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HYDROGEN ION EFFECTS AND THE VERTEBRATE LATE RECEPTOR POTENTIAL*

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SUMMARY

The role of H^+ effects in the late receptor potential was investigated in the bullfrog (*Rana catesbiana*) retina by isolation of the potential with aspartate and use of selected changes in the chemical environment. The amplitude of the late receptor potential increased with increases in buffer concentration; with substitution of ^2H_2O for H_2O; and with increasing pH. Increased buffer concentration also altered the Na^+ dependence of the late receptor potential. Upon illumination, changes in solution pH were observed indicative of H^+ uptake by the retina. The H^+ changes of the retina may be acting to reduce the amplitude of the late receptor potential.

INTRODUCTION

The role of H^+ in energy transduction and membrane potential changes is under investigation in photosynthetic and mitochondrial systems[1,2]. In the visual system, H^+ changes have been observed as a result of illumination in extracted solutions of rhodopsin[3,4,5] and in suspensions of rod outer segments[6,7,8]. Because H^+ changes may mediate membrane permeability changes[9], it is of some interest to investigate the relationship between the H^+ changes in the visual system and the late receptor potential. Since in the vertebrate retina it has not yet been possible to record the potential of a single receptor cell with multiple changes of solution, the aspartate technique of Sillman *et al.*[10,11] was used to isolate the late receptor potential with extracellular recording, and the H^+ effects were studied by changes in the composition of the perfusion fluid.

METHODS

The bullfrogs (*Rana catesbiana*) were dark adapted overnight and the retinas excised under dim red light. The retina was mounted in a chamber designed to permit continuous perfusion, exchange of solutions and the recording of electrical signals. The electrical signals were recorded with a d.c. preamplifier which was a.c. coupled to a Tektronix 502A oscilloscope (time constant, 1 s)[12]. The oscilloscope was in turn

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connected through a Vetter Model 2 FM adapter to a Sony 540 tape recorder. The stimulus consisted of the flash from a Honeywell 65C photographic strobe attenuated by Corning 3-72 and 5-56 filters ($\lambda > 440$ nm) and neutral density filters, and passed through a 0.2 cm diameter optic fiber to the preparation. Full flash intensity at the tip of the optic fiber (about 1.5 cm from the retina) was about $4 \cdot 10^{-5}$ J at 500 nm. This was typically attenuated by 2.4 or more log units. The perfusion fluid entering the chamber was changed at 15-min intervals during which the retina was stimulated at 2-min intervals. Exchange of solutions was complete within 10 min. The experiments were conducted at 24 °C.

In the buffer experiments the pH of the perfusion fluid was kept constant and the concentration of buffer used as the variable. The solutions used for the buffer experiments are presented in Table I. As the buffer concentration was changed, the other components were adjusted to maintain constant concentrations. The only ion concentration varied was that of Cl^- but Sillman *et al.*[10] had shown that Cl^- concentration has no significant effect on late receptor potential by substitution with aspartate. We have reaffirmed their observation over our range of concentration changes by substitution with SO_4^{2-}. Another change involved decreased osmolarity in the case of phosphate and increases for Tris, asparagine and borate. Increasing osmolarity with NaCl, however, led to decreasing late receptor potentials and increasing the 12 mM borate solution with 43.2 mosM sucrose had no effect on late receptor potentials amplitude[13]. Since the observation of decreasing late receptor potential amplitude (or no effect) when the osmolarity is increased is the opposite of that observed on increasing the concentration of the majority of buffers, it may be assumed that within the limits of the buffer changes, volume effects are not responsible for the changes in late receptor potential amplitude. The larger osmolarity changes in the case of Tris may, in fact, be responsible for its smaller effect on late receptor potential amplitude. By similar reasoning, since Tris results in a decrease in extracellular resistance at increased concentration, while the other buffers result in no change or a slight increase, the similarity of the results independent of the particular buffer used would suggest that changes in extracellular resistance are not responsible for the observed effects. In addition, substitution of Cl^- with SO_4^{2-} or aspartate[13] represents a resistance change quite analogous to that of increased buffer but shows no significant effects. Finally to check the possibility that the increased buffer may act to change Ca^{2+} concentration, the buffer effect was tested in the absence of added Ca^{2+} and at constant free Ca^{2+} concentrations (C. Gedney and S. E. Ostroy, unpublished). Under both conditions increased buffer concentration still resulted in increased late receptor potential amplitude suggesting that the buffer has no static effect on free calcium concentrations.

In the deuterium oxide experiments the perfusion solution consisted of 12 mM phosphate Ringer's solution with H_2O or ^2H_2O as the solvent. In the pH experiments the total buffer concentration was 12 mM with the pH adjusted by varying the buffer components.

To directly measure changes in H^+ concentration the retina was placed in 1 ml of unbuffered Ringer's solution at approx. pH 6.5 and subjected to intense light flashes. The pH was monitored with a Beckman Model 39030 combination electrode connected either to a Beckman Research pH meter with a strip chart recorder or to a high input impedance preamplifier with an oscilloscope.

TABLE I

COMPOSITION OF SOLUTIONS USED IN THE BUFFER EXPERIMENTS

Values are expressed in mM.

Solution	Na^+	K^+	Ca^{2+}	Mg^{2+}	Cl^-	HPO_4^{2-}	$H_2PO_4^-$	$H_2BO_3^-$	H_3BO_3	*Asn*	*Tris*	*Asp*	*Glucose*	*pH*
Normal Ringer's	116	2.4	0.1	0.1	96.8	10	2	0	0	0	0	0	20	7.4
2.4 mM phosphate	116	2.4	0.1	0.1	104.4	2	0.4	0	0	0	0	10	20	7.4
12 mM phosphate	116	2.4	0.1	0.1	86.8	10	2	0	0	0	0	10	20	7.4
24 mM phosphate	116	2.4	0.1	0.1	64.8	20	4	0	0	0	0	10	20	7.4
54 mM phosphate	116	2.4	0.1	0.1	9.8	45	9	0	0	0	0	10	20	7.4
2.4 mM borate	116	2.4	0.1	0.1	108.2	0	0	0.6	1.8	0	0	10	20	8.1
12 mM borate	116	2.4	0.1	0.1	105.8	0	0	3	9	0	0	10	20	8.1
24 mM borate	116	2.4	0.1	0.1	102.8	0	0	6	18	0	0	10	20	8.1
60 mM borate	116	2.4	0.1	0.1	93.8	0	0	15	45	0	0	10	20	8.1
6 mM asparagine	116	2.4	0.1	0.1	105.8	0	0	0	0	6	0	10	20	8.8
12 mM asparagine	116	2.4	0.1	0.1	102.8	0	0	0	0	12	0	10	20	8.8
24 mM asparagine	116	2.4	0.1	0.1	96.8	0	0	0	0	24	0	10	20	8.8
60 mM asparagine	116	2.4	0.1	0.1	78.8	0	0	0	0	60	0	10	20	8.8
2.4 mM Tris	116	2.4	0.1	0.1	110.0	0	0	0	0	0	2.4	10	20	8.1
12 mM Tris	116	2.4	0.1	0.1	114.8	0	0	0	0	0	12	10	20	8.1
24 mM Tris	116	2.4	0.1	0.1	120.8	0	0	0	0	0	24	10	20	8.1
60 mM Tris	116	2.4	0.1	0.1	138.8	0	0	0	0	0	60	10	20	8.1

RESULTS

The H^+ changes associated with illumination of the retina were measured directly with a H^+ sensitive electrode. A sample experiment, is shown in Fig. 1 and indicates a maximum change of about + 0.01 pH unit in the first flash which de-

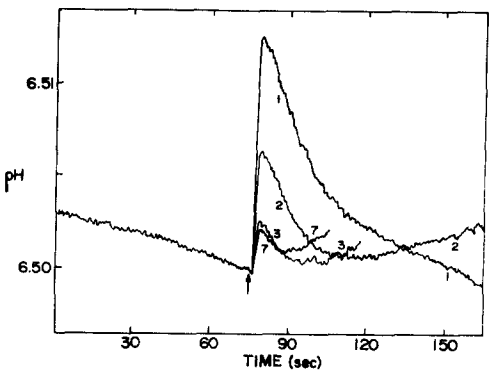

Fig. 1. Direct measurement of pH change upon illumination of an excised retina. Flashes 1, 2, 3 and 7. Arrow indicates time of flash. Differences in drift prior to the flash were insignificant and are represented by a single trace.

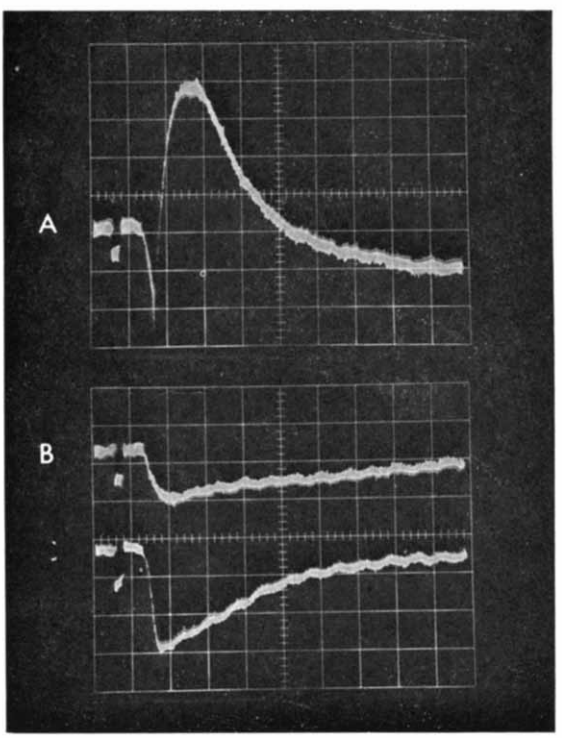

Fig. 2. The effect of buffer concentration on late receptor potential. Each signal is preceded by a 50 μV–50 ms calibrator pulse. A. Normal Ringer's–12 mM phosphate buffered solution. B. 12 mM phosphate-buffered Ringer's solution containing aspartate. C. 54 mM phosphate-buffered Ringer's solution containing aspartate. For a second measurement the perfusion is returned to 12 mM phosphate solution with aspartate (B) then again returned to 54 mM phosphate-buffered Ringer's solution containing aspartate. The results were also obtained in the absence of aspartate.

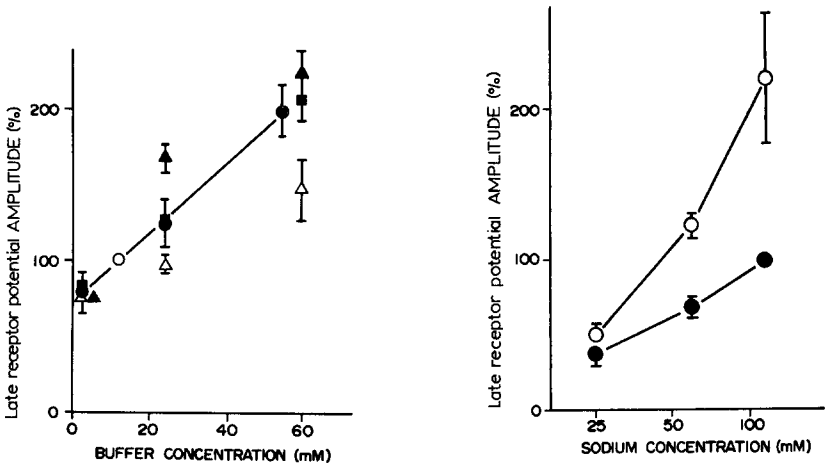

Fig. 3. Buffer concentration and late receptor potential amplitude. The points represent the average of two separate perfusions with at least three different retinas, except Tris which involves measurements from only the first perfusions. ●, phosphate; ■, borate; ▲, asparagine; and △, Tris. The late receptor potential amplitude at 12 mM of the respective buffer was taken as 100 % (○).

Fig. 4. The dependence of the buffer effect on log of Na^+ concentration. Each point represents data from at least three different retinas. 12 mM (●) and 60 mM (○) borate buffered Ringer's (pH 8.1) was used. Na^+ concentration was reduced by replacement with Li^+.

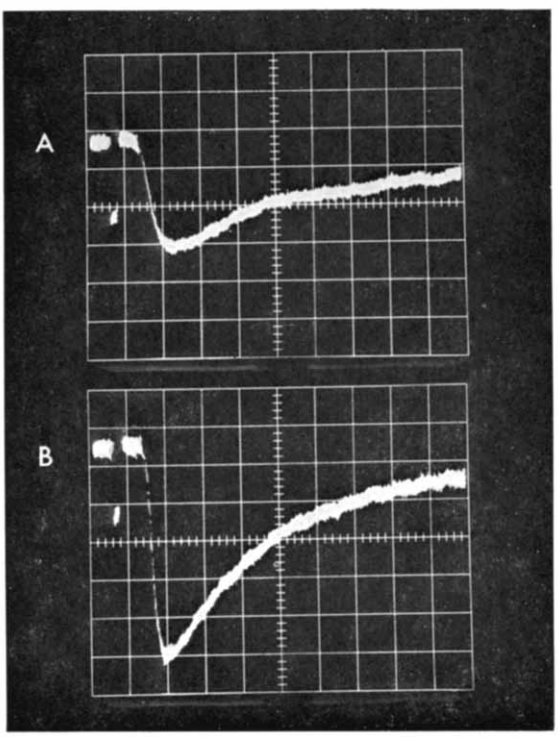

Fig. 5. Effect of ^2H_2O substitution on late receptor potential. Each signal is preceded by a 50 μV–50 ms calibrator pulse. A. H_2O Ringer's 12 mM phosphate (pH 7.4). B. ^2H_2O Ringer's 12 mM phosphate (pH 7.4). The signals shown represent the second of three perfusion repetitions. The results were the same with each repetition.

creased with each succeeding flash to little or no signal, consistent with the bleaching of the rhodopsin.

A representative experiment illustrating the effect of changing buffer concentration on the amplitude of the late receptor potential is shown in Fig. 2. The amplitude of the late receptor potential increases with increased buffer concentration. To avoid secondary effects dependent on the chemical nature of the particular buffer, the relationship between buffer concentration and late receptor potential amplitude was investigated with four different buffer systems. The buffer systems included monobasic and dibasic phosphate (anionic); borate–NaOH (anionic); asparagine–NaOH (zwitterionic) and Tris–HCl (cationic). The effect of changing buffer concentration on late receptor potential amplitude is presented in Fig. 3. In all cases increasing buffer concentration results in increased late receptor potential amplitudes, though Tris was found to be less effective than the others and not completely reversible.

To determine whether the buffer acts by interfering with the events producing or maintaining the late receptor potential, the effect of buffer on the Na^+ dependence of the late receptor potential was investigated. The results are shown in Fig. 4 and indicate that high buffer alters the normal Na^+ dependence of the late receptor potential. The late receptor potential amplitudes in 12 and 60 mM buffer are similar at 25 mM Na^+, but quite different at higher Na^+ concentrations.

The effect on the late receptor potential of substitution of $^2H^+$ for H^+ was determined and a representative experiment is illustrated in Fig. 5. The amplitude of the late receptor potential is increased in ^2H_2O solution but no time course changes are evident.

The effect of changing the pH of the perfusion fluid on the amplitude of the late receptor potential was measured over a range from pH 6.4 to 9.0. The results are shown in Fig. 6 and indicate an increased late receptor potential amplitude at increased pH. Though it does not affect the general trend of the results, the small

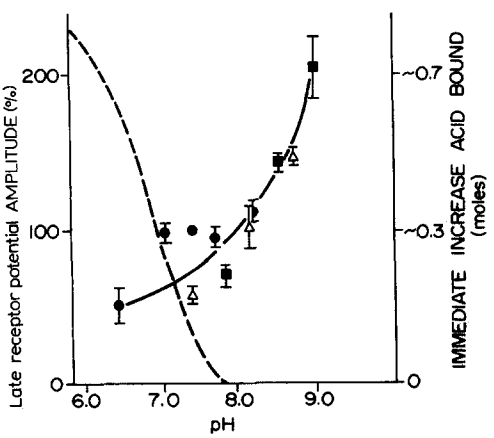

Fig. 6. The effect of pH on late receptor potential amplitude. Late receptor potential amplitude was measured in Ringer's solutions buffered with 12 mM phosphate (●), 12 mM borate (■) or 12 mM Tris (△) at different pH values within their buffering ranges. The response measured before and after test perfusion at pH 7.4 in 12 mM phosphate was taken as 100%. Rhodopsin H^+ uptake (------) from Radding and Wald[3].

phosphate effects from pH 7.2 to 7.4 suggest that in that case both pH and phosphate specific effects may be occurring.

DISCUSSION

The measurement of pH changes upon illumination of the retina (Fig. 1) confirms *in situ* the previous results which showed pH changes from rhodopsin solutions[3,4] and the rod outer segment suspensions[6-8]. If one assumes that 50% of the photopigment is bleached and that the pH change is approx. + 0.01 at pH 6.5 in 1 ml, then it is possible to approximate the H^+ uptake from the external solution per molecule of bleached rhodopsin. It is about $4.3 \cdot 10^{12}$ H^+ per $5 \cdot 10^{14}$–$5 \cdot 10^{15}$ rhodopsin molecules or 1 H^+ per 100–1000 molecules bleached. Since at this pH the solution[3] and rod outer segment results[7] indicate an uptake of one H^+ per molecule of rhodopsin, the rod cell or retina must be supplying most of the H^+ bound by the photopigment. The processes involved in the H^+ uptake could include: H^+ uptake by the photopigment; pH increases inside the rod cell; H^+ release by rod cell buffer components including the plasma membrane proteins; and H^+ movement across the plasma membrane. Conceivably the pH and protein ionization changes may be involved in the receptor process.

The additional experiments were intended to determine the relation between the H^+ changes and the late receptor potential. Three independent techniques were used to determine the effects The late receptor potential amplitude increased with increasing buffer (Figs 2 and 3), with the substitution of ^2H_2O for H_2O (Fig 5), and with increasing pH (Fig. 6). In addition, buffer affected the Na^+ dependence of the late receptor potential (Fig. 4). Because, of necessity, the experiments involve indirect measurements of the effect of H^+ changes on the late receptor potential, the site and mechanism of action of the chemical changes cannot be unequivocally determined at this time and must await further work. However, there is an explanation that is consistent with all of the experiments, *i.e.* that the H^+ changes of the retina act to reduce the amplitude of the late receptor potential. If the increased concentrations of buffer act to limit the H^+ changes, the results which show a larger late receptor potential at high concentrations of buffer (Figs 2 and 3) are consistent with this suggestion. In addition, since deuterium is similar to hydrogen it can participate in any process involving H^+ but its mobility in solution is lower than that of hydrogen. One mode of action of ^2H_2O could therefore be the reduction of the rate of H^+ change (Fig. 5). In the same vein, the increased late receptor potential amplitude with increasing pH could be the result of smaller H^+ changes caused by rhodopsin at the higher pH values (see rhodopsin change in Fig. 6). The H^+ uptake by the photopigment has been measured in both rhodopsin solutions and rod outer segment preparations and is most likely the cause of the H^+ changes measured in the retina.

Overall the results show that H^+ changes do occur upon illumination in the vertebrate retina. Chemical environments which are known to affect hydrogen ion changes including buffer, ^2H_2O and pH are found to affect the amplitude of the late receptor potential. In addition, buffer alters the Na^+ dependence of the late receptor potential. The various chemical environments may act by reducing the H^+ changes induced by illumination, suggesting that H^+ changes may be reducing the amplitude of the late receptor potential.

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