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Clearance of extracellular potassium: evidence for spatial buffering by glial cells in the retina of the drone

A. R. GARDNER-MEDWIN, J. A. COLES and M. TSACOPOULOS

Physiology Department, University College London, London WC1E 6BT (U.K.) and (J.A.C. and M.T.) Experimental Ophthalmology Laboratory, Clinique Universitaire d'Ophthalmologie, 22 Rue Alcide-Jentzer, 1211 Geneva 4 (Switzerland)

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Work with ion-selective microelectrodes on the retina of the honeybee drone has shown that potassium is released from photoreceptors during activity and enters glial cells. Measurements of the extracellular voltage gradients indicate that, in this preparation, currents flowing through the glial cells in the 'spatial buffer' pattern account for a large fraction of the glial K^+ entry in the active region of the tissue.

It has frequently been suggested that glial cells might play a role in reducing the changes of extracellular K^+ concentration around active nerve cells[6,8]. Direct evidence for this has been produced recently by Coles and Tsacopoulos[1], who were able to demonstrate increases of K^+ activity within glial cells in the retina of the honeybee drone when the photoreceptors were stimulated with flashes of light. It is clear that the role of glia is important in this preparation; but it is unclear by what mechanism K^+ enters the glia. In 1966, Orkand et al.[6] proposed a mechanism, called the 'spatial buffer', in which K^+ ions passively enter the glial syncytium in a region where neurons are active and, to maintain electroneutrality, other K^+ ions leave the syncytium in a remote region where neurons are not active. The current returns through the extracellular space and extracellular potential gradients are generated. Alternative possibilities[1,3] include K^+/Na^+ exchange and KCl entry across the glial membranes. In the present experiments we have measured the gradients both of potential and of K^+ activity within the drone retina preparation. From these we can infer the size of the spatial buffer currents. The K^+ entry which we calculate on the basis of this single mechanism is a substantial fraction of the K^+ which leaves the photoreceptors and enters the glial cells. In this preparation of the drone retina it therefore appears that the spatial buffer mechanism, acting through the glia, is a significant factor in the clearance of K^+ around the active neural elements.

The drone retina consists of two types of cells: (1) photoreceptors arranged in groups of 9 cells forming 'retinulae' that lie almost parallel to each other and extend from near the corneal surface for a distance of about 400 μm; and (2) glial (or

'pigment') cells which occupy most of the space between the retinulae[7]. The glial cells make gap junction contacts one with another through the tissue, while the photoreceptors in adjacent retinulae are electrically isolated from each other[7]. The tissue has the advantages that there are just the two types of cells with a simple anatomical arrangement, that each can be impaled with ion-selective electrodes[1], and that the photoreceptors can be simply and uniformly stimulated with flashes of light.

The experimental procedure is to remove the head and to cut through the retina

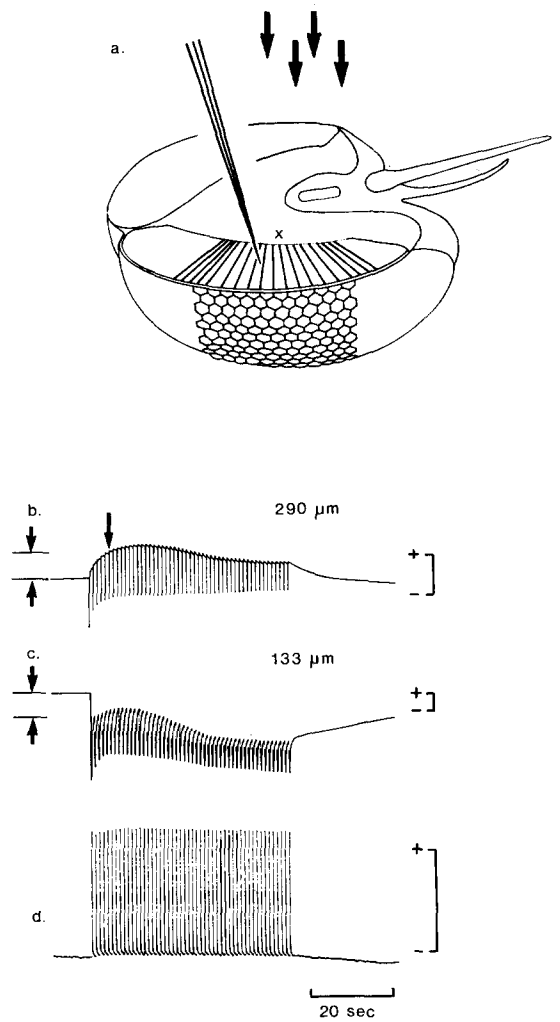

Fig. 1. a: the preparation — the back of the head is sliced off through the retinae and parallel to some of the ommatidia, which are indicated by radial lines. The retina is bounded distally by the corneal facets and proximally by the basement membrane through which the photoreceptor axons pass. Oxygenated Ringer solution (as in ref. 1, but with MOPS buffer) flows over the cut surface. Diffuse flash illumination is from above (arrows). b–d: records of EC voltage changes produced by 50 flashes, 1 per sec. b, c: from within the retina at depths (b) 290 μm; (c) 133 μm. d: from proximal to the basement membrane (site x in Fig.1a). Arrows indicate measurements made on the records 5 sec after the start of stimulation. Voltages measured relative to the bath. Calibrations: 2 mV, 20 sec.

parallel to the photoreceptors. The tissue is then held beneath the surface of rapidly flowing oxygenated bee Ringer[1]. Electrode penetrations and flash stimulation are made through the cut surface (Fig. 1a).

Previous work[1], has shown that flash stimulation leads to a fall of K^+ activity (a_K) in the photoreceptors and rises of a_K in the extracellular (EC) space and in the glial cells. The inferred efflux of K^+ from the photoreceptors is such that in the small EC volume it would cause an enormous increase of a_K (about 500 mM) if it were not for the influx into glial cells[1]. In fact, the rise of EC a_K is only 5–20 mM and the bulk of the released K^+ is accounted for by the rise of a_K within the glia[1]. Thus the clearance of K^+ into the glia is necessary, at least under these circumstances, for normal retinal function.

Our new observations suggest that the entry of K^+ into glia is partly a consequence of the fact that photoreceptors are only activated within a limited region of the tissue, bounded on one side by the cut surface and on the other side by anoxic tissue at a depth of 200–300 μm from the oxygenated surface. The limits of oxygenation have been demonstrated recently by Tsacopoulos et al.[9], using polarographic microelectrodes. It is only within this range of depths that intracellular penetrations of functional photoreceptors could be made, and it is within about the same zone that rises of EC K^+ activity, measured with ion-selective microelectrodes[1], were observed during flash stimulation (Fig. 2a).

We measured the EC potential changes induced by flash stimulation (1 sec^{-1}) at different depths through the tissue (Fig. 1b,c,). The responses consisted of negative transients following each flash and a maintained potential change that was maximally negative about 100 μm deep and that usually reversed to positivity below about 200 μm (Fig. 1b). The negative transients were probably associated with current flow into the photoreceptors since they were accompanied by positive transient potentials in the region of the photoreceptor axons (Fig. 1d). The maintained potential change had no correlate in the region of the axons. This maintained change was measured 5 sec after the onset of stimulation (arrows; Fig. 1b, c) and plotted as a function of depth (Fig. 2b). It can be seen that the region of the tissue with maximal elevations of EC K^+ concentration during stimulation (Fig. 2a) is the region with maximal negativity (Fig. 2b). This negativity is about 10 times greater than a diffusion potential in free aqueous solution for the corresponding gradient of K^+ concentration by K^+/Na^+ substitution[4]. It follows therefore that there are currents in the EC space approaching the active region, from both the surface and the deeper tissue (Fig. 2c). These currents must enter cells in the active zone and complete their paths through cytoplasm: this can only be through the glial cells (Fig. 2c) since the photoreceptors run parallel to the surface.

In order to relate the currents quantitatively to the intracellular data we have used graphs such as Fig. 2b to measure the voltage gradients 50 μm above and below the site with maximal EC negativity. The 100 μm zone between these boundaries will have contained a large fraction of the cells from which the intracellular data was obtained[1]. We estimated the EC current flowing into this zone[3] by taking the product of the measured voltage gradients (54 $\pm$ 14 mV/mm; the sum of the 2 gradients on either side of the zone; mean $\pm$ S.E.; 7 experiments) with the conductivity of the EC

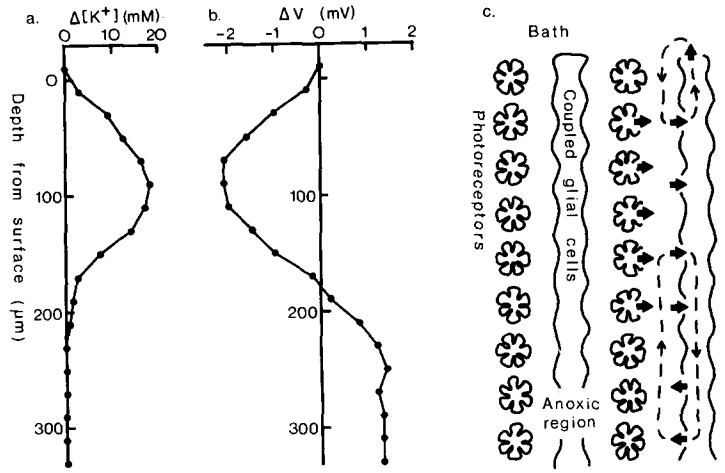

Fig. 2. a, b: rises of EC K^+ concentration (a) and EC voltage changes (b) produced 5 sec after the onset of stimulation, plotted as functions of depth within the preparation.A double-barreled K^+-sensitive micropipette[1] containing Corning resin 477317 was advanced 400 μm into the retina and was then withdrawn in steps. At each depth a train of 20 flashes (1 per sec) was given and the changes from the baseline before stimulation are plotted. The apparent K^+ concentration changes were inferred from the electrode calibration in Ringer with different K^+/Na^+ substitutions. The apparent baseline K^+ concentration was higher in the deep tissue (16 mM at 300 μm) than more superficially (3.20–6.0 mM at 0–200 μm). Similar results were obtained as the electrode was advanced, at those electrode sites that did not appear to correspond to cell penetrations. c: schematic interpretation of results such as Fig. 2a,b. The diagram is a section in the plane of an electrode track and perpendicular to the retinal ommatidia (indicated by the rosette-like photoreceptor clusters, or retinulae). The glial cells, coupled by gap junctions, fill most of the intervening space, but for schematic purposes they are shown as syncytia running vertically between columns of retinulae. The volume of the EC space is greatly exaggerated. Broken lines show the directions of current flow and the heavy arrows show K^+ flux across the photoreceptor and glial membranes during stimulation.

fluid (assumed to be 0.026 Ω^{-1} cm^{-1}, the value measured for the Ringer fluid), the EC space fraction (assumed to be 5%[1]) and the geometrical factor (λ^{-2}) due to tortuosity of the EC space (assumed to be 0.4, as measured for mammalian brain[5]). This gives 2.7 $\pm$ 0.7 $\mu A.mm^{-2}$, or 27 $\pm$ 7 μA per mm^3 of tissue within the 100 μm thick zone of tissue. Since the conductance of glial membranes is principally for K^+ [8], the amount of K^+ entering the glial cells can be directly calculated as current/F, where F = 96,500 Coulombs/mol. 27 μA thus corresponds to 0.3 nmol/sec K^+ entry per cu.mm (or μl) of tissue. Since the glial cells occupy about half the tissue volume[1], this corresponds to 0.6 nmol/μl/sec (0.6 mmol/l/sec) per unit glial cell volume. Some fraction of the entering K^+ will be carried away from the region by currents in the cytoplasm (Fig. 2c). In this preparation K^+ is unlikely to be the principal mobile ion carrying current in the glial cytoplasm since a_K in the glia is small (52 mM)[1] compared to the total osmolarity (590 mM). Thus, nearly all the entering K^+ can be expected to build up

locally in glial cytoplasm. It should be noted that in other preparations K^+ probably *is* the principal current carrier in glial cytoplasm[8] and in these preparations the spatial buffer mechanism may operate to clear significant quantities of K^+ through the glia without significant changes of cytoplasmic concentration. A further correction in calculating the cytoplasmic concentration change might be required if there were substantial shifts of water between compartments: but measurements with EC tracer ions[2] indicate only small changes (less than 5 % in EC space). We therefore expect, as a result of the spatial buffer current flow, an intracellular concentration rise at a rate of the order of 0.5 mM/sec. This compares with the measured rates of rise of glial K^+ concentration (assuming equality of the EC and IC K^+ activity coefficients) of 0.3–2.0 mM/sec (mean 0.9), from Table II of Coles and Tsacopoulos[1]. The data and the assumptions involved are not sufficiently accurate to permit very precise comparison between these two figures. But they do indicate that spatial buffer currents probably account for a substantial fraction of the entry of K^+ into the glial cytoplasm. This does not rule out the possible existence of other mechanisms making significant contributions to glial K^+ entry as well.

In this preparation the shift of K^+ into the glial cells associated with the spatial buffer currents is a result of the uneven K^+ elevation due to equilibration with Ringer fluid at the tissue surface and to the anoxia of photoreceptors deep within the tissue. In vivo, an uneven stimulation and elevation of EC K^+ concentration would presumably arise whenever the drone was confronted with features in the visual environment. The results support the idea that K^+ clearance from EC space by the spatial buffer mechanism might be a significant aspect of glial function[6]. In view of recent work indicating the importance of current-mediated transport in the dispersal of K^+ through *vertebrate* brain tissue[3], it may be reasonable to generalise this conclusion tentatively to a wide range of nervous systems.

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