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The Effects of Carbon Dioxide, Oxygen and pH on Spreading Depression in the Isolated Chick Retina

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In the isolated chick retina, the propagation velocity of Spreading Depression (SD) was approximately doubled and the frequency of spontaneous waves was substantially increased with solutions bubbled with 5% CO_2 instead of air, at constant pH (7.5–7.6). There was no effect on SD of raised pO_2. Large changes of pH (to 6.4 and 9.4) produced, respectively, decreases and increases of velocity; but there was no clear pH dependence with changes less than 0.5 pH units. The resting $[K^+]_o$ and the elevation during SD, measured with K^+ sensitive micro-electrodes, were not consistently altered with 5% CO_2 when there was faster conduction. The effect of raised pCO_2 is opposite to that observed previously in rat cortex in vivo, which afforded evidence for a similarity between SD and the disturbance in the aura phase of migraine attacks. The effects in vivo must presumably be due to factors not acting in the isolated chick retina.

INTRODUCTION

Interference with the carbon dioxide/bicarbonate buffer system has been shown to affect propagation of Leão's spreading depression (SD) both in vivo in mammals[3,5,6] and in vitro in the chick retina[8,10]. In intact animals these effects might be indirectly mediated through changes in the local blood flow and oxygen availability; but this cannot be the case in the isolated retina studied by Martins-Ferreira et al.[8]. Raised bicarbonate concentration in the superfusate (with constant pCO_2 and raised pH) speeded the propagation of waves of spreading depression circling around the periphery of the retina. Raised CO_2 levels (with constant bicarbonate and lowered pH) tended to slow propagation[10] and to raise the threshold for elicitation of SD towards the end of the experiment[8]. In order to characterize these effects further, with changes more comparable to those used in vivo, we have systematically varied pCO_2, PO_2 and pH in experiments on the isolated chick retina. Our interest in these effects stems from the possibility that the effects of CO_2 and O_2 on spreading depression may be related to their therapeutic action in the aura phase of migraine[2,3,5].

METHODS

Chicks (Brown Leghorn; 1–21 days old) were anaesthetized with ether and decapitated. The eyes were removed and sectioned close to the equator. The vitreous was removed with a Pasteur pipette and the posterior eyecup was mounted in the apparatus (Fig. 1) or stored in saline.

Individual waves of SD were initiated by touching the periphery of the retina gently with a sharp pin, under a dissecting microscope (×7) and cool illumination from a fiber optic light source. The SD wave was visible within a few seconds as an expanding white region with a distinct line of sharp contrast at the wavefront[7]. After recovery from the SD wave the pinprick was usually still visible and a site within ca. 50 μm would be used to elicit the next wave after a regular interval (usually 6–9 min). Propagation velocity was measured using a graticule in the microscope to time the wavefront between points 2.8 mm

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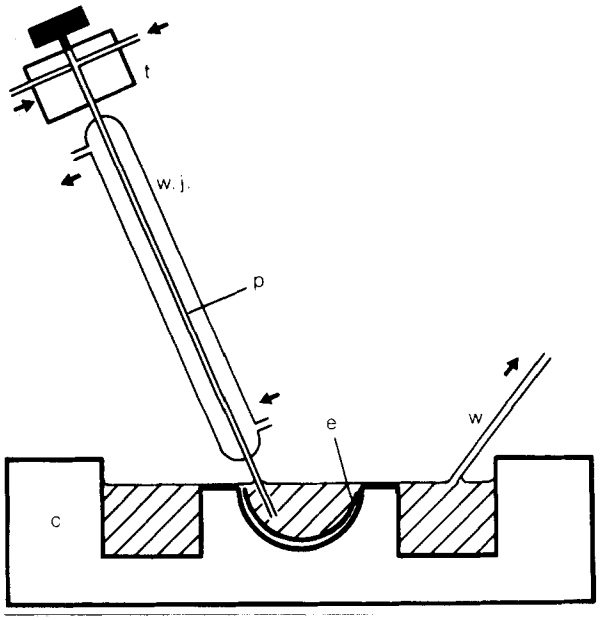

Fig. 1. Apparatus. The eyecup (e) was located in a central depression of a perspex chamber (c) and irrigated with saline from an apparatus consisting of a P.T.F.E. pipe (p), a heated water jacket (w.j.) and a two-way tap (t). Saline was drawn off to waste from a peripheral collecting well (w).

apart near the center of the retina, at least 2 mm from the point of SD initiation. A switch was pressed as the wavefront passed the graticule lines, causing a digital printer to record the time to a resolution of 0.1 s. Occasionally spontaneous SD waves occurred before the regular time for initiation: these were timed over the usual distance in the same region of retina, with the graticule rotated to be perpendicular to the wavefront. Wrinkling of the retina and white patches sometimes developed after several hours. Observations were terminated if these invaded the region of study.

Saline equilibrated with gases at room temperature was warmed to 28 °C (± 1 °C), passed over the retina at a rate of ca. 1 ml·min^{-1} and sucked to waste by a peristaltic pump (Fig. 1). The principal saline compositions are given in Table I. When bubbled with the stated gases, they each gave a pH in the range 7.5–7.6 at room temperature. Other gases were occasionally used, as indicated in the Results. Tissue preparation and initial recording were normally carried out in solution S1, bubbled with air. Solution S2, which was buffered with bicarbonate and 5% CO_2, is closer in composition to chick cerebrospinal fluid[1]; but S1 was preferred at the start because it produced fewer spontaneous SD waves during the setting up procedure. Solution S1 differs from S2 in having less bicarbonate, more phosphate buffer, and extra Na_2SO_4 and glucose to maintain Na^+ concentration and osmolarity. Osmolarity was accidentally overcompensated and is slightly higher (1.5%) in S1 than in S2. Solution S3 (pH 7.55 when bubbled with 100% O_2) was similar to S1, but with no bicarbonate.

Double-barreled K^+ sensitive microelectrodes (3–6 μm diameter) were constructed and calibrated by techniques described elsewhere[4]. These were advanced into the retina until they depressed and then suddenly penetrated a structure presumed to be the inner limiting membrane.

RESULTS

Preliminary experiments showed that waves of spreading depression could be elicited reliably at intervals of 6–9 min. With regular stimulation at this rate in phosphate-buffered saline (S1) equilibrated with air (pH 7.55), spontaneous SD waves were rare: 6/93 observed waves when all the observations under these conditions are pooled. The spontaneous waves usually originated from the cut edge of the retina. They were also rare with O_2-equilibrated solutions (S3) at the same pH (0/17 observed waves; not significantly different) but were significantly more com-

TABLE I

Composition of the principal solutions

Concentrations are in mM. The gases shown are those most commonly used with each solution, giving pH 7.5–7.6. Variations are described in the text.

Solution	*NaCl*	*KCl*	*$CaCl_2$*	*$MgSO_4$*	*$NaHCO_3$*	*NaH_2PO_4*	*Na_2HPO_4*	*Na_2SO_4*	*Glucose*	*Gas*
S1	107	7	1.8	0.9	1.5	1.0	5.0	11.9	28.3	Air
S2	107	7	1.8	0.9	34.5	0.17	0.83	—	7.2	95%O_2+5%CO_2
S3	107	7	1.8	0.9	—	1.0	5.0	12.7	29.0	O_2

mon ($P < 0.1\%$: χ^2-test with Yates correction) with bicarbonate buffered solutions (S2) equilibrated with 95% O_2 + 5% CO_2, pH 7.5 (26/64 observed waves) and with 85% O_2 + 15% CO_2, pH 7.0 (8/13 observed waves). The frequencies with 5 and 15% CO_2 are not significantly different from each other, but each is significantly greater ($P < 1\%$) than the results quoted above for 100% O_2, as well as for air. We therefore conclude that low levels of CO_2 and bicarbonate at constant pH tend to reduce the tendency for the isolated retina to produce spontaneous waves of SD.

Fig. 2A shows results from an experiment in which propagation velocity was compared in solutions equilibrated with air (S1), 95% O_2 + 5% CO_2 (S2) and 100% O_2 (S3), all at pH 7.55. The propagation velocity was highest in the bicarbonate—CO_2 buffered solution. In experiments on 7 retinae using this experimental design, with solution changes maintained for 10–20 min, the mean velocities and standard errors of the means were 2.21 ± 0.10 mm/min (n = 56) for air, 2.24 ± 0.21 mm/min (n = 17) for O_2 and 4.26 ± 0.29 (n = 30) for 95% O_2 + 5% CO_2. The velocities for air and O_2 are not significantly different but that for 95% O_2 + 5% CO_2 is significantly greater ($P < 0.1\%$; Student's t-test) than those for either air or O_2.

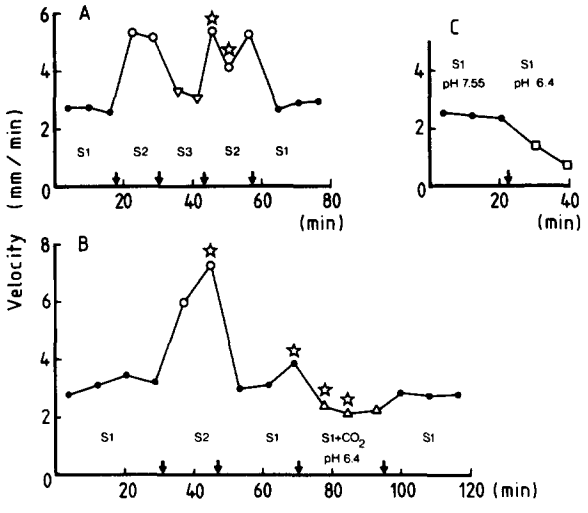

Fig. 2. The effect of solution changes on the propagation velocity of SD waves. A–C are from separate retinae observed initially in solution S1 equilibrated with air (pH 7.55). Changes were to: (A) S2 (95% O_2 + 5% CO_2; pH 7.55) and S3 (100% O_2; pH 7.55); (B) S2 (95% O_2 + 5% CO_2; pH 7.55) and S1 (95% O_2 + 5% CO_2; pH 6.4); and (C) S2 (air; acidified with HCl to pH 6.4.). Solution changes are shown by arrows and spontaneous SD waves by asterisks. The changes in C were irreversible, with the retina remaining refractory to SD.

Retinae that were kept through most of an experiment in solution S2, equilibrated with 95% O_2 + 5% CO_2, gave a wide range of velocities in this solution (mean 4.84 ± 0.50 (S.E.M.) mm/min; total range 1.3–7.5 mm/min). Changes to the same solution equilibrated with 85% O_2 + 15% CO_2 (pH 7.0) produced, on average, a decrease in velocity; but this was not significant (mean difference between measurements before and ca. 6 min after a change 0.8 ± 0.49 (S.E.M.) mm/min; n = 7).

When the bathing solution was changed from S1 equilibrated with air to the identical solution equilibrated with 95% O_2 + 5% CO_2 (without raising the bicarbonate concentration), pH fell from 7.55 to 6.4. A small but significant fall in propagation velocity occurred, instead of the rise observed with the same gas mixture at constant pH (Fig. 2B). The mean velocity at pH 6.4 under these conditions (1.67 ± 0.12 mm/min, n = 17) was significantly less than the control values in air ($P < 1\%$). The phosphate buffered solution (S1) was also acidified to pH 6.4 by addition of HCl and equilibrated with air; it then produced a marked drop in propagation velocity (Fig. 2C) followed by irreversible deterioration of the retina associated with overall whitening and refractoriness for SD. Changes in pH of this solution over the range 7.2–7.8 and changes of Ca^{2+} concentration to 0.5 and 1.5 mM had no clear effect on propagation velocity. More pronounced alkalinization with the high bicarbonate solution (S2) bubbled with 100% O_2 (pH 9.4) caused an increase in propagation velocity, but substantially less than when the same solution was bubbled with 95% O_2 + 5% CO_2 (pH 7.55). It appears that although velocity is pH-sensitive at constant pCO_2, rather extreme values are required to equal or to counter the effects of 5% CO_2.

The marked differences between the low bicarbonate solutions (S1 and S3) and the high bicarbonate solution (S2) at constant pH might have been due to the compensatory changes made in sulphate and glucose rather than to the changes of bicarbonate and pCO_2. This was ruled out in control experiments with a solution identical to S2 except for 69 mM sucrose in place of 34.5 mM $NaHCO_3$. Changing from S1 to this solution equilibrated with air or O_2 (pH 7.5–7.6) produced no change in velocity (mean velocity 2.18 ± 0.37 (S.E.M.) mm/min; n = 5).

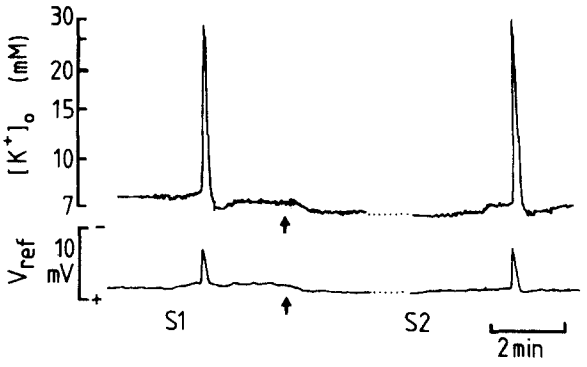

Fig. 3. Measurements of extracellular K^+ activity changes during SD. Records of potassium signal (upper trace) and voltage (lower trace) from a double-barreled K^+-sensitive microelectrode during SD waves in a retina superfused with solution S1 (air; pH 7.55) and then S2 (95% O_2 + 5% CO_2; pH 7.55). Calibrations for the K^+ signal are the K^+ concentrations in calibration solutions ((NaCl) + (KCl) = 150 mM) giving the indicated voltages. Ca. 6 min of the continuous recording is omitted. Arrows indicate the solution change.

Since rises of potassium concentration in extracellular space are probably involved in SD propagation[3], we measured $[K^+]_o$ with ion-selective microelectrodes in two retinae and looked for differences under a pair of conditions that gave markedly different propagation velocities (Fig. 3). Baseline $[K^+]_o$ in the tissues was normally in the range 7–9 mM and changed somewhat unpredictably when a change was made from S1 equilibrated with air to S2 equilibrated with 95% O_2 + 5% CO_2. On average there was an increase of $[K^+]_o$ (+0.57 ± 0.26 mM, n = 14; $P < 5\%$). The K^+ concentration during a wave of SD was elevated for ca. 15 s, reaching peak values during this time of 25–35 mM, with a negative potential shift ca. 5 mV. There was no clear difference between the characteristics of the $[K^+]_o$ rise or the negative potential shift in the two solutions (Fig. 3).

DISCUSSION

The propagation velocity of SD and the incidence of spontaneous waves were both increased when a bathing solution equilibrated with room air was changed to one equilibrated with 95% O_2 + 5% CO_2 at the same pH (7.55). No such effect was observed with 100% O_2. This sensitivity to CO_2 was not observed by Martins Ferreira et al.[8] in comparisons of retinae maintained from the start of an experiment in 100% O_2 and 95% O_2 + 5% CO_2. This may be because of a difference in pH between the two conditions compared by Martins Ferreira et al.[8]. We measured the pH of their solution when equilibrated with these gases and obtained values of 9.2 (100% O_2) and 7.5 (95% O_2 + 5% CO_2). From our results with altered pH, the velocity tends to be raised by a substantial alkaline shift of the bathing medium, as well as by increased pCO_2. This pH effect essentially confirms a separate result obtained by Martins Ferreira et al.[8], in which they varied bicarbonate with constant pCO_2 and will consequently have varied the pH of their solutions. When bicarbonate is held constant and both pCO_2 and pH are altered, as in some of our experiments and in the study of Rodrigues and Martins Ferreira[10], the two effects are opposed. The effects we obtained with 5% CO_2 were substantially larger, however, than those due to pH changes over the range 7.0–9.4. We found no evidence in our study for any effect on SD of an elevation of pO_2 above the level in air.

The direct effect of raised pCO_2 on SD in the chick retina, at constant pH, is in the wrong direction to account for the effects observed in the rat in vivo. In the rat, 10–15% inspired CO_2 raised the threshold for eliciting SD and there was a slowing or failure or propagation[3,5]. Since the pH of cerebrospinal fluid falls, however, with CO_2 inspiration (ca. 0.25 pH units for 10% inspired CO_2)[9], the pH-dependence described above might contribute to the observed changes. In the chick retina, however, we needed large falls of pH (> 0.5 units) to slow SD significantly, even without the opposing effect of raised pCO_2. Since we are essentially unable to confirm the results obtained in the rat with the chick retina preparation, in vitro, we conclude that either the two tissues are intrinsically different in respect of SD sensitivity to CO_2, or else the effect in the rat is principally an indirect one, for example due to changes in blood flow.

The mechanism by which CO_2 speeds SD propagation in the chick retina is unknown. A rise in pCO_2 lowers cytoplasmic pH[11] and may influence cell volume and the dynamics of K^+ release and build-up. We found no marked change, however, in the rise of extracellular $[K^+]_o$ during SD under these conditions.

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REFERENCES

1 Anderson, D. K. and Hazelwood, R. L., Chicken cerebrospinal fluid: normal composition and response to insulin administration, *J. Physiol. (Lond.)*, 202 (1969) 83–95.

2 Dexter, S. L., Rebreathing aborts migraine attacks, *Brit. Med. J.*, 284 (1982) 312.

3 Gardner-Medwin, A. R., Possible roles of vertebrate neuroglia in potassium dynamics, spreading depression and migraine, *J. exp. Biol.*, 95 (1981) 111–127.

4 Gardner-Medwin, A. R. and Nicholson, C., Changes of extracellular potassium activity induced by electric current through brain tissue in the rat, *J. Physiol. (Lond.)*, 335 (1983) 375–392.

5 Gardner-Medwin, A. R. and Skelton, J. L., Leão's spreading depression: evidence supporting a role in the migraine aura. In F. Clifford Rose and W. K. Amery (Eds.), *Cerebral Hypoxia in the Pathogenesis of Migraine*, Pitman Press, Bath, 1982, pp. 127–131.

6 Lehmenkühler, A., Speckmann, F. J. and Caspers, H., Cortical spreading depression in relation to potassium activity, oxygen tension, local flow and carbon dioxide tension. In M. Kessler (Ed.), *Ion and Enzymatic Electrodes in Biology and Medicine*, University Park Press, Baltimore, MD, 1976, pp. 311–315.

7 Martins-Ferreira, H. and de Oliveira Castro, G., Light scattering changes accompanying spreading depression in isolated retina, *J. Neurophysiol.*, 29 (1966) 715–726.

8 Martins-Ferreira, H., de Oliveira Castro, G., Struchiner, C. J. and Rodrigues, P. S., Circling spreading depression in isolated chick retina, *J. Neurophysiol.*, 37 (1974) 773–784.

9 Messeter, K. and Siesjö, B. K., Electrochemical gradients for H^+ and HCO_3^- between blood and CSF during sustained acid-base changes. In B. K. Siesjö and S. C. Sørensen, (Eds.), *Ion Homeostasis of the Brain*, Munksgaard, Copenhagen, 1971, pp. 190–200.

10 Rodrigues, P. S. and Martins-Ferreira, H., Influencia do pH na velocidade de propagacao dal depressao alastrante na retina isolada, *Proc. Acad. Brasileira de Ciencias* 47 (1975) 366–367.

11 Thomas, R. C., The effect of carbon dioxide on the intracellular pH and buffering power of snail neurones, *J. Physiol. (Lond.)*, 255 (1976) 715–735.