The Effect of some Sodium Substitutes on the Receptor Potential of the Crayfish Photoreceptor Cell

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Receptor potential, Na⁺-substitutes, ion dependence, photoreceptor cell, Crustacea

Isolated crayfish retinas were perfused with four solutions in which Li⁺, NH₄⁺, Tris H⁺ and glucose were substituted for the sodium ions in the physiological salt solution.

The changes of the extracellularly recorded receptor potential (ReP) evoked by short or long stimuli were measured. The changes in the shape of ReP by test solutions were different for each Na-substitute.

For lithium ions as a Na-substitute (Tab. I and Fig. 3) the plateau value _h_ was considerably decreased (to ~20%) contrary to the peak-amplitude _h_ which even slightly increased.

Ammonium ions show quite a different effect than all the other substitutes. The ReP is decreased strongly and irreversibly (Tab. II and Fig. 4).

When Tris (hydroxymethyl-ammoniummethane-hydrochloride) is substituted for Na, _h_ decreased to about 60 per cent and the plateau is even more reduced (to 20 per cent; Tab. III). Only the recovery value for _h_ (50% smaller) is markedly different contrary to our former experiments where choline was used as Na-substitute (decrease 20%).

Glucose as a substitute for sodium chloride caused strongly decreased peak-amplitude _h_ 26% (Tab. IV and Fig. 9). Increased osmotic pressure due to excess glucose causes irreversible damage of the ReP (Tab. V). All the changes except those produced by NH₄⁺ were reasonably reversible.

The results can be explained by the following assumptions:

a) the maximum of the ReP is caused mainly by an increase in the permeability of the cell membrane for sodium. Ca- and Mg-ions also contribute to it to a certain degree.

b) The plateau value of the ReP to long light stimuli is determined:

1. by the sodium concentration gradient,
2. by active transport processes,
3. by the Ca²⁺ and Mg²⁺-gradients,
4. the chloride gradient may perhaps contribute to this value.

It is generally accepted that the receptor potential of the invertebrate retina is caused by ion currents across the visual cell membrane. In this process positive ions permeating from the external medium into the cell play a decisive role.

In earlier publications we have reported ion substitution experiments in the crustacean retina

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form a somewhat different task in contributing to the receptor potential of the crustacean retina than is the case in the retina of Limulus.

Material and Methods

The present paper deals with four series of experiments during which three kinds of monovalent ions, Li⁺, NH₄⁺, Tris H⁺ and glucose were substituted for the sodium ions in the physiological salt solution. The receptor potential (ReP) of the retina of the crayfish Astacus leptodactylus Eschscholtz was measured by extracellular electrodes.

Preparation of the retina, measuring apparatus, and experimental procedure have been described in detail in previous papers (Stieve 5, 6).

Van Harreveld’s solution (Van Harreveld 4) with a pH of about 7.7 was used as saline. The temperature during the experiments was about 15 °C. During several series of experiments the following substitutes for the Na-ions of the solution were used: Lithium chloride, ammonium chloride, Tris (tris-hydroxymethyl-amino-methane-hydrochloride) and glucose. In the following physiological Van Harreveld’s solution will be named “normal” solution or physiological saline while the solution containing different substitute ions will be referred to as “test” solution.

The concentration of the substitute ions was isotonic-equivalent to the concentration of the sodium ions in the physiological solutions:

- 207.3 mM/l for Li⁺ and NH₄⁺ ions,
- 207.2 mM/l for Tris solution,
- 414.6 mM/l for glucose as substitute for NaCl and the sodium ions of NaHCO₃. The concentration of the chloride ions in the solution containing glucose was 36.7 mM/l. The pH-values of all test solutions used were between 7.1 and 7.6.

Procedure

The retina was illuminated by white light at regular time intervals, and the electrical response to light, the receptor potential (ReP) of the visual cells, was registered.

Every 10 min the retina was stimulated for about 6 ms and every 30 min a stimulus of about 1 s duration was applied.

Apart from these light stimuli the preparation was kept in the dark during the whole experiment. The duration of the experiments was 3 to 5 hours. The time of experiment t was counted from the beginning of the pre-period. All potentials occurring in the retina are expressed as polarity of the distal against the proximal electrode. In all figures the negative voltages are plotted in upward direction.

The following measured values were determined for the characterisation of the ReP’s and served as basis for the evaluation:

a) for short stimuli (τ about 6 ms; Fig. 1 a):
- \( h_{\text{max}} \) — the amplitude of the maximum (mV),
- \( t_e \) — the latency — the time at which stimulus began until the first visible increase of the ReP (ms),
- \( t_{\text{max}} \) — the peak-amplitude-time — the time from begin of the stimulus until the maximum is reached (ms),
- \( t_2 \) — the time in which the ReP decreases from \( h_{\text{max}} \) to \( h_{\text{max}}/2 \) (ms).

b) for long stimuli (τ about 1 s, Fig. 1 b):
- \( h_{\text{max}} \) — the amplitude of the maximum (mV),
- \( h_0 \) — the plateau value — the amplitude at stimulus end (mV),
- \( t_{\text{max}} \) — the peak-amplitude-time (ms),
- \( h_1 \) — the amplitude 500 ms after stimulus’ end (mV).

Fig. 1. Scheme of receptor potential. a. after short stimulus, b. after long stimulus, to explain the measured experimental data.

The shape-quotient \( h_{\text{max}}/h_e \) was also determined.

In the following the stimulus duration τ will be quoted as a subscript of the respective measured quantity (e. g. \( h_{\text{max}} = h_{\text{max}6} \) after a stimulus of 6 ms duration). For the evaluation of all experiments of a series, the measured quantities of the ReP’s were compared as relative values, i. e. they were expressed in per cent of the value of a reference potential recorded in the same experiment at the end of the pre-period (immediately before addition of the test saline).

After a pre-period of 60 min, during which the retina was perfused with normal saline, the preparation was exposed to the test saline for 60 or more min in the main period. The experiments ended with another 60 min perfusion with normal saline in the after-period. For the evaluation, the last ReP of the pre-period (a-value) was compared with the ReP after 60 min perfusion with test saline (b-value) and with the ReP after 60 min perfusion again with normal saline (c-value). If main period or after-period lasted longer than 60 min, the RePs after 120 min and 180 min (e. g. for the main period; values \( b_2 \) and \( b_3 \)) were also evaluated.
The adaptation sensitivity of the retina under the influence of the different substitute ions for the sodium ions was measured by comparing the potential recorded 5 or 10 min after a light adapting stimulus of 1000 ms duration with a reference potential caused by a short stimulus of 6 ms duration applied shortly before the light adapting stimulus.

**Results**

**Lithium ions**

The total number of experiments where lithium ions were substituted for Na-ions in the physiological solution was eight.

Fig. 2 shows RePs recorded after short and long stimuli in one experiment of this series. In Table I the measured values of the experiments after both short and long stimuli are compiled.

After 6 ms stimuli the ReP changes only insignificantly — the apparent increase of the maximal amplitude $h_{\text{max}}$ lies within the statistical deviation of the results. The latency $t_1$ also shows no significant change, however it appears to decrease rather than increase.

The times $t_{\text{max}}$ and $t_2$ become shorter ($t_2$ by about 50 per cent). This reduction in time is marked only during the first 60 min of perfusion with Li-saline. The changes are fully reversible.

After long stimuli (duration about 1000 ms) the maximal amplitude $h_{\text{max}}$ of the RePs is not significantly changed. The plateau value $h_\alpha$ decreases to a large extent in the course of the plot, so that the shape quotient $h_{\text{max}}/h_\alpha$ rises to about 600 per cent.

Parallely $h_\alpha$ decreases and remains at ~ the 70 per cent level by the end of the experiment.

Regarding the adaptation sensitivity under the influence of Li-ions, we found that $h_{\text{max}}$ was insignificantly decreased while $t_2$ remained unchanged. After 60 min dark adaptation $t_2$ increased by about 50 per cent.

Contrary to our results, where the amplitude of the RePs was not changed while the plateau value was considerably decreased, SMITH et al. observed total (but also reversible) disappearance of the ReP of the ventral eye of *Limulus* when Li was substituted for sodium.

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**Table I.** Measured quantities of the RePs in sodium-free Li-saline. Peak amplitude ($h_{\text{max}}$), plateau-value ($h_\alpha$), shape-quotient ($h_{\text{max}}/h_\alpha$), peak-amplitude-time ($t_{\text{max}}$), and amplitude 500 ms after stimulus end ($h_\beta$) for long stimuli ($r$ about 1000 ms) and peak amplitude, latency ($t_1$), peak-amplitude-time, and decrease-time ($t_2$) for short stimuli ($r$ about 6 ms). a-values: pre-period; b-values: perfusion with Li-saline; c-values: after-period. (A 81 — A 88).

<table>
<thead>
<tr>
<th>Time [min]</th>
<th>$h_{\text{max}}$ [mV]</th>
<th>$h_\alpha$ [mV]</th>
<th>$h_{\text{max}}/h_\alpha$</th>
<th>$t_{\text{max}}$ [ms]</th>
<th>$h_\beta$ [mV]</th>
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</thead>
<tbody>
<tr>
<td>a</td>
<td>60</td>
<td>1.3 ± 0.3</td>
<td>0.7 ± 0.2</td>
<td>1.9 ± 0.1</td>
<td>117 ± 9</td>
</tr>
<tr>
<td>b&lt;sub&gt;1&lt;/sub&gt;</td>
<td>120</td>
<td>108 ± 16</td>
<td>20 ± 5</td>
<td>567 ± 48</td>
<td>74 ± 7</td>
</tr>
<tr>
<td>b&lt;sub&gt;2&lt;/sub&gt;</td>
<td>180</td>
<td>113 ± 15</td>
<td>19 ± 4</td>
<td>640 ± 37</td>
<td>76 ± 10</td>
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<tr>
<td>b&lt;sub&gt;3&lt;/sub&gt;</td>
<td>240</td>
<td>111 ± 16</td>
<td>25 ± 6</td>
<td>542 ± 71</td>
<td>75 ± 10</td>
</tr>
<tr>
<td>c</td>
<td>300</td>
<td>92 ± 6</td>
<td>47 ± 6</td>
<td>209 ± 21</td>
<td>72 ± 6</td>
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<table>
<thead>
<tr>
<th>Time [min]</th>
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<th>$t_\alpha$ [ms]</th>
<th>$t_{\text{max}}$ [ms]</th>
<th>$t_2$ [ms]</th>
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<td>a</td>
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<td>89 ± 4</td>
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<tr>
<td>b&lt;sub&gt;1&lt;/sub&gt;</td>
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<td>109 ± 16</td>
<td>92 ± 14</td>
<td>76 ± 6</td>
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<tr>
<td>b&lt;sub&gt;2&lt;/sub&gt;</td>
<td>170</td>
<td>118 ± 14</td>
<td>117 ± 17</td>
<td>94 ± 11</td>
</tr>
<tr>
<td>b&lt;sub&gt;3&lt;/sub&gt;</td>
<td>230</td>
<td>125 ± 20</td>
<td>117 ± 25</td>
<td>95 ± 16</td>
</tr>
<tr>
<td>c</td>
<td>290</td>
<td>93 ± 6</td>
<td>147 ± 24</td>
<td>110 ± 9</td>
</tr>
</tbody>
</table>
Fig. 2. Receptor potentials of an isolated crayfish retina in sodium-free Li-saline recorded after short (τ about 6 ms) and long (τ about 1000 ms) stimuli (A 81). Times of photographs: a-values: pre-period; b-values: perfusion with test-saline; c-values: after-period.

Fig. 3. Plot of measured quantities (peak-amplitude $h_{\text{max}}$ for short stimuli and $h_{\text{max}1000}$ for long stimuli; shape-quotient $h_{\text{max}}/h_0$; decrease-time $t_2$) of the ReP's recorded during perfusion with sodium-free Li-saline. Last value of pre-period: 100 per cent reference value. Abscissa: time in min (A 81).

Fig. 4 shows the RePs after short stimuli. Table II comprises the results of the experiments after short and long stimuli.

In a solution with ammonium chloride the effect on the retina is well-defined.

The ability to react electro-physiologically disappears and remains very low even after perfusion with the normal physiological solution (c-value: $h_{\text{max}}$ 16 per cent), so that the effect can be referred to as irreversible. The latency $t_1$ is hardly changed in the presence of ammonium ions; however it rises

Table II. Measured quantities of the ReP's in sodium-free NH$_4$-saline. a-values: pre-period; b-values: perfusion with NH$_4$-saline; c-values: after period. (A 94—A 104).

<table>
<thead>
<tr>
<th>Stimulus duration $\tau = \text{ca. 1000 ms}$</th>
<th>$h_{\text{max}}$</th>
<th>$h_e$</th>
<th>$h_{\text{max}}/h_e$</th>
<th>$t_{\text{max}}$</th>
<th>$t_2$</th>
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<tbody>
<tr>
<td>time [min]</td>
<td>1.1 ± 0.3 mV</td>
<td>0.7 ± 0.2 mV</td>
<td>2.1 ± 0.2</td>
<td>121 ± 17 ms</td>
<td>0.42 ± 0.13 mV</td>
</tr>
<tr>
<td>a</td>
<td>b</td>
<td>c</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>60</td>
<td>120</td>
<td>180</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>13 ± 2%</td>
<td>64 ± 15%</td>
<td>83 ± 24%</td>
<td>194 ± 38%</td>
<td>1 ± 1%</td>
<td></td>
</tr>
<tr>
<td>7 ± 1%</td>
<td>106 ± 11%</td>
<td>59 ± 3%</td>
<td>40 ± 6%</td>
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<tr>
<td>21 ± 5%</td>
<td>31 ± 6%</td>
<td>154 ± 18%</td>
<td>73 ± 12%</td>
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<table>
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<tr>
<th>Stimulus duration $\tau = \text{ca. 6 ms}$</th>
<th>$h_{\text{max}}$</th>
<th>$t_e$</th>
<th>$t_{\text{max}}$</th>
<th>$t_2$</th>
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</thead>
<tbody>
<tr>
<td>time [min]</td>
<td>0.8 ± 0.2 mV</td>
<td>22 ± 2 ms</td>
<td>89 ± 8 ms</td>
<td>199 ± 20 ms</td>
</tr>
<tr>
<td>a</td>
<td>b</td>
<td>c</td>
<td></td>
<td></td>
</tr>
<tr>
<td>50</td>
<td>110</td>
<td>170</td>
<td></td>
<td></td>
</tr>
<tr>
<td>8 ± 2%</td>
<td>106 ± 11%</td>
<td>59 ± 3%</td>
<td>40 ± 6%</td>
<td></td>
</tr>
<tr>
<td>16 ± 3%</td>
<td>275 ± 40%</td>
<td>154 ± 18%</td>
<td>73 ± 12%</td>
<td></td>
</tr>
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</table>
Fig. 4. Receptor potentials recorded after short (τ about 6 ms) and long (τ about 1000 ms) stimuli in sodium-free NH₄-saline (A 99). For times of photographs see Fig. 2.

To about 270 per cent of the reference value when the preparation is again perfused with normal saline.

The times $t_2$ and $t_{max}$ become significantly shorter; $t_{max}$ diminishes to about 60 per cent, $t_2$ to about 40 per cent. In normal saline both values increase again.

The maximal amplitude $h_{max}$ of the ReP's caused by long stimuli (τ about 1000 ms) becomes drastically reduced to about 7 per cent in the presence of ammonium ions. As had been the case for short stimuli the changes are practically irreversible too; $h_{max}$ reaches only 21 per cent of the reference value at the end of the after-period.

The plateau value $h_e$ (13%) as well as the shape-quotient $h_{max}/h_e$ (~65%) decrease; $h_e$ becomes as low as about 1 per cent of its reference value.

In the last phase of the experiment the ReP becomes flat-topped owing to longer peak-amplitude-times.

Under the influence of ammonium ions the ReP of the retina is very much reduced almost to cessation of electro-physiological reaction.

For that reason statements regarding the effect of ammonium ions on the adaptation sensitivity cannot be made.

The changes occurring in the presence of ammonium were irreversible within the duration of the experiments.

Smith et al. found that after replacement of external sodium by NH₄⁺ the dark potential of the ventral eye of Limulus was hardly changed while the receptor potential was very much decreased or disappeared completely. The changes were reversible.

NH₄⁺ was described as substitute for both sodium and potassium in the squid giant axon by Binstock and Lecar.

Tris

In 7 experiments the retinae were perfused up to 3 hours with a saline containing Tris (tris-hydroxymethyl-aminomethane-hydrochloride) instead of NaCl. The experiments were extended over such a long period in order to observe possible long-term changes.

Fig. 6 shows ReP's recorded after long and short stimuli during one experiment of this series and Fig. 7 the course of the experiments. In Table III the results of this series are compiled.

After short stimuli (τ about 6 ms) the amplitude $h_{max}$ diminishes to about 60 per cent and, upon washing out of the test saline, remains 20 per cent lower than the reference value.
saline. At the end of experiment $t_c$, $t_{\text{max}}$, and $t_2$ remain about 30 per cent lower than the respective reference values. The longer $c$-value of $t_2$ seems to be connected with adaptation sensitivity.

After long stimuli ($r$ about 1000 ms) the amplitude $h_{\text{max}}$ is reduced less than after short stimuli. The decrease of the plateau-value $h_e$, however, is considerable (to about 20 per cent) and leads to a 4-fold magnification of the shape quotient $h_{\text{max}}/h_e$.

After washing out of the test solution, $A_{\text{max}}$ reaches

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**Table III.** Measured quantities of the ReP's in sodium-free Tris-saline. $a$-values: pre-period; $b$-values: perfusion with Tris-saline; $c$-values: after period. (A 106—A 112).

<table>
<thead>
<tr>
<th>time [min]</th>
<th>$h_{\text{max}}$</th>
<th>$h_e$</th>
<th>$h_{\text{max}}$</th>
<th>$t_{\text{max}}$</th>
<th>$h_a$</th>
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<tbody>
<tr>
<td>$\alpha$</td>
<td>60</td>
<td>$1.6 \pm 0.3$ mV</td>
<td>$0.9 \pm 0.2$ mV</td>
<td>$2.1 \pm 0.2$</td>
<td>$156 \pm 21$ ms</td>
</tr>
<tr>
<td>$\beta_1$</td>
<td>120</td>
<td>$70 \pm 11%$</td>
<td>$19 \pm 4%$</td>
<td>$409 \pm 70%$</td>
<td>$67 \pm 5%$</td>
</tr>
<tr>
<td>$\beta_2$</td>
<td>180</td>
<td>$66 \pm 10%$</td>
<td>$19 \pm 4%$</td>
<td>$384 \pm 67%$</td>
<td>$72 \pm 7%$</td>
</tr>
<tr>
<td>$\beta_3$</td>
<td>240</td>
<td>$74 \pm 9%$</td>
<td>$22 \pm 4%$</td>
<td>$330 \pm 43%$</td>
<td>$77 \pm 8%$</td>
</tr>
<tr>
<td>$\gamma$</td>
<td>300</td>
<td>$80 \pm 8%$</td>
<td>$54 \pm 2%$</td>
<td>$149 \pm 56%$</td>
<td>$70 \pm 4%$</td>
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<table>
<thead>
<tr>
<th>time [min]</th>
<th>$h_{\text{max}}$</th>
<th>$h_{\text{max}}$</th>
<th>$t_{\text{max}}$</th>
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<tr>
<td>$\alpha$</td>
<td>50</td>
<td>$1.0 \pm 0.3$ mV</td>
<td>$28 \pm 3$ ms</td>
<td>$109 \pm 14$ ms</td>
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<tr>
<td>$\beta_1$</td>
<td>110</td>
<td>$64 \pm 13%$</td>
<td>$87 \pm 9%$</td>
<td>$65 \pm 3%$</td>
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<tr>
<td>$\beta_2$</td>
<td>170</td>
<td>$61 \pm 13%$</td>
<td>$87 \pm 5%$</td>
<td>$69 \pm 6%$</td>
</tr>
<tr>
<td>$\beta_3$</td>
<td>250</td>
<td>$69 \pm 11%$</td>
<td>$90 \pm 4%$</td>
<td>$85 \pm 12%$</td>
</tr>
<tr>
<td>$\gamma$</td>
<td>290</td>
<td>$79 \pm 11%$</td>
<td>$66 \pm 5%$</td>
<td>$67 \pm 4%$</td>
</tr>
</tbody>
</table>

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**Fig. 6.** Receptor potentials recorded after short ($r$ about 6 ms) and long ($r$ about 1000 ms) stimuli in sodium-free Tris-saline (A 106). For time of photographs see Fig. 2.

The latency $t_r$ as well as the values of $t_{\text{max}}$ and $t_2$ are somewhat reduced during perfusion with Tris-saline. At the end of experiment $t_c$, $t_{\text{max}}$, and $t_2$ remain about 30 per cent lower than the respective reference values. The longer $c$-value of $t_2$ seems to be connected with adaptation sensitivity.

After long stimuli ($r$ about 1000 ms) the amplitude $h_{\text{max}}$ is reduced less than after short stimuli. The decrease of the plateau-value $h_e$, however, is considerable (to about 20 per cent) and leads to a 4-fold magnification of the shape quotient $h_{\text{max}}/h_e$.

After washing out of the test solution, $A_{\text{max}}$ reaches

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**Fig. 7.** Plot of measured quantities of the ReP's recorded during perfusion with sodium-free Tris-saline. Last value of pre-period: 100 per cent reference value. Abscissa: time in min (A 106).
80 per cent and $h_e \sim 50$ per cent of their reference values.

The peak-amplitude-time $t_{\text{max}}$ is somewhat reduced under the influence of Tris and remains so even after washing out of the test solution.

Contrary to the effects observed for short stimuli, where the RePs were not changed significantly, the ability of the retina to react electro-physiologically seems to be permanently affected (plateau-value) when long stimuli are applied.

Concerning the adaptation phenomena observed under the influence of Tris saline the ReP measured 5 min after a long stimulus is not significantly different from the reference potential recorded 10 min before the long stimulus. Peak-amplitude $h_{\text{max}}$ and plateau value $t_2$ remain about the same. After the long dark adaptation period (60 min) both values, particularly $t_2$, were considerably increased.

Millecchia et al.\textsuperscript{6} and Smith et al.\textsuperscript{5} substituted Tris for external sodium in experiments with the ventral eye of Limulus. The dark potential was hardly changed while the receptor potential disappeared but reappeared again in normal saline. Millecchia describes the changes as partial recovery.

Fulpius and Baumann\textsuperscript{7} found reduced amplitude of the plateau of the slow potential if Tris was substituted for external sodium in their experiments with honeybee drone retinula cells.

Glucose

In a series of 5 experiments the retinae were tested in a solution containing glucose instead of NaCl.

Fig. 8 shows the RePs measured after short and long stimuli in one experiment of this series. The course of this experiment is shown in Fig. 9. Table IV contains the results of the experiments.

The RePs caused by short stimuli ($\tau$ about 8 ms) during the presence of glucose in the saline are reduced in size ($h_{\text{max}}$ 26 per cent). The latency $t_1$ and the peak-amplitude-time $t_{\text{max}}$ are prolonged while $t_2$ decreases by a small amount. After perfusion with normal solution $h_{\text{max}}$ rises again and reaches about 78 per cent of the reference value. The reaction of the retina during the after-period shows that the changes are not fully reversible. The latency $t_1$, which had been markedly prolonged (about twice as long as in the pre-period) remains prolonged

Table IV. Measured quantities of the RePs in sodium-free glucose-saline. a-values: preperiod; b-values: perfusion with glucose-saline; c-values: after-period. (IB 3—IB 7).

\begin{table}[h]
\begin{tabular}{|c|c|c|c|c|c|c|}
\hline
\textbf{time} & \textbf{h} & \textbf{h} & \textbf{h} & \textbf{h} & \textbf{h} & \textbf{h} \\
\textbf{[min]} & \textbf{max} & \textbf{e} & \textbf{max}/\textbf{e} & \textbf{max} & \textbf{e} & \textbf{max} \\
\hline
\textbf{a} & 60 & 0.62 ± 0.14 mV & 0.21 ± 0.04 mV & 2.9 ± 0.1 & 120.0 ± 0.03 ms & 0.14 ± 0.03 mV \\
\textbf{b} & 120 & 75 ± 24% & 69 ± 14% & 122 ± 28% & 170 ± 13% & 55 ± 24% \\
\textbf{c} & 180 & 82 ± 13% & 87 ± 5% & 105 ± 14% & 129 ± 11% & 74 ± 21% \\
\hline
\textbf{Stimulus duration $\tau$ = ca. 1000 ms} & & & & & & \\
\hline
\textbf{time} & \textbf{h} & \textbf{t} & \textbf{t} & \textbf{t} & \textbf{t} & \textbf{t} \\
\textbf{[min]} & \textbf{max} & \textbf{e} & \textbf{max} & \textbf{e} & \textbf{max} & \textbf{e} \\
\hline
\textbf{a} & 55 & 0.57 ± 0.13 mV & 32.6 ± 7.0 ms & 97.0 ± 19.1 ms & 188.6 ± 25.1 ms & \\
\textbf{b} & 110 & 26 ± 14% & 229 ± 24% & 155 ± 13% & 91 ± 17% & \\
\textbf{c} & 165 & 78 ± 19% & 131 ± 11% & 131 ± 6% & 85 ± 4% & \\
\hline
\end{tabular}
\end{table}
during the after-period; the $t_{\text{max}}$ value rises by about 50 per cent; the $t_{\text{amp}}$-value which had not become significantly shorter under the influence of the glucose saline decreases further in the after-period and finally remains about 15 per cent lower than the reference value. The changes show that the retina has been to a certain degree irreversibly damaged by the effect of the glucose-saline.

After long stimuli ($\tau$ about 1000 ms) the maximal amplitude $h_{\text{max}}$ is reduced to about 75 per cent of the reference value, the shape-quotient $h_{\text{max}}/h_c$ becomes somewhat larger, and $t_{\text{max}}$ is considerably increased (by about 70 per cent).

Table V. Measured quantities of the ReP’s in sodium-free hyperosmotic glucose-saline. a-values: pre-period; b-values: perfusion with hyperosmotic glucose-saline; c-values: after-period. (A122—A126).

<table>
<thead>
<tr>
<th>time [min]</th>
<th>$h_{\text{max}}$</th>
<th>$h_c$</th>
<th>$h_{\text{max}}/h_c$</th>
<th>$t_{\text{max}}$</th>
<th>$t_a$</th>
</tr>
</thead>
<tbody>
<tr>
<td>a 60</td>
<td>2.1 ± 0.4 mV</td>
<td>1.2 ± 0.1 mV</td>
<td>2.2 ± 0.5</td>
<td>151 ± 34 ms</td>
<td>0.87 ± 0.12 mV</td>
</tr>
<tr>
<td>b 90</td>
<td>8 ± 13%</td>
<td>13 ± 5%</td>
<td>64 ± 13%</td>
<td>429 ± 100%</td>
<td>7 ± 2%</td>
</tr>
<tr>
<td>c 160</td>
<td>49 ± 7%</td>
<td>51 ± 12%</td>
<td>110 ± 19%</td>
<td>146 ± 18%</td>
<td>27 ± 6%</td>
</tr>
</tbody>
</table>

Stimulus duration $\tau = \text{ca. 1000 ms}$

Regeneration of the retina after washing out of the glucose saline seems to be better for long than for short stimuli. Maximal amplitude $h_{\text{max}}$ and plateau value $h_c$ remain only insignificantly reduced; the peak-amplitude-time $t_{\text{max}}$ remains increased by about 30 per cent.

In an additional series of 7 experiments, a strongly hypertonic saline was used containing 829 mm/l glucose — twice as much as in the experiments described above. Table V shows the results of these experiments. The changes of the RePs are more marked than in the series with 414.6 mm/l glucose.

The ReP is diminished to a large extent, for long stimuli ($\tau$ about 1000 ms) to about 8 per cent of the reference value and for short stimuli ($\tau$ about 6 ms) it even vanishes completely. The peak-amplitude-time $t_{\text{max}}$ is considerably prolonged (more than 400 per cent) for long stimuli.

After-perfusion with normal physiological solution (c-values) causes an increase of the ReP to about half of its reference value. The latency $t_1$ increases to twice the value of the pre-period.

The changes clearly confirm that the retina has been markedly and irreversibly damaged by the hyper-osmotic glucose saline. Owing to these damages the adaptation sensitivity in the presence of glucose could not be measured.

In earlier experiments with Eupagurus (Stieve 1), glucose as substitute for sodium chloride caused irreversible decrease of maximum amplitude and plateau value of the ReP to about 50 per cent and considerable increase of latency and peak-amplitude-time.
These findings are in accordance with those of Mil-LECCHIA et al., who stated constant dark potential and greatly reduced receptor potential if sucrose was sub-
stituted for external sodium in experiments with the ventral eye of Limulus.

FULPIUS and BAUMANN found reduced amplitude of the plateau of the slow potential if sucrose was sub-
stituted for external sodium in their experiments with honeybee drone retinula cells. These changes were
reversible.

**Discussion**

Ionic mechanisms are assumed to cause the re-
ceptor potential following a light stimulus both in vertebrate and invertebrate photoreceptors.

The electrical effects observed upon photoreceptor illumination can be explained by the cation channel hypothesis.

It is assumed that the ReP in invertebrate photo-
ceptor cells is caused by an increase of the permeability of the visual cell membrane to certain cations and that sodium fluxes are most probably mainly responsible for the receptor potential.

In addition to earlier experiments where choline was used as substitute for sodium, the present ex-
periments were performed to find out whether and to what degree the specific effect of sodium on the ReP could be determined.

Tris and glucose as well-known and often used physiological substances and Li⁺ and NH₄⁺ because of their chemical similarity to sodium were chosen as substitute ions.

In evaluating the results the effects caused specifically by each of the ions had to be separated from those caused by the lack of sodium. Therefore the values recorded in the after-period (c-values) were particularly informative because at that time only traces of the substitute could be in the preparation (not more than 0.5 per cent²).

The adaptation sensitivity under the influence of all substitute ions will not be discussed further. As can be seen in Table VI, no significant changes were re-
corded in the presence of Li⁺ and Tris (contrary to earlier experiments with choline, which has a marked influence on the adaptation sensitivity), while the changes under the influence of NH₄⁺ and glucose could hardly be judged because they fell into the range of rapidly and irreversibly decreasing values of the maximum amplitude of the ReP's.

In the following we will try to interpret the results subsequently for each species of substitute ions.

**Lithium-ions**

Li⁺ is regarded as near-perfect substitute for so-
dium ions in the frog nerve membrane because of its negligible effect on the action potential. HILLE et al. found that Li⁺ permeates through the sodium channels of the squid giant axon almost as easily as sodium itself. CARPENTER stated that in the frog muscle Li⁺ can enter the passive sodium channels but cannot be actively transported out again.

In our experiments the amplitude $h_{\text{max}}$ of the ReP's was not significantly increased whereas the plateau value $h_e$ was considerably decreased. The changes were fully reversible (contrary to the findings of SMITH et al. and FULPIUS and BAU-
MANN, see results). The changes concerning the plateau value in our experiments may be explained

<table>
<thead>
<tr>
<th>medium</th>
<th>number of experiments</th>
<th>$h_{\text{max}}$ [%]</th>
<th>$t_2$ [%]</th>
<th>$t_{\text{DA}}$ [min]</th>
<th>number of measurements</th>
<th>$h_{\text{max}}$</th>
<th>$t_2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>phys. solution</td>
<td>5</td>
<td>63,8 ± 4,9%</td>
<td>128,8 ± 10,8%</td>
<td>10</td>
<td>5</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>glucose-saline</td>
<td>5</td>
<td>30,7 ± 7,8%</td>
<td>189,5 ± 38,2%</td>
<td>10</td>
<td>5</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>phys. solution</td>
<td>7</td>
<td>97,9 ± 4,9%</td>
<td>71,3 ± 4,2%</td>
<td>5</td>
<td>7</td>
<td>7</td>
<td></td>
</tr>
<tr>
<td>Tris-saline</td>
<td>7</td>
<td>92,4 ± 13,2%</td>
<td>71,8 ± 7,1%</td>
<td>5</td>
<td>7</td>
<td>7</td>
<td></td>
</tr>
<tr>
<td>phys. solution</td>
<td>8</td>
<td>130,0 ± 15,1%</td>
<td>266,6 ± 42,9%</td>
<td>60</td>
<td>7</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>phys. solution</td>
<td>8</td>
<td>107,8 ± 4,3</td>
<td>77,7 ± 9,3%</td>
<td>5</td>
<td>8</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>Li-saline</td>
<td>8</td>
<td>92,2 ± 9,5</td>
<td>75,0</td>
<td>5</td>
<td>8</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>NH₄-saline</td>
<td>9</td>
<td>96,8 ± 6,8%</td>
<td>123,8 ± 14,4%</td>
<td>60</td>
<td>8</td>
<td>7</td>
<td></td>
</tr>
<tr>
<td>phys. solution</td>
<td>9</td>
<td>104,3 ± 4,8%</td>
<td>63,9 ± 3,8%</td>
<td>5</td>
<td>9</td>
<td>9</td>
<td></td>
</tr>
<tr>
<td>NH₄-saline</td>
<td>9</td>
<td>56,9 ± 10,4%</td>
<td>125,7 ± 14,4%</td>
<td>5</td>
<td>9</td>
<td>7</td>
<td></td>
</tr>
</tbody>
</table>
under the assumption that active transport mechanisms take part in the forming of the plateau value.

It may be possible that due to the receptor potential the sodium concentration gradient decreases and that the pump activity prevents an excessive decrease. Another possibility is that the pump is electrogenic. The latter, however, is not very probable because the electrogenicity of the pump should have reversed polarity as compared with the pump electrogenicity during the after-hyperpolarisation.

**Ammonium-ions**

According to **Binstock and LeCrae** 8 NH$_4^+$ does not much affect the physiological reaction in the squid giant axon.

The conductance changes observed in voltage clamp experiments described by the above authors show approximately the same time course in ammonium solutions as in normal physiological solutions. Ammonium carries the early transient current with about 0.3 times the permeability of potassium, the maximum amplitude and plateau-value in our experiments latency $t_1$ and peak-amplitude-time $t_{\text{max}}$ increase, these quantities are decreased under the influence of Tris and remain low also in the after-period.

Plateau value is decreased in the presence of Tris as well as of choline but these changes were reversible after the washing-out of choline, whereas the values remained lower at about 80 per cent in the after-period of the Tris experiments.

Apparently these changes in the Tris experiments may be due mainly to the absence of sodium ions but overlapping of several effects cannot be excluded. One might assume that specific effects (reaction of rhodopsin with amino-group of Tris) could be responsible for the long-term changes (c-period) observed.

At present, almost nothing is known about the ion channels in the photoreceptor membrane. One
possible assumption is that rhodopsin contributes to the formation of these channels and that they may become partially blocked by reaction of rhodopsin with a molecule of the size of Tris.

**Glucose-saline**

Glucose is known as a hardly permeating substance and non-charge carrier. Generally our results are in accordance with those of other authors (see results).

Apparently glucose as substitute for sodium chloride causes longer latency and decrease of the time $t_2$ of the ReP response. The low ionic strength of the glucose solution is probably responsible for these changes as well as for the prolonged peak-amplitude-time. The fact that the shape-quotient remains almost constantly at 100 per cent throughout the experiments (which had not been the case for Li$^+$) suggests that the changes are perhaps due not only to the lack of sodium, but may have something to do with the absence of chloride too.

Lack of sodium does explain the decrease of the maximum amplitude, but one would expect a greater reduction of the plateau value.

A revised interpretation of earlier experiments$^1$, where lack of chloride in the saline had caused an increase of the plateau value, might suggest that chloride influences the plateau value, perhaps because of increased membrane permeability to chloride ions in that phase. Under this assumption lack of chloride as well as of sodium at the same time might have an antagonistic effect on the plateau value which leads to a constant shape-quotient.

The role of chloride for the ReP will be dealt with in a later publication.

**Final remarks**

Summarizing the results we may say that none of the ions tested was a physiologically indifferent substitute for sodium concerning its role for the ReP. Each substitute influenced maximum amplitude and plateau value of the RePs to a certain degree and the actions of all substitutes differed from one another (as opposed to Millecchia et al.$^6$, who found similar effects for all sodium substitutes they used).

The different findings concerning the single substitutes however might be explained if certain assumptions are made:

1. The maximum amplitude of the ReP seems to be mainly determined by sodium. When sodium is replaced by Li$^+$, the value of $h_{\text{max}}$ does not change much. In the presence of the other substitutes to which the photoreceptor cell membrane is probably less permeable, $h_{\text{max}}$ is reduced to about 50 per cent. It does not disappear completely because other cations (probably Ca$^{2+}$ and/or Mg$^{2+}$) can replace sodium to a certain degree.

2. The second assumption is based on the fact that the plateau value is greatly decreased in the presence of all substitutes except glucose. It seems that the effect of sodium on the plateau value is specific and not transferable to Li$^+$ or the other cations used. This suggests that the active transport of sodium contributes to the plateau value, perhaps by preventing a true great decrease in the sodium concentration gradient. The observation that the plateau of the ReP is more strongly reduced by ouabain$^3$ or cyanide$^6$ poisoning than the maximum of the ReP is consistent with this assumption.

Erl er$^7$ also assumes a contribution of an active ion transport to the plateau of the ReP of the Balanus photoreceptor, because of the dependence of the height of the plateau from the state of dark adaptation.

3. As the shape-quotient remains constant only in the presence of glucose it is suggested that lack of sodium and chloride may act antagonistically on the plateau value of the ReP in a way discussed above.

Referring to our initial question concerning the specific effect of sodium on the ReP it seems reasonable to assume that maximum amplitude $h_{\text{max}}$ and plateau value $h_e$ are mainly determined by the sodium concentration gradient and the membrane permeability to sodium, but that active sodium transport phenomena might be involved in the formation of the plateau value as well. The dark potential seems not to be correlated much with the effect of sodium ions.

We wish to express our gratitude to Mr. R. Backbier, Miss G. Böttcher, Mr. H. Breuer, Miss L. Paulssen and Mrs. Chr. Wirth for their technical help.

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