Effects of Anoxia, Ammonia and Carbon Dioxide on Light-Induced Responses in Isolated Carp Retina

341

Effects of Anoxia, Ammonia and Carbon Dioxide on Light-Induced Responses in Isolated Carp Retina

Koroku Negishi

Department of Neurophysiology, Neuroinformation Research Institute (NIRI),

University of Kanazawa School of Medicine, Kanazawa 920

(Accepted 13 March 1984)

Abstract

Isolated carp retinas were used to examine the effects of anoxia (N_2), cyanide (HCN), ammonia (NH_3) and carbon dioxide (CO_2) on the horizontal cell membrane potential as well as on the transreceptoral, intraretinal and transretinal responses recorded by means of two glass microelectrodes. Responses were induced by a focal stimulus (0.5 mm dia., 620 nm) and diffuse background illumination (white light). Two or three of the responses were simultaneously recorded. The following results were obtained : (i) The horizontal cell membrane potential was affected rapidly by anoxia, HCN and NH_3 ; the membrane potential was initially depolarized and then hyperpolarized, accompanied by abolition of S-potentials. (ii) The intraretinal response (composed of proximal PIII and PII components of the electroretiongram) was abolished by N_2 (anoxia), HCN or NH_3, while the focal transreceptoral response (distal PIII) remained almost unchanged or, as in the case of NH_3 application, increased. This suggests that synaptic transmission from the receptors to proximal (horizontal and bipolar) cells is readily interrupted, while the electrogenesis of receptor cells persists under a brief period of anoxia or exposure to low concentrations of HCN or NH_3 in O_2. (iii) The focal intraretinal response (proximal PIII) was enhanced in amplitude by the presence of negative potential shift (PII -dominant potential) caused by diffuse background illumination. This negative potential was selectively abolished by the agents used, particularly by NH_3. (iv) CO_2 (10%) in O_2 gradually hyperpolarized horizontal cells and reduced both transreceptoral and intraretinal responses as well as S-potentials with the same time sequence, indicating that CO_2 supresses primarily the receptor activity, reducing retinal excitability in general. (v) Although the effects of the gases used are assumed to be due to complex changes in both aerobic metabolism and intracellular pH, the site most susceptive to respective gases appears to be different in the retina.

The rapid and drastic effects of carbon dioxide (CO_2) and ammonia (NH_3) on the membrane potential of horizontal cells were first observed by Laufer et al[1] in the isolated retina of marine fishes (*Gerridae, Centropo-* *midae* and *Mugilidae*) ; CO_2 hyperpolarized while NH_3 initially depolarized and then hyperpolarized horizontal cells, accompanied by abolition of S-potentials. These observations were confirmed later and reported

together with the effects of anoxia and metabolic inhibitors on horizontal cells by the Caracas research group[2)-7)]. Anoxia and certain metabolic inhibitors such as cyanide (HCN), azide (N_3H) and carbon monoxide (CO) initially depolarized and then hyperpolarized, accompanied by abolition of S-potentials in a way similar to NH_3. These authors concluded that the horizonal cell is very sensitive to the above gases and metabolic inhibitors, suggesting that the function of this class of cells is highly dependent upon aerobic metabolism.

Such interpretation was based on the following findings : (i) NH_3 was found to abolish light-induced responses except for a "receptor potential" in the fish retina[3)]. (ii) N_3H was used to differentiate the distal PIII subcomponent from the proximal PIII of the frog electroretinogram[8)]. Findings (i and ii) support the view that the receptor cells are more resistant than the other retinal cells to NH_3 and N_3H. (iii) Anoxia, NH_3, metabolic inhibitors and CO_2 strogly influenced the resting membrane potential of horizontal cells, whereas the resting potential of cells which did not give rise to S-potentials in the same fish retina was only slightly affected by these gases[9)10)].

Contrary to the foregoing view, Drujan et al.[11)] demonstrated that the S-potential was able to spread laterally through an anoxic area of the retina. Along with other findings in tissue and single cell respiration experiments[12)], they concluded that the oxygen-dependence of the S-potentials reflects the aerobic metabolism of photoreceptor cells.

The present series of experiments was performed to reappraise the effects of anoxia, HCN, NH_3 and CO_2 on horizontal cells of the carp retina, and to further clarify the site susceptible to anoxia and these agents. In addition to the intracellular recording of horizontal cells, the transreceptoral, intraretinal and transretinal light-induced responses were simultaneously recorded. A part of the results obtained from the present series of

anoxia experiments was reported elsewhere[6)]

Materials and Methods

The experiments were performed on the isolated retina of the carp (*Cyprinus carpio*). Before eye enucleation the fish was maintained in darkness for about 1 hr. The retina was then removed under dim room light (about 3 lx with white light) and kept, receptor side-up, in a moist chamber, having 1 outlet and 3 inlets. Oxygen (O_2) was passed continuously through the chamber and served as the control gas medium. The gas exchange system used was the same as that described previously[5)6)]. For application of cyanide (HCN) or of ammonia (NH_3) to the isolated retina, a minute amount of these gases was added to the control gas stream passing through the chamber by means of a by-pass system. We were unable to measure and control the concentrations of HCN and NH_3 in the chamber ; very low concentrations were assumed to need for producing changes in the membrane potential. In fact, the NH_3 concentration applied was close to our threshold for smelling it.

Photic stimulation

A 500 W Xenon arc lamp with color temperature of 6000°K was used as the source of light. Red (620 nm) light was focused on a diaphragm mounted in front of an electromagnetic shutter driven by an electronic stimulator. After collimation, the beam passed through the monochromatic interference filter (halfband width of 10-14 nm) and a series of neutral density filters which reduced the light intensity by 0.5-log steps up to 3.5 log units, and was focused on to the retinal surface as a circular spot of 0.5 mm dia. by means of an apochromatic objective. The illumination level of the focal stimulus was lower by about 2 log units than that necessary to produce the maximal amplitude of S-potentials. The duration of the stimulus was 300 msec, and it was delivered every 1.5 sec. To examine the effect of background illumination on the focal light-induced responses, a 6-V pilot lamp, placed 10 cm away

from the preparation, was used to illuminate diffusely the entire retina ; the illumination level was approximately 2 lx at the receptor surface.

Recording methods

Glass-microelectrodes, filled with 2.5 M KCl, of 10-25 MΩ resistance were used to record the membrane potential of horizontal cells through a direct-coupled amplifier, as well as extracellular light-induced responses through RC-coupled amplifiers. For simultaneous recordings of 2 or 3 types of light-induced responses, two microelectrodes were used ; one was placed at the receptor surface, and the other was located at the outer plexiform layer (OPL) or inserted into a horizontal cell. Both microclectrodes were directed towards the center of the focal stimulus area (0. 5 mm dia.). A common indifferent electrode was an Ag-AgCl-wick placed beneath the retina at the vitreal side. Detailed methods of the simultaneous recording were described elsewhere[6].

In the present report, a light-induced change in the field potential between the microelectrode tips at the receptor surface and at the OPL is called the "transreceptoral response" (Trc) ; it was recorded through a differential RC-coupled amplifier system (time constant, 2. 0 sec). On the other hand, a light-induced change in the field potential between the tip of the intraretinal microelectrode (at OPL) and the indifferent Ag-AgCl electrode (at the vitreal side) is named as the "intraretinal response" (Irt), and was fed into a single-ended amplifier system (t.c., 1.0 sec). In addition, a light-induced responses, recorded with the surface microelectrode and the indifferent electrode (at the vitreal side), is termed the "transretinal response" (Trt), which was fed into another single ended amplifier system (t. c., 1.0 sec). It may be noted that when a focal light stimulus was used the transreceptoral response corresponds to the distal PIII and the intraretinal response to the proximal PIII of Murakami and Kaneko[8], and that when a

diffuse stimulus was used the transreceptoral response is approximately similar to the cornea-negative component PIII and the intraretinal response to the cornea-positive component PII of Hanitzsch[13]. Furthermore, the transretinal response is synonymous with the slow surface potential[14)15], and the amplitude of this response was approximately equal to an algebraic summation of those of the transreceptoral and intraretinal responses. All of the date from the experiments were stored on a 4-channel FM magnetic tape (TEAC R-410). The extracellular responses were displayed positivity-upward at the receptoral side in respect to the vitreal side.

Results

Intracellular recordings of horizontal cells

The membrane potential of horizontal cells in the carp retina was drastically influenced by anoxia, HCN, NH₃ and CO₂. Figure 1 summarizes the results obtained in the present experi-
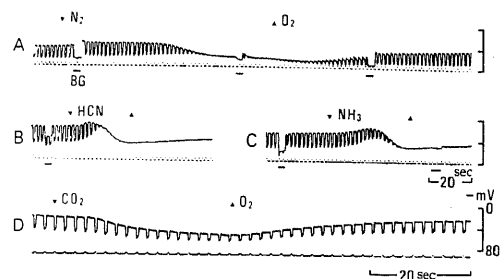

Fig. 1. Effects of anoxia, HCN, NH₃ and CO₂ on the horizontal cell membrane potential. Downward deflections are hyperpolarizing S-potentials in response to focal light stimuli of red light (a circular spot of 0.5 mm dia.). Periods of exposure of the isolated retina to various gases are indicated with triangles (▼ and ▲), whereas periods of background illumination are indicated with lines (BG) below the stimulus-signal tracing. Record A : Control gas medium (O₂) was gradually replaced by N₂ and reintroduced. Records B and C : A minute amount of HCN or NH₃ was mixed into the gas stream of O₂ during the period marked with triangles. Record D : O₂ was replaced by a gas mixture of CO₂ (10%) in O₂ and then reintroduced. Potential (0-40-80 mV) scales are shown at the right-side end of each record, and time (20 sec) scales indicated below records C and D.

ments. Anoxia (record A), HCN (B) and NH_3 (C) initially depolarized and then rapidly hyperpolarized the membrane potential of the cells, accompanied by abolition of the S-potentials (A-C), whereas 10% CO_2 in O_2 (D) reversibly hyperpolarized the cell and diminished the S-potential.

Extracellular recordings of light-induced potentials

A surface slow response, recorded trans-retinally at the receptor surface and induced by a focal spot of light, is dominantly positive,
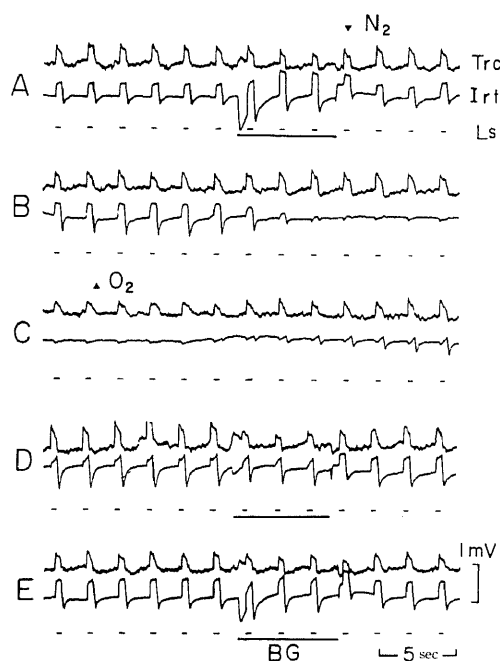
whereas a response produced by a non-focal, surround or diffuse illumination is predominantly negative[14]−[17]. In our recordings, the amplitude of the positive, focal trans- (Trt) or intra-retinal (Irt) response was enhanced during diffuse background illumination (BG) which prodused a negative potential shift (Figs. 2-5A, in the control state).

The focal intraretinal (Irt) response was abolished by exposure of the retina to N_2 (anoxia ; Fig. 2) or to low concentrations of HCN (Fig. 3) or of NH_3 (Fig. 4), although the focal transreceptoral (Trc) response was only slightly reduced or, as in the case of NH_3 application, enlarged at least initially. The background illumination effect on the focal intraretinal response also disappeared during a brief period of anoxia, and recovered more slowly than the focal intraretinal response. Notice that background illumination reduced both focal transreceptoral and intraretinal responses even after their amplitudes had recovered with O_2 (Fig. 2D).

The effect of HCN was similar to that of anoxia on the focal transreceptoral and intraretinal responses. In the experiment shown in Fig.3, the transretinal (Trt) response was recorded simultaneously with both transreceptoral and intraretinal responses. After HCN application, large shifts occurred in all

Fig. 2. Anoxia experiment with simultaneous recording of the transreceptoral (Trc) and intraretinal (Irt) responses. Dashed lines (Ls) signal focal light stimuli. Records A-E are taken from a continous recording of a whole course of anoxia experiment. For illustration, unnecessary parts are cut out between the pieces of the recordings illustrated. Data-arrangement in Figs. 3-6 are made in the same way as in Fig. 2. Elapsed time between records A and B, B and C, C and D, and D and E is 80,15,10 and 40 sec, respectively. The control gas medium (O_2) was gradually replaced by N_2 (▼ in A). The application period of N_2 was about 100 sec in this case. Time (5 sec) and potential (1 mV) scales are indicated at the right end of record (E).
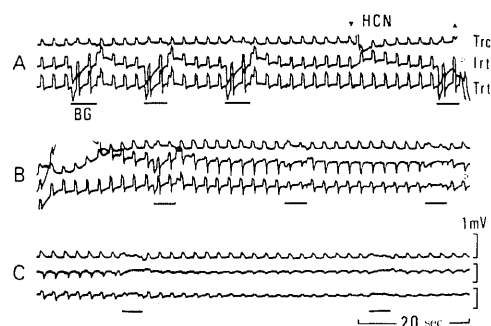

Fig. 3. HCN experiment with simultaneous recording of the transreceptoral (Trc), intraretinal (Irt) and transretinal (Trt) responses. A minute amount of HCN was mixed into the O_2-stream during the period marked with triangles (▼ and ▲ in A). Notice different amplifications for the three responses. Records A to C are continuous (▷).

tracings, probably due to a change in the d.c. potential (between A and B). The trans-receptoral response was slightly increased in amplitude, while both intra- and trans-retinal responses were markedly influenced by this agent. The positive potential of the intraretinal response was diminished, accompanied by enlargement of a negative transient at the offset of the focal stimuli (off-transient). This kind of off-transient was also seen intraretinally in the anoxia experiment (Fig. 2B and D).

NH₃ was observed to cause a transient enlargement of the intra- and trans-retinal responses and prolonged enlargement of the transreceptoral response (Fig. 4A). Sub-sequently, the amplitude of the transretinal response was reduced considerably due to abolition of the positive intraretinal response and to appearance of a negative intraretinal response, although the enlargement of the transreceptoral response persisted (A). Twenty sec later (B), the transreceptoral response gradually returned to the original amplitude and the intraretinal response became positive, whereas the transretinal response was still small because of poor recovery of the positive intraretinal response. Further 30 sec later (C), the intra- and trans-retinal responses recovered still better, whereas diffuse background illumination caused a positive potential shift in all tracings and markedly reduced all types of response, indicating that the negative intra-retinal potential shift in response to diffuse background still did not recover in this period.
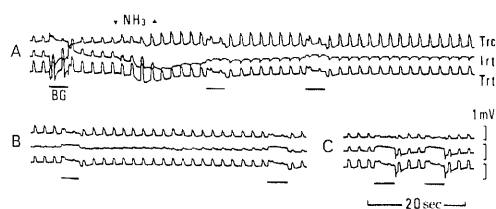
Differing from the effects of anoxia, HCN and NH₃, an exposure of the retina to a gas mixture of 10% CO₂ in O₂ reduced the amplitude of both focal transreceptoral and intraretinal responses, and also diminished the negative intraretinal potential shift caused by diffuse background illumination (Fig. 5A). All these reductions occurred with the same time sequence, indicating that CO₂ acts primarily on the transreceptoral response or reduces generally retinal excitability, and that it does not interrupt synaptic transmission at the outer plexiform layer with this concentration.

Relationship between S-potentials and extracellular responses

In some experiments, the transretinal response and the horizontal cell membrane potential were simultaneously recorded. Figure 5B shows the coincident changes caused by CO₂. Following introduction of 10% CO₂ in O₂ (Fig. 5B-1), the amplitude of the trans-retinal response and of the S-potentials was gradually reduced with the same time course, while the horizontal cell membrane potential (Vh) hyperpolarized (from 30 to 45 mV). Replacement of CO₂ by O₂ reversed these

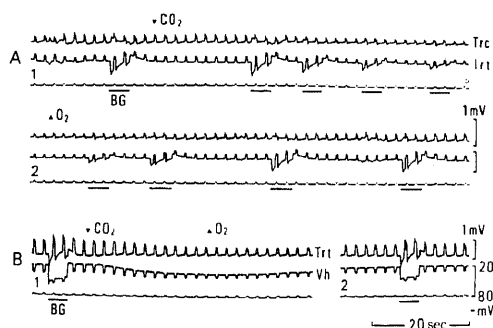Fig. 5. CO₂ experiment. Record A : Simultaneous recording of the transreceptoral (Trc) and intra-retinal (Irt) responses. O₂ was gradually replaced by a gas mixture of 10% CO₂ in O₂ (▼ in A-1), and reintroduced (▲ in A-2). Record B : Simultaneous recording of the transretinal response (Trt) and the S-potential (Vh). Elapsed time between records B-1 and 2 was 15 sec. Potential scale (20-80 mV)at the right-end of record (B-2) is given for the horizontal cell membrane potential in d. c. recording.

Fig. 4. NH₃ experiment with simultaneous record-ing of the transreceptoral (Trc), intra- (Irt) and trans-retinal (Trt) responses. Elapsed time bet-ween records A and B, and between B and C was 20 and 30 sec, respectively.

effects. In Fig. 6, the first application of NH_3 produced an enlargement of the amplitude of both transretinal response and S-potentials (from A to B), the enlargement of the S-potentials being associated with a depolarization of the resting potential (from 40 to 20 mV). On the other hand, the second application of NH_3 caused a further slight enlargement of both responses and an additional slight depolarization, but subsequently resulted in a rapid diminution of the transretinal response, a rapid abolition of the S-potentials and in a sharp hyperpolarization (from 20 to 60 mV) of the horizontal cell (B). Records C, D and E were obtained from different horizontal cells during the recovery state, 3, 5 and 10 min respectively, after the second application of NH_3. The enhancement of the focal transretinal response by diffuse background illumination was observed only in the control state (A). After the first application of NH_3, even though the S-potentials in response to both focal and diffuse light stimuli and the focal transretinal responses were markedly enlarged, background illumination did not cause a negative transretinal shift and reduced the focal response (A-B). Furthermore, although the focal transretinal response and S-potentials returned to the original amplitude, background illumination still reduced the focal

transretinal response (E). In these periods (after NH_3 application and in its recovery), therefore, the negative intraretinal potential shift in response to background illumination (P II) was selectively abolished, as already shown in Fig. 4.

Discussion

Possible changes in extra- and intra-cellular pH

The agents used in the present experiments can cause pH changes in the retinal tissue. HCN and CO_2 should decrease while NH_3 should increase the tissue pH. CO_2 reduced while NH_3 enhanced the amplitude of the transreceptoral response, possibly due to in part a primary effect of the opposite pH shifts on the receptor cells. However, HCN and NH_3 showed a similar effect to that of anoxia on the horizontal cell mambrane potential; all of them initially depolarized and subsequently hyperpolarized the cells, and abolished the activities in the layer proximal to the receptor cells. On the other hand, CO_2 caused purely a hyperpolarization of horizontal cells and reduced all the responses with the same time course, but did not block synaptic transmission from the receptors to second order cells at this concentration of CO_2. Therefore, the initial depolarization of horizontal cells and the synaptic blockage at the OPL, caused by HCN and NH_3, appear not only due to a pH change in the tissue but also due to an impairment in aerobic metabolism.

CO_2 and NH_3 have been shown to decrease or increase the intracellular pH of snail neurons much more rapidly and largely than to change the extracellular pH [18]. Recently, with the same intracellular pH-sensitive microelectrode developed by the above author, Spray et al.[19] have demonstrated that the gap junctional conductance is a simple and sensitive function of intracellular pH. In their experiments, CO_2 is used to reduce the intracellular pH in electrically coupled cell pairs from blastomeres of amphibian and teleost embryos at the cleavage stage.
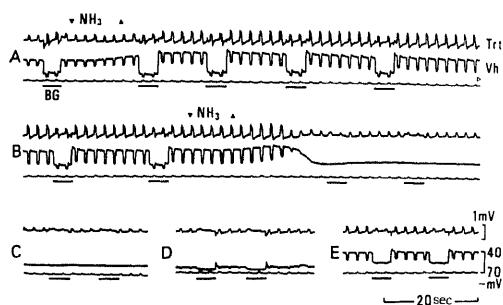

Fig. 6. NH_3 experiment with simultaneous recording of the transretinal response (Trt) and S-potential (Vh). Records A-B were continuous ($\triangleright$), whereas records C to E were obtained 3, 5 and 10 min after the second application of NH_3 ($\blacktriangledown$ in B), respectively; the membrane potentials were recorded from different horizontal cells.

In the fish retina, horizontal cells in a given layer are electrically coupled at gap junctions between them[20][21]. If CO$_2$ or NH$_3$ is assumed to primarily alter the intracellular pH of horizontal cells, the lateral spread of S-potentials (reflected in a hyperpolarization caused by background illumination in the present recordings) would be readily altered ; CO$_2$ reduces while NH$_3$ increases the spread potential. However, the situation is not simple as will be discussed, and the present experiments are not appropriately designed for the purpose to explore electrical coupling at gap junctions between horizontal cells. CO$_2$ may primarily act on cones and Müller cells to reduce the intracellular pH, because carbonic anhydrase activity has been found densely in the laminated segment of cones and in Müller cells but negative in rod, horizontal, bipolar and ganglion cells[22]. Certainly, CO$_2$ reduced (Fig. 5) while NH$_3$ increased the amplitude of the transreceptoral response (Fig. 6) ; a possible intracellular pH shift in cones might be linked with such response changes.

Anoxia sensitivity at the outer plexiform layer

The results shown in Fig. 1 with the carp retina confirmed earlier findings, reported on the retina of marine fishes, that the horizontal cell membrane potential is strongly influenced by anoxia, HCN, NH$_3$ and CO$_2$[1]−[7].

From the results shown in Figs. 1A and 2, it could be stated that the receptor cells were still responding to the focal stimulus, when the intraretinal responses and the S-potential disappeared during the course of O$_2$-deprivation. The anoxic abolition of the intraretinal responses means that the retinal layer proximal to the receptor cell layer became inactive. Drujan et al.[11] showed, with a double chamber system and with focal and non-focal light stimuli (see their Figs. 5 and 6), that the S-potential is able to spread laterally through an anoxic area of the retina. Therefore, they concluded that the oxygen-dependence of the S-potential is located at the receptor cell level

rather than at the horizontal cell level. However, taking into consideration the present finding (Figs. 1A and 2) that the transreceptoral response remained while the proximal responses (proximal PIII, PII and S-potential) were abolished during the course of O$_2$-deprivation. The site most susceptible to anoxia appears to be located at the outer plexiform layer. If this is the case, it could be reasonably assumed that the focal S-potential in the experiment by Drujan et al.[11] was readily abolished due to an anoxic blockage of synaptic transmission from the receptors to horizontal cells. The fact that the S-potential spreads laterally through an anoxic part indicates that the membrane properties of the somata at gap junctions beween horizontal cells are not altered significantly during such a brief period of O$_2$-deprivation. A possible evidence, for the view that synaptic transmission is the earliest event influenced by hypoxia, was presented by Eccles et al.[23] in the cat spinal reflex, and also by Hubbard and Løyning[24] in cat diaphragm-phrenic nerve preparations. According to the latter author's view, Trifonov and Ostrovskii[25] assumed the synaptic region at the outer plexiform layer in the carp retina to be the anoxia-sensitive site ; the results presented here supports this assumption.

On the basis of the viwe of Trifonov[25] that a depolarization of horizontal cells is due to transmitter release from receptor terminals, an L-aspartate or L-glutamate-like substance has been assumed to be an excitatory synaptic transmitter of receptors on horizontal cells[27]−[30]. Either amino acid, applied diffusely over the receptor surface, depolarizes horizontal cells, abolishes the S-potentials and isolates the transreceptoral response (distal PIII) from the other components of ERG[29][31]−[33]. On the other hand, elevated Mg^{++} (20 mM) and lowered Ca^{++} (0.5 mM) in a perfusing solution have been shown to hyperpolarize horizontal cells, block the S-potentials and to isolate the distal PIII, also causing a synaptic brockage from receptor terminals to horizontal cells[27][34].

The synaptic blockage with L-glutamate or L-aspartate has been assumed to be due to a depolarization-inactivation, whereas that with high Mg^{++} plus low Ca^{++} to be due to a stop of transmitter release from receptor terminals. If these assumptions are adaptable to interpretation of the present results, it would be conceivable that anoxia, metabolic inhibitors and NH_3 may initially facilitate transmitter release from receptor terminals (slight depolarization of horizontal cells) and subsequently stop the release (large hyperpolarization). Therefore, the oxygen dependence of the horizontal cell membrane potential may reflect the aerobic metabolism of the receptor cells, which couples with a transmitter-release mechanism in their synaptic terminals. However, a possibility that the postsynaptic membrane of apical dendrites of horizontal cells becomes inactive under hypoxia could neither be rejected completely nor be proved in any way with the present results.

Center-surround organization of the trans-retinal response

The amplitude of the focal transreceptoral response was slightly reduced by diffuse background illumination while the focal intraretinal response was markedly enhanced (Fig. 2A). This effect is due to an antagonistic center-surround organization of the surface potential[14)15], and it was observed in the transretinal response (record A in Figs. 3, 4 and 6). Therefore, it can be stated that a neural mechanism participating in the background illumination effect or in the center-surround phenomenon of the transretinal response takes place at a level proximal to the receptor cell layer, as already pointed out by Murakami and Sasaki[16)17].

In the present series of experiments, the focal stimulus was monochromatic at 620 nm while the diffuse background illumination was white, delivered from a 6-V pilot lamp. Therefore, the focal light stimulated mainly the cone system but the diffuse light activated both cone and rod systems. However, additional experiments,

in which white focal and diffuse lights or red focal (620 nm) and diffuse (600 nm) lights were combined in the same way as in the experiments illustrated, showed the same enhancing effect of the diffuse background on the focal transretinal response. Therefore, the neural mechanisms participating in the enhancing effect cannot be due to interactions between the cone and rod systems. It is obvious that the enhancing effect of the diffuse background illumination on the focal response requires the induction of a negative shift (PII-dominant) in the intraretinal potential.

Selective abolition of the transretinal negative potential by NH_3

In the present experiments, we were unable to control a quantity of NH_3 applied. The initial effect of this vapor at smaller doses was to enlarge the focal transretinal response (P III) and S-potential (see the first application in Fig. 6A). The initial enlargement of the transretinal response (see also Fig. 6A) is assumed to be in part due to an enlarged transreceptoral response (Fig. 4A), to an enlarged intraretinal response, or due to those of both responses. The final effect of NH_3 at larger doses was to abolish all kinds of activity in the proximal layer (S-potential, P II and proximal P III), while the transreceptoral response (distal PIII) remained almost unchanged (as seen at the beginning of Fig. 4B). It is of interest to point out that the enlargement of the focal transretinal response was coincident in time with that of the focal S-potential following the first NH_3 application (Fig. 6A-B), but during this period diffuse background illumination reduced the focal transretinal response, which had been enhanced by the background before the NH_3 application. This indicates that NH_3 interferes selectively with a neural process participating in generation of the negative intraretinal potential shift (PII) in response to diffuse background illumination and its enhancing effect on the focal intraretinal response. This selective

abolition by NH$_3$ persists during the recovery state of the responses (Fig. 6E). If the origin of a part of PII (*b*-wave) is Müller cells, as suggested by recent workers[35)-37)], the K$^+$-sensitivity of those cells might intimately be related to changes in the intracellular *p*H.

References

1) Laufer, M., Svaetichin, G., Mitaral, G., Fatechand, R., Vallecalle, E. & Villegas, J. : The effect of temperature, carbon dioxide and ammonia on the neuron-glia unit, p. 457-463. *In* R. Jung & H. Kornhuber (ed.), The Visual System : Neurophysiology and Psychophysics, Elsevier, Amsterdam, 1961.

2) Svaetichin, G., Negishi, K., Fatehchand, R., Drujan, B. D. & Selvin de Testa, A. : Nervous function based on interaction between neuronal and non-neuronal elements, p. 243-266. *In* E. D. P. de Robertis & R. Carrea (ed.), Progress in Brain Research, Vol. 15 Biology of Neuroglia, Elsevier, Amsterdam, 1965.

3) Fatehchand, R., Svaetichin, G., Negishi, K. & Drujan, B. D. : Effects of anoxia and metabolic inhibitors on S-potentials of isolated fish retina. Vision Res., **6**, 271-283 (1966).

4) Negishi, K. & Svaetichin, G. : Oxygen dependence of retinal S-potential producing cells. Science, **152**, 1621-1623 (1966).

5) Negishi, K. & Svaetichin, G. : Effects of anoxia, CO$_2$ and NH$_3$ on S-potential producing cells and on neurons. Pflügers Arch. ges. Physiol., **292**, 177-205.

6) Negishi, K. & Sugawara, K. : Evidence for the anoxia sensitivity of the synaptic region at the outer plexiform layer in the fish retina. Vision Res., **13**, 983-987 (1973).

7) Negishi, K., Svaetichin, G., Laufer, M. & Drujan, B. D. : Polarographic and electrophysiological studies of retinal respiration. Vision Res., **15**, 527-533 (1975).

8) Murakami, M. & Kaneko, A. : Differentiation of PIII subcomponents in cold-blooded vertebrate retina. Vision Res., **6**, 627-636 (1966).

9) Svaetichin, G., Negishi, K., Drujan, B. D. & Muriel, C. : S-potentials and retinal automatic control systems. Proc. Ist European Biophys. Cong., Baden, Y II B/3, 77-88 (1971).

10) Negishi, K. : Recent trend in studies of retinal S-potential. F. Ophthal. Jpn., **22**, 349-366 (1971).

11) Drujan, B. D., Svaetichin, G. & Negishi, K. : Retinal aerobic metabolism as reflected in S-potential behavior. Vision Res. Suppl., **3**, 151-159 (1971).

12) Drujan, B. D. & Svaetichin, G. : Characterization of different classes of isolated retinal cells. Vision Res., **12**, 1777-1784 (1972).

13) Hanitzsch, R. : Fast intraretinal potentials of the isolated mammalian retina. Vision Res., **12**, 781-791 (1972).

14) Brindley, G. S. : Responses to illumination recorded by microelectrodes from the frog's retina. J. Physiol., Lond., **143**, 339-352 (1956).

15) Motokawa, K., Oikawa, T., Tasaki, K. & Ogawa, T. : The spatial distribution of electric responses to focal illumination of the carp retina. Tohoku J. Exp. Med., **70**, 151-164 (1959).

16) Murakami, M. & Sasaki, Y. : Analysis of spatial distribution of the ERG components

in the carp retina. Jpn. J. Physiol., **18**, 326-336 (1968).

17) Murakami, M. & Sasaki, Y. : Localization of the ERG components in the carp retina. Jpn. J. Physiol., **18**, 337-349 (1968).

18) Thomas, R. C. : Intracellular *p*H of snail neurons measured with a new *p*H-sensitive glass micro-electrode. J. Physiol., Lond., **238**, 159-180 (1974).

19) Spray, D. C., Harris, A. L. & Bennett, M. V. L. : Gap junctional conductance is a simple and sensitive function of intracellular *p*H. Science, **211**, 712-715 (1981).

20) Naka, K. -I. & Rushton, W. A. H. : The generating and spread of S-potential in the fish (*Cyprinidae*). J. Physiol., Lond., **192**, 437-461 (1967).

21) Kaneko, A. : Electrical connections between horizontal cells in the dogfish retina, J. Physiol., Lond., **213**, 95-105 (1971).

22) Parthe, J. : Histochemical localization of carbonic anhydrase in vertebrate nervous tissue. J. Neurosci. Res., **6**, 119-131 (1981).

23) Eccles, R. M., Løyning, Y. & Oshima, T. : Effects of hypoxia on the monosynaptic reflex pathway in the cat spinal cord. J. Neurophysiol., **29**, 315-332 (1966).

24) Hubbard, H. J. and Løyning, Y. : The effects of hypoxia on neuromuscular transmission in a mammalian preparation. J. Physiol., Lond., **185**, 205-223 (1966).

25) Trifonov, Yu. A. & Ostrovskii, M. A. : Characteristics of synaptic transmission between photoreceptors and horizontal cells. Neirofiziologiya, **2**, 79-86 (1970).

26) Trifonov, Yu. A. : Study of synaptic transmission between photoreceptors and horizontal cells by means of electric stimulation of the retina. Biofizika, **13**, 809-817 (1968).

27) Dowling, J. E. & Ripps, H. : Aspartate isolation of receptor potentials in the skate retina. Biol. Bull. (Woods Hole)., **141**, 384-385 (1971).

28) Dowling, J. E. and Ripps, H. : Adaptation in skate photoreceptors. J. gen. Physiol., **60**, 698-719 (1972).

29) Murakami, M., Ohtsu, K. & Ohtsuka, T. : Effects of chemicals on receptors and horizontal cells in the retina. J. Physiol., Lond., **227**, 899-913 (1972).

30) Ehinger, B. : Connections between retinal neurons with identified neurotransmitters. Vision Res., **23**, 1281-1291 (1983).

31) Sillman, A. J., Ito, H. & Tomita, T. : Studeis on the mass receptor potential of the isolated frog retina. I. General properties of the response. Vision Res., **9**, 1435-1442 (1969).

32) Sugawara, K. & Negishi, K. : Effects of some amino acids on the horizontal cell-membrane potential in the isolated carp retina. Vision Res., **13**, 977-981 (1973).

33) Sugawara, K. & Negishi, K. : Effects of some amino acids on light-induced responses in the isolated carp retina. Vision Res., **13**, 2479-2489 (1973).

34) Kaneko, A. & Shimazaki, H. : Effects of external ions on the synaptic transmission from photoreceptors to horizontal cells in the carp retina. J. Physiol., Lond., **252**, 509-522 (1975).

35) Miller, R. F. & Dowling, J. E. : Intracellular responses of the Müller (glial) cells of mudpuppy retina : Their relation to *b*-wave of the electroretinogram. J. Neurophysiol., **33**, 323-341 (1970).

36) Newman, E. A. : Current source-density analysis of the *b*-wave of frog retina. J. Neurophysiol., **43**, 1355-1366 (1980).

37) Tomita, T. & Yanagida, T. : Origins of the ERG waves. Vision Res., **21**, 1703-1707 (1981).

剝離鯉網膜の光応答に及ぼす無酸素，アンモニアおよび二酸化炭素の作用

金沢大学医学部附属神経情報研究施設情報伝達研究部門
根岸晃六．金沢大学十全医学会雑誌，第93巻，第2号，341 – 351．(昭59)．

抄　　録　　剝離鯉網膜を用い，無酸素(N_2)，シアン化水素(HCN)，アンモニア(NH_3)および二酸化炭素(CO_2)の水平細胞膜電位および各種網膜光応答に及ぼす作用を2本の誘導ガラス電極を用いて検索した．光応答は焦点（径0.5 mm，620 nm）および全野照射（白色）により誘発した．2ないし3種の光応答を同時記録することにより，次の所見が得られた．（i）水平細胞網膜電位は無酸素（N_2），HCN および NH_3 に大きく影響を受けた．膜電位は初め脱分極，続いて過分されS電位は消失した．（ii）内網膜性応答（網膜電図の proximal PIII と PII要素を含む）は上記のガスで消失したが，焦点径受容器層応答(distal PIII)は殆んど不変か，またはとくに NH_3 投与の場合には，むしろ増大した．このことは光受容器細胞から水平および双極細胞へのシナプス伝達は容易に遮断されるが，光受容器細胞の電気現象の発生機構は短時間の無酸素および低濃度の HCN または NH_3 への曝露に対してかなりの抵抗性を持っていることを示す．（iii）焦点内網膜性応答（proximal PIII）の振幅は全野照射背景光が陰性電位変化（PII要素）を誘発する場合に増大された．この陰性電位の発生は上記ガス，とくに NH_3 によって選択的に消去された．（iv）CO_2（酸素中10%）は水平細胞を過分極し，同じ時間経過でS電位，経受容器層および内網膜性応答を縮小させた．このことは CO_2 が光受容器細胞の活動を原発的に抑制し，網膜全層に亘る興奮性を減弱するように働いていることを示す．（v）これらガスの網膜電気現象に及ぼす作用は，好気性代謝の障害と細胞内水素イオン濃度の変化に基ずく錯雑な影響の結果であり，それぞれ網膜内での機作部位が異なると考えられる．