Dependence of the b-wave on the potassium concentration in the isolated superfused rabbit retina

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Abstract. The amplitude of the b-wave of the isolated superfused rabbit retina is drastically reduced with increasing potassium concentration (10 and 20 mM respectively) in the perfusate like in frog retina. These results are in agreement with the idea of the glial origin of the b-wave, but an influence of potassium on synaptic transmission remains a possibility. For these results the conditions for tissue survival are imperative. When the retina was superfused with a plasma saline mixture kept at 35°C, b-wave amplitudes for different preparations varied between 300 μV and 900 μV and loss of sensitivity was tolerated till 15% in one preparation. The temperature quotient for the amplitude of b-wave was 4–6 between 35°C and 25°C, for the peak time about two.

Introduction

The idea that the b-wave originates in the glial Müller cells (Miller & Dowling, 1970) has found general favour although some inconsistencies have been noted (Oakley, 1975; Karwoski & Proenza 1977; Dick & Miller, 1978; Kline et al., 1978). Recently, Fujimoto and Tomita (1981) have shown, that the Müller cells must be assumed to be highly asymmetrical, and this may explain these inconsistencies.

A further discrepancy concerns the dependence of the b-wave on the extracellular potassium concentration in frog and rat retina. In the frog retina the b-wave diminishes with increasing potassium concentration (Miller, 1973), in the rat retina the b-wave enlarges with increasing K+ up to 20 mM and only diminishes when the K+-concentration exceeds 30 mM (Winkler, 1973). Here we show, that when cellular functions are well maintained, the rabbit retina behaves like that of the frog, and that therefore there may be no species differences needing consideration. For this result the care of the isolated retina is essential. The technique is therefore described in detail.

Method

The means of preserving cellular function in the isolated rabbit retina has been described previously (Hanitzsch & Bornschein, 1965; v. Lützow, 1966; Hanitzsch & Trifonow, 1968). A piece of retina of 7 mm diameter was
spread out on nylon tissue in a chamber and superfused by a nutrient solution containing plasma. In the present experiments the original technique of preparation and the original chamber were used, but the composition of the saline solution in the nutrient solution was changed. The nutrient solution consists of 40% horse plasma and 60% saline solution or 50% horse plasma and 50% saline solution.

The saline solution previously used was a modified Tyrode solution buffered with phosphate. Under these conditions the optimal temperature for the superfused preparation was 30–31°C (v. Lutzow & Bornshein, 1966); using a high perfusion rate of 20 ml/min a special supply of oxygen was not necessary. This was convenient, because solutions with a high plasma content easily foam-bubbled with oxygen. To maintain the retina approximately at body temperature, the saline solution must be changed to a carbonate buffered solution consisting of 126 mM NaCl, 4 mM KCl, 1.8 mM CaCl₂, 0.9 mM MgSO₄, 24 mM NaHCO₃ and 40 mM glucose. The perfusate was equilibrated with 95% O₂, 5% CO₂ and the pH adjusted to 7.5–7.6. The addition of oxygen seemed to be unnecessary at the high perfusion rate still used. The temperature was 35°C, unless otherwise stated. The preparation was discarded, when the loss of sensitivity exceeded 15% or the pH dropped. Routinely the experiment can be performed with 250 ml perfusate recirculating over approximately 3 hours with one piece of retina, after which time another piece of retina (mostly from the second eye) and freshly prepared perfusate should be used.

In a first series of experiments the influence of temperature was studied. The preparation was cooled down from 35°C to 25°C and warmed up again to 35°C. For this series the plasma content of the solution was 40%.

In a second series of experiments the K⁺-content of the solution was increased to 10 and 20 mM respectively and checked against the standard concentration of 4 mM. K⁺ was replaced with Na⁺ in solutions with a relative high sodium content (see Table 1). All solutions were isosmotic. Usually one solution with increased K⁺-content was tested twice on one piece of retina, then the next piece was taken. In this series often two pieces of retina from one eye were used, the second piece kept with pigmentepithelium, choroid and sclera intact in a moist chamber at 17°C. The plasma content of the solution was 50% in this series. 6 pieces of retina were studied at 35°C, one at 33°C and two at 30°C.

One further experiment was done without any plasma in the solution at a temperature of 30°C.

Stimulation and DC-recording device were conventional (Hanitzsch, 1978). The retina was dark adapted, the stimulus interval was 1 min. The reference intensity was 40 lx (0 log units).

Results

Figure 1 shows a typical example of three experiments done to examine the influence of temperature on the ERG of the isolated superfused rabbit