The reversal potential at the desensitized endplate

BY SIR BERNARD KATZ, F.R.S. AND R. MILEDI, F.R.S.

Department of Biophysics, University College London,
Gower Street, London WC1E 6BT

(Received 16 August 1977)

It has been reported that desensitization of the motor endplate, during prolonged application of acetylcholine, is associated with a marked shift in the ‘reversal potential’, interpreted as a change in ion selectivity of the permeable endplate channels. The matter has been re-examined by direct determination of reversal potentials, avoiding the linear extrapolation on which previous estimates were based. The present results show no significant alteration of the reversal potential ascribable to desensitization.

Kuba & Koketsu (1976) have reported that ‘desensitization’ of the endplate, that is the progressive decline of response to prolonged application of acetylcholine (ACh) is accompanied by a marked shift of the reversal potential $E_{reversal}$ (the null-point for ACh-induced endplate current flow). These authors observed a displacement of $E_{reversal}$ towards a more negative level, from approximately $-10$ to $-35$ mV, when the ACh effect had been reduced to 37%. They interpreted this as indicative of altered ion selectivity of the endplate channels, involving a greater depression of sodium than potassium conductance, and they inferred that desensitization is a process which appears to affect the properties of the ion channel rather than the earlier steps of the ACh-receptor interaction. However, the values of $E_{reversal}$ obtained by Kuba & Koketsu can be queried, for they have been derived by linear extrapolation of the current–voltage relation during ACh ionophoresis. Such estimates can be seriously misleading, as has recently been pointed out by Mallart, Dreyer & Peper (1976; cf. Dionne & Stevens 1975), and it was thought necessary, therefore, to re-examine the matter by direct determination of reversal potentials.

METHODS

The experiments were made on endplates of frog sartorius muscles, at a temperature of 5–7 °C. The muscles were kept in Ringer solution (NaCl 115.6 mm, KCl 2.0 mm, CaCl$_2$ 1.8 mm) to which neostigmine methylsulphate ($2 \times 10^{-6}$ g/ml) and 2 mm Na-phosphate buffer at pH 7 was added. Two intracellular microelectrodes were inserted in the endplate region, one containing 3 M KCl for recording membrane potential, the other filled with 4 M K acetate for passing current to displace the membrane potential. Depolarization steps were required for driving the membrane p.d. beyond zero, to inside-positive values.
Sir Bernard Katz and R. Miledi

To avoid contraction artefacts, glycerol pre-treatment was used, the procedure of Eisenberg, Howell & Vaughan (1971) being adopted in thirteen experiments (including all those in which impulse-evoked e.p.p.s were recorded), while in seven others the bath was made hypertonic by the addition of 200 mm NaCl. Neither procedure was wholly successful, but after electrodes had been inserted and preliminary trials made, the resting potential fell usually to low levels at which the residual contractility was inactivated. The average value of the potential, in the absence of current or ACh application, was $-45 \text{ mV}$ (s.e. $\pm 2 \text{ mV}$ in 31 series of observations, with an extreme range of $-75$ to $-20 \text{ mV}$). As there was no difficulty, even starting from the lowest levels, in determining a clear-cut reversal potential, none of these observations was rejected. The mean value of $-45 \text{ mV}$ was, in fact, fairly close to that ($-50 \text{ mV}$) at which the initial level of

**Figure 1.** Reversal potentials during desensitization, using ACh potential as test response. *A–C*, 3 different endplates. *A1* and *B1* during desensitization; *A2* and *B2* during partial recovery (ACh test dose was reduced during recovery). *C1* and 2, Response to rapidly repeated ACh-pulses at different membrane potentials. Note: while phasic responses decline progressively, the ‘null-point’ stays at constant level. Vertical scales show membrane p.d. (inside minus outside potential). Time scale applies to all records except *C2* which was taken at 2.5 times lower speed.
the membrane potential was stabilized in Kuba & Koketsu’s (1976) experiments. Desensitization was produced in most experiments by steady ionophoretic application of ACh, the drug pipette being placed close to and approximately midway between the positions of the voltage and current electrodes. Three different

![Image](http://rspb.royalsocietypublishing.org/)

**Figure 2.** Desensitization tested on impulse-evoked e.p.ps. A–C, three different endplates. A, showing reversal potentials before (A1), during ionophoretic ACh application (A2), and during partial recovery (A3). In B and C, desensitization was produced by bath-applied ACh (2.3 × 10⁻⁶ M). B1 and C1 before, B2 and C2 during desensitization. Note different voltage scales, showing membrane p.d. in all except the separate 3 bottom records. Membrane p.d.s in the latter: B1, −31.5 mV; B2, −35 mV; C2, −29 mV.

procedures were followed: (i) the test stimulus was provided by an ACh pulse delivered from the same drug pipette (cf. Katz & Thesleff 1957). The depression of the response was followed during application of the desensitizing dose, and the test pulses could if necessary be increased in strength and duration so as to restore the amplitude of the ACh potential to a convenient size. (ii) In other experiments
the test response was provided by the impulse-evoked e.p.p. Desensitization was then produced (a) by local ionophoresis or (b) by bath application of ACh. In case (a), the resulting desensitization (of the whole endplate) was less severe, but onset and partial recovery could be followed in a single run without withdrawing the micro-electrodes. In case (b), the electrodes were reinserted after the ACh concentration had been raised sufficiently; no attempt at ‘wash-out’ tests was made.

![Graph showing reversal potentials for e.p.ps (A) and ACh-potentials (B) at 2 endplates. A1 and B1 before; A2 and B2 during desensitization; A3 and B3 during partial recovery. In B, the ACh pipette was brought closer after B1, and the test dose was reduced between B2 and B3. Ordinates: amplitude of test response. Abscissae: level of membrane p.d.](http://rspb.royalsocietypublishing.org/)

The value of $E_{epe}$ was found in each case by displacing the membrane potential until the response (ACh potential or e.p.p.) reversed sign, and then by ‘bracketing’ the null-point as closely as possible so that only a minimum interpolation was needed to determine the reversal potential.
Reversal p.d. at desensitized endplate

Results

Figures 1–3 illustrate the method of determining reversal potentials at different stages of desensitization and recovery, using ACh potentials (figures 1 and 3B) or e.p.ps (figures 2 and 3A) as test responses. There was no substantial change in the ‘null-point’ during desensitization, regardless of the procedure or the tests which were used. Perhaps the most direct demonstration of this lack of effect is provided by the experiments of figure 1C in which rapidly repeated ACh pulses were applied, the responses to successive pulses declining progressively, but all of them passing through a null-point at the same membrane potential.

With a steady dose of ACh, more intense desensitization could be produced. The depression of the response, at comparable membrane potential, varied in the different experiments and depended on the procedure. With ACh-test responses (twelve experiments), the average reduction in amplitude was to 26 % of the control response, ranging between 44 % and 4 %. Desensitized ACh-potentials had a slower time course; their initial rate of rise, which is indicative of the response of the nearest receptor sites, suffered greater reduction than revealed by amplitude measurements.

Table 1. Effect of desensitization on reversal potential

A. Twelve experiments using ACh pulses for test responses

<table>
<thead>
<tr>
<th></th>
<th>mean ( E_{\text{re}} )</th>
<th>s.e. ( \pm ) mV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>mean ( E_{\text{re}} )</td>
<td>mean ( E_{\text{re}} )</td>
</tr>
<tr>
<td>Desensitized</td>
<td>mean ( E_{\text{re}} )</td>
<td>mean ( E_{\text{re}} )</td>
</tr>
<tr>
<td>( \Delta E ) (Desensitized minus Control)</td>
<td>mean ( E_{\text{re}} )</td>
<td>mean ( E_{\text{re}} )</td>
</tr>
</tbody>
</table>

For all 20 experiments together, the mean desensitization was to 33 % of the control response, and the mean value of \( \Delta E \) = 0.35 mV (s.e. \( \pm \) 0.35 mV).

When impulse-evoked e.p.ps were used for the test response, desensitization was less intense, amounting on the average to 43 % of the control amplitude (range 56 to 27 %; in three experiments with ionophoretic ACh application the mean was 49 %, while bath application (five experiments) was more effective in reducing the response, on the average to 38 %).

Measurements of the reversal potential are summarized in table 1.

Discussion

Under the conditions of the present experiments, no substantial shift of the reversal potential was observed during desensitization. Any changes which were seen amounted at most to a few millivolts, hardly outside the limits of error. There
appeared to be some difference between results with ACh-potentials and those with e.p.ps as test responses. In the e.p.p. experiments the reversal potentials were slightly more positive (close to zero membrane potential), and there was a small negative shift (mean $-1.7$ mV) of $E_{\text{enc}}$ during desensitization. As to the latter observation, it was noted — in the three experiments with ionophoretic desensitization — that the negative shift persisted and even increased during the recovery period. It cannot therefore be attributed to desensitization as such.

One possible explanation for these differences may be offered. It arises from the fact that, whereas the e.p.p. is a response of a relatively large area, extending over the whole length of the endplate (several hundred micrometres in the frog), the ACh-potential is a much more localized response and involves only a small part of the endplate surface. This might affect the results in two ways: (a) reversing the e.p.p. along the whole length of the junction might require a more positive voltage displacement, at the point of recording, than reversing a focal ACh-potential nearby; (b) to desensitize the whole of the endplate effectively would need a larger ACh dose than is required to desensitize a focal response produced by the same drug pipette. The more intense ACh dose is likely to cause a greater influx and intracellular accumulation of sodium, which in turn would bias the e.p.c. towards a more negative value.

In comparing the present results with those of Kuba & Koketsu (1976), it has to be pointed out that the experimental conditions differed in certain respects. Thus, in order to be able to find the reversal potential, the muscles were subjected to glycerol pre-treatment or immersed in hypertonic solution. Membrane potentials were lower; neostigmine was used to reduce the ACh dose requirement; amplitudes of voltage rather than current responses were measured. Some caution may, therefore, be needed in generalizing the present conclusions, but it is clear that a direct determination of ACh reversal potentials is a safer procedure than extrapolating current-voltage relations of unproven linearity (cf. Mallart et al. 1976).†

References


