 *	104 *
c	180	22 *	$30 \pm 8\%$	87 *	132 *

* Only one of the six experiments performed yielded receptor potentials with a distinct transient maximum; for other experiments only plateau values were measurable.

3.1 pH 5.5

Saline buffered with phosphate, pH 5.5, markedly reduced the peak and plateau amplitudes of the ReP, an effect which was only partially reversible; the effect on the plateau, h_e, was relatively stronger than on the peak amplitude, h_{max}. The peak time, t_{max}, had decreased only at the end of the recovery period.

3.2 pH 9.5

Saline buffered with phosphate at pH 9.5 produced essentially the same effects as saline buffered at pH 5.5 (reduction of the peak- and plateau-amplitudes of the ReP) with one exception; the peak time, t_{max}, increased at the end of the after period to about 130 per cent. However, the results pertaining to the peak are limited to one experiment, for in the others the ReP did not exhibit a maximum.

The effects of the three buffers at various pH's on the ReP magnitude can be compared:

The results obtained must be compared cautiously because 1. only two experiments at each pH-value with glycine buffered saline were performed, 2. in phosphate-buffer no short stimuli were applied, and 3. in five of six experiments at pH 9.5 in phosphate-buffer the RePs did not show a distinct transient maximum. However, despite these limitations, certain trends are apparent.

Fig. 4 shows the magnitudes of the maximum amplitude, $h_{max_{1000}}$ of the RePs obtained in Tris-, glycine-, and phosphate-buffer salines at the four different pH values. The diagram permits of comparison of the action of three different buffers at pH 3.5, 5.5, and 9.5. (Experimental data at pH 7.5 in the corresponding buffer were used as reference values.)

Generally, it can be stated that the magnitudes of the transient, h_{max}, and the plateau, h_e, of the

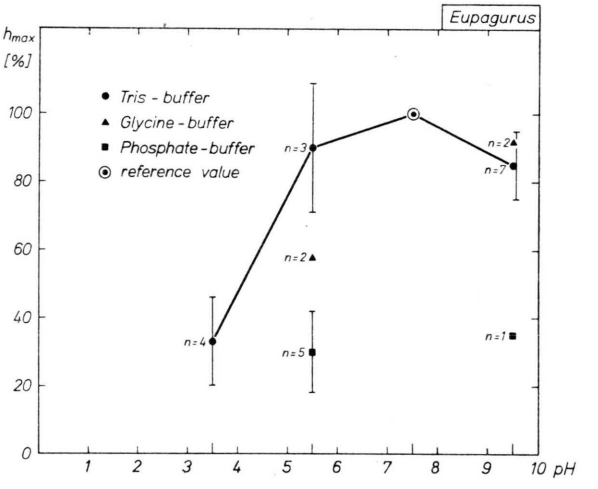

Fig. 4. The effect of pH on $h_{max_{1000}}$ of the ReP ($h_{max_{500}}$ for phosphate-buffered-saline). The data (b-values) are normalized with respect to the last value obtained during the pre-period.

ReP are much lower in phosphate-buffer than in Tris- and glycine-buffered-salines at all pH-values tested. One effect which was common for all experiments, independent of pH and buffer used, was an irreversible prolongation of the latency, t_l. Although the effects of pH on the characteristics of the ReP were reproducible, the reversibility of these effects was generally poor.

This poor reversibility of the pH-induced changes of the ReP is in striking contrast to the relative good reversibility of those changes of the ReP induced by other ionic alterations of the extracellular environment under the same experimental conditions (see ref. 18 – 22).

1. Acidic salines

The maximal amplitude, h_{max}, decreased at pH 5.5 in all buffer solutions. In Tris the decrease was

irreversible, while in glycine the reversibility was better, although the decrease was more marked. The most remarkable (but to some extent reversible) reduction of the maximal amplitude occurred in phosphate-buffer. The shape-quotient, h_{max}/h_e, remained about the same in Tris and glycine, but was temporarily increeased to almost twice its value in phosphate-buffer. The peak-amplitude time, $t_{max_{1000}}$, was unchanged or slightly decreased. Comparisons at pH 3.5 are not possible because at this pH only experiments with Tris-buffer were performed. The changes observed at this pH, however, were similar to the above, but more drastic (also t_2 was distinctly shortened).

2. Alkaline salines

All buffers at pH 9.5 reduced the ReP-amplitude, h_{max}. The reduction was small in Tris- and glycine- but very marked in phosphate-buffer. The changes were irreversible. The shape-quotient was virtually unaffected by Tris and decreased irreversibly by glycine because the amplitude, h_e, increased. This effect was even more marked in phosphate saline, which was indicated by the disappearance of h_{max}. Generally the peak-amplitude time, t_{max}, did not change significantly in any of the three buffers. Only in Tris-buffer $t_{max_{20}}$ and $t_{max_{1000}}$ were both slightly decreased.

The changes of the RePs recorded under the conditions of our experiments can be summarized:

1. *At different pHs* (acidic compared to alkaline) the maximal amplitude, h_{max}, was reduced in all cases with one exception (Tris pH 5.5). The shape-quotient, h_{max}/h_e, was not changed or increased (for pH 3.5) in the acidic range, while in the alkaline range (pH 9.5) it was unchanged or decreased (for glycine- and phosphate-buffers).
2. Regarding the effects of the *different buffer solutions* the decrease of h_{max} and the influence on h_{max}/h_e was stronger in phosphate-buffered saline than in Tris-buffered saline, while the effects in glycine-saline lay in between.
3. *The reversibility* of the observed effects of the three buffer solutions was generally poor (as compared to the influence of other substances on the ReP). It was worst in Tris-saline and best pronounced in glycine-buffered saline.

Discussion

The observed effects of pH on the ReP may consist primarily of pH-dependent reactions of the following systems of photoreceptor cells which govern or influence the response to light:

1. The visual pigment;
2. systems which determine membrane properties, especially membrane conductivity and light-induced changes;
3. other systems in the visual cell which secondarily influence the processes controlling the generation of the ReP.

It is unlikely that only one of the above systems is responsible for the observed pH dependence of the ReP. The observed effects of pH seem to be of a complex nature. For instance, the binding of protons on the membrane surface or in ionic channels (which is probably taking place) cannot alone account for the maximum in the pH dependence curve of the ReP (Fig. 4).

In the photochemical conversion of vertebrate rhodopsin [11], the life time of metarhodopsin I is maximal between pH 6.5 and 7.5. Since it is probable that the reaction, meta I → meta II, is linked to the conductivity change of the photoreceptor membrane, one would expect a maximum of the ReP amplitude between pH 6.5 and 7.5, which was observed in the experiments described above. However, Ward and Ostroy [2] did not find such a pH dependence in their experiments on the vertebrate retina. The amplitude of the ReP increased with increasing pH and the pH dependence curve exhibited no maximum or plateau in the range of pH investigated (between 6.5 and 9.0). This leads to the conclusion that the height of the vertebrate ReP is not primarily governed by the pH dependence of the visual pigment reactions but, rather, by other properties of the photoreceptor cell (membrane?), at least when pH changes extracellularly.

The effects on the ReP observed in our experiments in acidic pH's are similar to the results of Hille [15] who found that the conductivity of the node of Ranvier in frog nerve quickly and reversibly decreased in the pH range from 7.5 to 4.0. This change exhibited a pK of 5.2, leading to the conclusion that the inside of the Na^+ channel has acidic polar groups. The effects of alkaline pH on the ReP in our experiments are not in accord with the results of Hille. This could mean that the possible Na^+

channels of invertebrate photoreceptor membranes could have characteristics different than those of vertebrate axons, an inference supported by the fact that tetrodotoxin does not block Na^+ influx in invertebrate photoreceptors [22]. The discrepancy could also be explained by assuming that alkaline salines affect rhodopsin or other properties of the invertebrate photoreceptor membrane in such a manner as to produce the observed maximum in the ReP magnitude-pH curve.

The relatively poor reversibility of the effects of pH on the ReP agrees well with the findings of Ward and Ostroy [2] in vertebrate photoreceptors. This poor reversibility could be explained, for instance, by permanent changes in the molecular structure of the cell membrane.

In addition to the above described effects, secondary effects could possibly influence the ReP, for instance by affecting ATP-ase system. The ouabain-sensitive sodium pump is generally accepted to be identical with sodium-potassium activated ATP-ase which exists in cell membranes across which a concentration gradient for sodium and potassium exists. ATP-ase activity is found both in the intact vertebrate retina and in a suspension of rod outer segments [24–28]. The optimal pH for ATP-ase activity in homogenates of *Sepia* retinas is 7.1 and the activity curve has quite a sharp maximum [29]. The observed effect of pH on the RePs and its irreversibility may be caused, to some extent, by affecting ionic pump activity or another enzyme systems.

For instance the activity of another membrane bound enzyme(s), 5′AMP- and 5′GMP-nucleotidase(s), in bovine ROS has a maximum at pH 8.5 [30].

Two aspects of the results obtained in our experiments were relatively unexpected.

1. The transient and plateau magnitudes were about equally affected by the changes of pH. This is in striking contrast to all the other experiments we have done, in which the external environment of visual cells was altered [19, 21]. Furthermore pH changes to values below or above 7.5 did not cause marked pH-characteristic changes of the ReP. The only (small) differences worth mentioning concern the relative influence on the amplitudes of the transient and the steady state value of the ReP and quantitative differences on the rate of repolarization of the ReP in alkaline and acidic salines. In solutions of alkaline pH one would expect, especially in phosphate buffer, a lowered concentration of free calcium ions. Reduction in $[Ca^{2+}]_{out}$ causes h_e to increase and this may be responsible for the fact that, in alkaline phosphate saline, transient maxima of the ReP were rarely observed. On the other hand, lowering of the concentration of free calcium causes an increase of t_2 (Stieve and Wirth [19]) which is clearly opposite to the results described above. The observed shortening of t_2 must have other reasons. The concentrations of phosphate used in our experiments are within the range of concentrations which have been used by different investigators [2, 17, 31].

2. The relatively poor reversibility of the pH effects is also in contrast to most of the results of previous ion substitution experiments. In the present experiments the reversibility was poorest when Tris was the buffer. In former experiments [21] where all the Na^+-ions in the extracellular solution were substituted by Tris at pH 7.5 the ReP showed only small changes and good reversibility. This observation rules out a specific damaging action of Tris on the cell membrane at pH 7.5.

In contrast to results obtained in previous ion substitution experiments, the effect of pH in lenthening the latent period of the ReP and in shortening t_{max} at the same time, is surprising. The increase in latency suggests that the processes leading to the opening of ionic channels are slowed and that the change in conductivity is smaller and of shorter duration. This interpretation is in agreement with the fact that the increase in latency and shortening of t_{max} are relatively small compared to the changes in voltages of the ReP.

The differences in the effects of the buffer systems used are not easy to explain. Hille did not find differences in the action of various buffers on the conductivity of the nerve membrane [15]. The differences of the buffer-effect in our experiments are quantitative; Tris has a small effect on ReP magnitude, which is virtually not reversible, whereas glycine has a large effect on ReP magnitude, and this effect is much more reversible. It is possible that the buffering capacity of buffers used varied and may not, in all cases, have controlled the pH in the close vicinity of the cell membrane. Ward and Ostroy [2] observed (for bullfrog retina) that the ReP amplitude increased with increasing buffer concentration.

The relative effects of the three buffers used are similar at low and high pH. Since the buffering

capacity of Tris and phosphate saline at low pH is considerably lower than glycine buffered saline, it would appear that the observed differences are not attributable to differences in buffering capacity.

The effects of pH, described above (Fig. 4) resemble the general shape of enzyme activity *versus* pH curves. This could be attributed to a pH dependence of the activity of some membrane protein which controls the ReP magnitude. However, it is most probable that a number of other effects contribute to the shape of this relation.

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[1] E. W. Abrahamson and S. E. Ostroy, Progr. Biophys. Mol. Biol. **17**, 181 [1967].
[2] J. A. Ward and S. E. Ostroy, Biochim. biophysica Acta [Amsterdam] **283**, 373 [1972].
[3] J. K. Wong and S. E. Ostroy, Arch. Biochem. Biophysics **154**, 1 [1973].
[4] J. Fukami, Folia Ophthalmol. Jap. **11**, 519 [1960].
[5] C. M. Radding and G. Wald, J. gen. Physiol. **39**, 909 [1956].
[6] F. Erhardt, S. E. Ostroy and E. W. Abrahamson, Biochim. biophysica Acta [Amsterdam] **112**, 256 [1966].
[7] G. Falk and P. J. Fatt, J. Physiology **183**, 211 [1966].
[8] D. G. McConnell, C. N. Rafferty, and R. A. Dilley, J. biol. Chemistry **243**, 5820 [1968].
[9] C. N. Rafferty and D. G. McConnell, J. Cell Biol. Abstr. **43**, 109 a [1969].
[10] H. M. Emrich, Z. Naturforsch. **26 b**, 352 [1971].
[11] H. Stieve, M. Wilms, and G. Nöll, Z. Naturforsch. **28 c**, 600 [1973].
[12] R. Hubbard and R. C. C. St. George, J. gen. Physiol. **41**, 501 [1958].
[13] T. Hara and R. Hara, Nature [London] **214**, 572 [1967].
[14] G. Wald, J. gen. Physiol. **51**, 125 [1968].
[15] B. Hille, J. gen. Physiol. **51**, 221 [1968].
[16] B. Deuticke, personal communication [1973].
[17] W. Sickel, Handbook of Sensory Physiol. **Vol. VII/2**, p. 667, ed. M. G. F. Fuortes, Springer-Verlag, Berlin 1972.
[18] H. Stieve, Z. vergleich. Physiol. **47**, 457 [1964].
[19] H. Stieve and Chr. Wirth, Z. Naturforsch. **26 b**, 457 [1971].
[20] H. Stieve, H. Bollmann-Fischer, and B. Braun, Z. Naturforsch. **26 b**, 1311 [1971].
[21] H. Stieve, H. Gaube, and T. Malinowska, Z. Naturforsch. **27 b**, 1535 [1972].
[22] H. Stieve and T. Malinowska, Z. Naturforsch. **28 c**, 149 [1973].
[23] H. T. S. Britton and R. A. Robinson, J. chem. Soc. [London] **1931**, 1456.
[24] J. B. Federovich and M. A. Ostrowskij, Biofisika **13**, 449 [1968].
[25] S. L. Bonting, L. L. Caravaggio, and M. R. Canady, Exp. Eye Res. **3**, 47 [1964].
[26] R. N. Frank and T. H. Goldsmith, Arch. Biochem. Biophysics **110**, 517 [1965].
[27] Y. Secogutti, J. cellular comparat. Physiol. **56**, 129 [1960].
[28] D. G. McConnell, J. Cell Biol. **27**, 459 [1965].
[29] J. J. H. H. M. de Pont and S. L. Bonting, Comparat. Biochem. Physiol. **39 B**, 1005 [1971].
[30] H. Hibbs, personal communication 1973.
[31] A. L. Hodgkin and R. D. Keynes, J. Physiology **128**, 28 [1955].