Effects of Barium and Bicarbonate on Glial Cells of Necturus Optic Nerve

Studies with Microelectrodes and Voltage-sensitive Dyes

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ABSTRACT We have studied the effects of Ba++, a known K+ channel blocker, on the electrophysiological properties of the glial cells of Necturus optic nerve. The addition of Ba++ reversibly depolarized glial cells by 25–50 mV; the half maximal depolarization was obtained with a Ba++ concentration of ~0.3 mM. In the presence of Ba++, the sensitivity of the membrane to changes in K+ was reduced and there was evidence of competition between K+ and Ba++ for the K+ channel. These effects, which were accompanied by a large increase in the input resistance of the glial cells, indicate that Ba++ blocks the K+ conductance in glial cells of Necturus optic nerve.

With the K+ conductance reduced, we were able to investigate the presence of other membrane conductances. We found that in the presence of Ba++, the addition of HCO3- caused a Na+-dependent hyperpolarization that was sensitive to the disulfonic stilbene SITS (4-acetamido-4'-isothiocyanostilbene-2, 2'-disulfonic acid). Removal of Na+ resulted in a HCO3- dependent, SITS-sensitive depolarization. These results are consistent with the presence in the glial membrane of an electrogenic Na+/HCO3- cotransporter in which Na+, HCO3-, and net negative charge are transported in the same direction. In Cl- free solutions, the Ba++-induced depolarization increased, suggesting a small permeability to Cl-.

Using voltage-sensitive dyes and a photodiode array for multiple site optical recording, the distribution of potential changes in response to square pulses of intracellularly injected current were recorded before and after the addition of Ba++. In the presence of Ba++, both the amplitude and rise time of the potential increased and the decay of amplitude as a function of distance decreased. Such results indicate that Ba++ increases the membrane resistance more than the resistance of the intercellular junctions.

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INTRODUCTION

Since the early work of Kuffler and colleagues (e.g., Kuffler et al., 1966) studies of the membrane properties of glial cells have provided insight into their physiological role. Such studies have shown that K⁺ channels of glial cells play a role in K⁺ homeostasis within the neuronal microenvironment (see review of Coles, 1985). In many preparations, the K⁺ conductance of the glial membrane is so prominent that it is difficult to demonstrate additional ionic conductances in these cells (Ransom and Carlini, 1987). Thus the blocking of K⁺ channels might help to determine whether other ionic fluxes contribute to glial function.

One preparation that has proved particularly suitable for the study of glial cells is the optic nerve of *Necturus maculosus* (Kuffler et al., 1966). The nerve is 100 μm in diameter and consists of large glial cells interlaced with small unmyelinated axons. The glial cells are connected via gap junctions to form a syncytium. Their membrane behaves as a K⁺ electrode over a large range of K⁺ concentration.

Ba⁺⁺ has been shown to block K⁺ channels in a variety of excitable (Nishi and Soeda, 1964; Eaton and Brodwick, 1980; Armstrong et al., 1982) and nonexcitable (Burckhardt et al., 1984) membranes. Here we present evidence that Ba⁺⁺ decreases the K⁺ conductance in glial cells of *Necturus* optic nerve. With the K⁺ conductance reduced, we were able to examine the effects of ion substitutions, most notably HCO₃⁻, on the glial membrane potential. We also used voltage-sensitive dyes and a system for multiple-site recording to monitor the effects of Ba⁺⁺ on the glial syncytium.

Some of the results have been communicated in preliminary form (Aston et al., 1986, 1987).

METHODS

The dissection of the optic nerve, the experimental arrangement for electrical recording with intracellular electrodes (Kuffler et al., 1966; Aston et al., 1987), and the arrangement for optical recording using voltage-sensitive dyes (Salzberg et al., 1983) have been described elsewhere. Briefly, *Necturus* was decapitated, the optic nerve was exposed and desheathed in situ, and removed through a window in the roof of the mouth. For intracellular recording the nerve was mounted at room temperature (20–23°C), in a 0.2-ml chamber containing a Ringer's solution of the following composition in millimolar: 110 NaCl, 3 KCl, 2 CaCl₂ and 5 HEPES, (adjusted to pH 7.4 with NaOH). The nerve was continuously superfused at ~1 ml/min via a six-way tap. The microelectrodes (10-30 MΩ) were filled with 2 M K-citrate or 3 M KCl and connected to a conventional high impedance amplifier (World Precision Instruments, New Haven CT). The bath was grounded via a AgCl electrode connected to the bath through a 3 M KCl agar bridge (0.25 mm in diameter) placed near the suction outflow. The output of the amplifier was recorded on either a Gould Inc. 220 (Oxnard, CA) or a Kipp and Zonen BD 41 (Bohemia, NY) chart recorder (DC to 10 Hz).

Solutions with a Ba⁺⁺ concentration of 2 mM or greater were Ca⁺⁺ free; solutions containing <2 mM Ba⁺⁺ were made to be 2 mM in divalent cations by the addition of CaCl₂. Solutions containing 10 mM HCO₃⁻ were bubbled with a mixture of 2.3% CO₂, 97.7% O₂; the added HCO₃⁻ was substituted for Cl⁻. For Na⁺-free solutions, N-methyl-D-glucamine chloride replaced NaCl, the pH of the HEPES buffer was adjusted with KOH, and the concentration of KCl was modified to maintain the total [K⁺] at 3 mM. The tonicity of Na⁺-containing solutions was kept constant by adjusting the amount of NaCl; for Na⁺-free solutions, the
concentration of N-methyl-D-glucamine chloride was adjusted. For Cl−-free solutions, NaCl
and BaCl2 were replaced by the corresponding methane-sulfonate salts, and KCl by K+ glu-
conate. All solutions had a pH of 7.4.

For optical recordings, the nerve was pinned on transparent Sylgard in a chamber placed
on the stage of a compound Zeiss UEM microscope (Carl Zeiss, Inc., Thornwood, NY) and
stained for 1 h in the Neaurus Ringer's solution containing 0.1 mg/ml of the pyrazo-oxonol
dye RH155 (Grinvald et al., 1980; commercially available as NK3041 from Nippon Kankoh-
Shikiso Kenkyusho, Okayama, Japan). This dye was chosen because it has shown a high affin-
ity for glial membrane in other preparations (e.g., Konnerth et al., 1987). The image of the
nerve was projected onto the surface of an array of 124 photodiodes located in the image
plane of a 20X water immersion objective. The light source used for imaging was a tungsten-
halogen lamp whose light was collimated, heat filtered, and rendered quasimonochromatic
with an interference filter (705 nm). The photocurrent outputs of the individual photodetec-
tors were converted to voltages (Salzberg et al., 1977), AC coupled, amplified, multiplexed,
and the resulting data were digitized and stored in a PDP11/34A computer. The stained
nerve was impaled with a microelectrode, and square pulses of current were applied through
an active bridge circuit.

RESULTS

Ba++ Depolarizes Glial Membranes

Fig. 1 (A–D) shows two experiments (A,B and C,D) that compare the depolarizations
induced by Ba++ and K+ on the glial membrane. Each experiment represents data
from a single impalement, and the rate of superfusion was constant throughout.
Both ions produced a reversible depolarization. In contrast to the K+-induced depo-
larization, the Ba++-induced depolarization took longer to plateau and reversed
more slowly. In addition, the Ba++-induced depolarization was accompanied by an
increase in membrane resistance (Figs. 7 and 8) whereas the K+-induced depolariza-
tion was accompanied by a decrease in the membrane resistance (Tang et al., 1985;
not shown here). Fig. 1 E shows the Ba++ dose-response curve obtained from a sin-
gle impalement of another nerve. The depolarization increased monotonically in the
range of [Ba++] from 0.02 to 5 mM, and showed a tendency to saturate at higher
values. In four experiments in which at least five different concentrations of Ba++
were used, a half maximal depolarization was obtained with a Ba++ concentration
between 0.2 and 0.5 mM.

Ba++ Decreases the K+ Sensitivity of the Membrane

The glial membrane normally behaves as a K+ electrode (Kuffler et al., 1966). Fig. 2
illustrates the sensitivity of the glial membrane potential to changes in bath [K+] in
the presence and absence of Ba++. In normal Ringer's solution a reduction in [K+]
from 3 to 1 mM produced a hyperpolarization (Fig. 2 A); when Ba++ was present,
this hyperpolarization did not occur (Fig. 2, B and C). At a nearly saturating concen-
tration of Ba++ (Fig. 2 C), the addition of 1 mM K+ caused a depolarization of a few
millivolts. The Ba++-induced increase in membrane resistance and reduction of the
sensitivity of the membrane potential to decreases in [K+] indicate that Ba++ blocks
the K+ conductance. When [K+] was raised from 3 to 10 mM the membrane poten-
tial depolarized and appeared, under the conditions of this experiment, to approach
the K⁺ equilibrium potential, E_K (Fig. 2, D–F). In other preparations the addition of 10 mM K⁺ in the presence of 2 mM Ba⁺⁺ produced a hyperpolarization of a few millivolts. Fig. 2 G summarizes the effects of changing [K⁺] in the presence of Ba⁺⁺. The overall effect can be interpreted as the result of competition between Ba⁺⁺ and K⁺ for the K⁺ channel (see discussion below).

**Effects of HCO₃⁻ in the Presence of Ba⁺⁺**

Fig. 3 illustrates the short and long term effects on the glial membrane potential of adding 10 mM HCO₃⁻ when 2 mM Ba⁺⁺ is present. In the short run the addition of HCO₃⁻ produced a rapid and reversible hyperpolarization (Fig. 3 A). The mean hyperpolarization was 27 mV (SD = 10 mV, n = 15). In many nerves the brief addition of HCO₃⁻ produced a membrane potential that was more negative than the original resting potential in Ba⁺⁺-free Ringer’s solution. When the cells were superfused for a longer time in HCO₃⁻, the initial HCO₃⁻-induced hyperpolarization returned toward the original baseline and then reached a new steady state (Fig. 3 B). The speed of the recovery and the value of the new steady state potential were quite variable. In Fig. 3 B the recovery was sharp and rapid and the membrane potential returned to very near the original baseline; in other nerves, the recovery was more gradual and the new steady state potential was negative to the original baseline. The results illustrated in Fig. 3 also occurred in the absence of external Cl⁻ and were 1
order of magnitude larger than the results obtained when HCO$_3^-$ was added in the absence of Ba$^{++}$ (Astion et al., 1987; not shown here).

Fig. 4 shows that the HCO$_3^-$-induced hyperpolarization in the presence of Ba$^{++}$ was Na$^+$-dependent and sensitive to SITS (4-acetamido-4'-isothiocyanostilbene-2, 2'-disulfonic acid). In low Na$^+$ solutions (12 mM) the HCO$_3^-$-induced hyperpolarizations were an average of 21% (SD = 5%, n = 6) of those observed in control solutions ([Na$^+$] = 112 mM). In the complete absence of Na$^+$, the HCO$_3^-$-induced hyperpolarizations were an average of 6% (SD = 4%, n = 4) of the controls. In the presence of SITS (0.1–1 mM) the HCO$_3^-$-induced hyperpolarizations were an average of 42% (SD = 5%, n = 3) of the controls. The addition of SITS had no measurable effect in the absence of HCO$_3^-$.

The reduction of bath [Na$^+$] produced a HCO$_3^-$-dependent, SITS-sensitive depolarization (Fig. 5). The depolarization caused by reducing [Na$^+$] from 112 to 12 mM in the absence of HCO$_3^-$ was an average of 23% (SD = 19%, n = 5) of the depolarization observed when the cell had been bathing in HCO$_3^-$ (10 mM) for a prolonged time. When Na$^+$ was completely removed the depolarization in the absence of HCO$_3^-$ was an average of 31% (SD = 24%, n = 3) of the result in the presence of HCO$_3^-$.

**Figure 2.** The sensitivity of membrane potential to external K$^+$ is reduced in the presence of external Ba$^{++}$. All records were obtained from the same impalement. The initial $V_{m}$ in millivolts, is shown at the left of each trace. A–C show the effect on the membrane potential of changing K$^+$ concentration from 3 to 1 mM in the presence of 0, 0.2, and 2 mM Ba$^{++}$. D–F show the effect of changing K$^+$ concentration from 3 to 10 mM in the presence of 0, 0.2, and 2 mM Ba$^{++}$. (G) Membrane potential (millivolts) vs. external K$^+$ concentration in the presence of different Ba$^{++}$ concentrations. Records A–F were used in constructing the graph. In addition, some of the points in the graph are average results from identical solution changes performed during the same impalement as A–F.
Figure 3. Short and long term effects of superfusing with HCO$_3^-$ in the presence of Ba$^{++}$ (2 mM). In these experiments external pH was kept constant and HCO$_3^-$ replaced Cl$^-$. All HCO$_3^-$-containing solutions were bubbled with 2.3% CO$_2$. (A) Reversible hyperpolarization caused by superfusing with a Ringer's solution containing 10 mM HCO$_3^-$ for a brief time. Initial $V_m$ was $-40$ mV. (B) Long term effect of 10 mM HCO$_3^-$. After the initial hyperpolarization the membrane potential slowly returned toward the baseline and a steady resting potential was reestablished. Initial $V_m = -47$ mV. A and B are from different nerves.

Figure 4. In the presence of Ba$^{++}$ (2 mM), the HCO$_3^-$-induced hyperpolarization is Na$^+$ dependent and SITS sensitive. (A) Na$^+$ dependence of the HCO$_3^-$-induced hyperpolarization. The middle record shows the effect of adding 10 mM HCO$_3^-$ when the cell had been bathing in a solution in which Na$^+$ had been completely removed. HCO$_3^-$ was added as choline-bicarbonate, NaCl was replaced with N-methyl-d-glucamine chloride, and the pH of the HEPES buffer was adjusted with KOH. The left and right records, taken before and after the middle record, are controls from the same impalement showing HCO$_3^-$-induced hyperpolarizations in the presence of 112 mM Na$^+$. The time between records is 20 min. Initial $V_m = -43, -41$, and $-42$ mV for the left, middle, and right records. In the presence of Ba$^{++}$, the addition of 10 mM choline had no effect (not shown). (B) SITS-sensitivity of the HCO$_3^-$-induced hyperpolarization. The middle record shows the effect of adding HCO$_3^-$ when the nerve had been bathing in 0.5 mM SITS. The left and right panels are SITS-free controls recorded before and after the middle panel. In this experiment there was a small artifact that occurred upon switching the tap. SITS had no effect on the membrane potential in the absence of HCO$_3^-$. The time between records is 30 min. Initial $V_m$ was $-42$ mV for the left and middle records, and $-43$ mV for the right record.
variable, but it was always greater in the presence than in the absence of HCO₃⁻. Fig. 5 C shows that in the presence of Ba²⁺ and HCO₃⁻, the addition of SITS (0.5 mM) produced a small depolarization (3–5 mV) and decreased the low Na⁺-induced depolarization by approximately one third. It was difficult to quantitate the effect of SITS more precisely because of slowly shifting baselines during the experiments. As discussed below, the results described in this section are most readily explained by the presence in the glial membrane of a Na⁺/HCO₃⁻ electrogenic cotransporter in

\[ \text{Na}^+ + \text{HCO}_3^- \rightarrow \text{Na}^+\text{HCO}_3^- \]

which the stoichiometry of HCO₃⁻ to Na⁺ is greater than 1 (Boron and Boulpaep, 1983).

**Membrane Sensitivity to Other Ions in the Presence of Ba²⁺**

Experiments in which the bath [Cl⁻] was varied in the presence of Ba²⁺ showed a small dependence of membrane potential on [Cl⁻]. However, these experiments were difficult to interpret because of the complex effects of changes in [Cl⁻] on the indifferent bath electrode. Attempts to circumvent this problem with the use of a K⁺-tetrakis bath electrode (Thomas and Cohen, 1981) were unsuccessful. Fig. 6
illustrates an experiment that suggests a small Cl⁻ permeability. The depolarization produced by Ba⁺⁺ was greater in Cl⁻-free Ringer's solution than in the normal solution. The average depolarization induced by adding 5 mM Ba⁺⁺ to the normal Ringer's solution was 28 mV (SD = 6 mV, n = 4) in contrast to 41 mV (SD = 4 mV, n = 4) when 5 mM Ba⁺⁺ was added to Cl⁻-free Ringer's solution (means are significantly different, P < 0.025, t test).

To test the possibility that the Ba⁺⁺ depolarization resulted from an influx of Ba⁺⁺ through Ca⁺⁺ channels we added up to 1 mM of Cd⁺⁺, a Ca⁺⁺ channel blocker (Hagiwara and Byerly, 1981), to the solutions containing Ba⁺⁺. The addition of Cd⁺⁺ had no effect on the Ba⁺⁺-induced depolarization, suggesting that Ba⁺⁺ does not have its effect via permeation through Ca⁺⁺ channels. The addition of 3 mM Ca⁺⁺ also had no effect on the Ba⁺⁺-induced depolarization, suggesting that the electrochemical gradient for Ca⁺⁺ did not directly contribute to the membrane potential in the presence of Ba⁺⁺.

Effect of Strophanthidin in the Presence of Ba⁺⁺

Previous experiments in Necturus (Tang et al., 1980) have shown that in the absence of Ba⁺⁺, the addition of strophanthidin (10⁻⁴ M), a reversible inhibitor of the Na⁺/K⁺ pump, had no effect on glial cell membrane potential. In the presence of Ba⁺⁺,
(2 mM), the addition of strophanthidin (50 or 100 μM) produced a reversible depolarization of ~3 mV (n = 4). This suggests a small direct contribution of the electrogenic Na⁺/K⁺ pump to the glial membrane potential in the presence of Ba⁺⁺.

**Optical Recording of Spread of Electrotonic Potentials**

Fig. 7 illustrates the distribution of electrotonic potentials recorded optically by means of a photodiode array and the voltage-sensitive dye RH155. This technique permits one to monitor changes in glial membrane potential by detecting the changes in optical properties (here, absorption) of membrane-bound dyes that behave as molecular voltage transducers (Salzberg et al., 1977, 1983; Grinvald et al., 1980; Konnerth et al., 1987). Fig. 7 A was obtained in normal Ringer's solution, and Fig. 7 B was obtained after the addition of 2 mM Ba⁺⁺. In the presence of Ba⁺⁺,

![Figure 7](image)

both the amplitude and rise time of the potential increased and the decay of amplitude with distance decreased. In addition, for any region of the nerve imaged onto a single photodetector, there was an increase in the voltage change produced by the current injection. Fig. 8 illustrates the output of the photodiode element adjacent to the current-passing electrode. In the presence of Ba⁺⁺, the input resistance (averaged over the region of the nerve imaged onto a single photodetector) increased and the membrane charged more slowly. Similar changes in input resistance and membrane charging were seen in conventional two electrode experiments.

**DISCUSSION**

**Mechanism of Ba⁺⁺ Action**

The mechanism of Ba⁺⁺ action has been best studied in giant axons of *Loligo* (Armstrong et al., 1982); in this preparation Ba⁺⁺ competitively inhibits the K⁺ conduc-
distance by reversibly occupying a voltage-dependent location in the $K^+$ channel. Our data (Fig. 2) are most simply explained by this competitive model.

According to the competitive model, changes in external $[K^+]$ alters both $E_K$ and the amount of $Ba^{++}$ bound to $K^+$ channels. The model can explain why decreasing $[K^+]$ often leads to a depolarization in the presence of $Ba^{++}$ (Fig. 2 C). Reduction of $[K^+]$ increases the number of $K^+$ channels blocked by $Ba^{++}$. An increase in the number of $Ba^{++}$-blocked channels, by itself, depolarizes the cell, and a decrease in $[K^+]$, by itself, hyperpolarizes the cell. In cells where the effect of increasing the number

![Figure 8](image)

**Figure 8.** Effect of $Ba^{++}$ on the electrotonic potential recorded optically from the region adjacent to the current electrode. (A) Electrotonic response detected optically by an element of the photodiode array adjacent to the current electrode. Depolarizing current injection was 50 nA with a 200-ms duration; same experiment as in Fig. 6. Control and $Ba^{++}$ records are superimposed at the same gain. $Ba^{++}$ record did not reach steady state during the duration of the current pulse. Initial $V_m$ was $-85$ mV in normal Ringer's solution and $-45$ mV in the Ringer's solution containing 2 mM $Ba^{++}$. Each record is the average of 16 sweeps. (B) Same as A, except that the current pulse had the opposite polarity.

of $Ba^{++}$-blocked channels outweighs the effect of decreasing $[K^+]$, one expects to observe a depolarization when the $[K^+]$ is reduced in the presence of $Ba^{++}$. Using similar reasoning, the competitive model can also explain why increases in $[K^+]$ occasionally produce a hyperpolarization, and hyperpolarizations due to $K^+$ withdrawal are inhibited to a greater extent than $K^+$-induced depolarizations.

**Evidence for Electrogenic $Na^+/HCO_3^-$ Cotransport**

A $Na^+/HCO_3^-$ electrogenic cotransporter has been described in which $HCO_3^-$ (or its equivalent), $Na^+$, and net negative charge move in the same direction (e.g., Boron
and Boulpaep, 1983; Yoshitomi et al., 1985; Wiederholt et al., 1985; Lopes et al., 1987; for review see Boron, 1986). When recording membrane potential, the cotransporter is indicated by the following: (a) addition of HCO₃⁻ causes a Na⁺-dependent, SITS-sensitive, hyperpolarization; (b) Removal of Na⁺ yields a HCO₃⁻-dependent, SITS-sensitive depolarization; and (c) The effects of adding HCO₃⁻ and removing Na⁺ are insensitive to changes in Cl⁻.

Our data are consistent with the criteria above for the presence in the glial membrane of an electrogenic Na⁺/HCO₃⁻ cotransporter. Figs. 3 and 4 show the hyperpolarization induced by the addition of HCO₃⁻. Fig. 4 shows that the hyperpolarization is Na⁺-dependent and SITS-sensitive. Fig. 5 demonstrates the depolarization induced by lowering bath [Na⁺]; it is HCO₃⁻-dependent and SITS-sensitive. We have previously shown that the membrane potential changes induced by HCO₃⁻ in the presence of Ba⁺⁺ (Fig. 3) are insensitive to removal of Cl⁻ (Astion et al., 1987).

The small SITS-induced depolarization was HCO₃⁻ dependent (contrast Figs. 4 B and 5 C). This suggests that in the presence of Ba⁺⁺, the electrogenic Na⁺/HCO₃⁻ cotransporter moves HCO₃⁻, Na⁺, and net negative charge inward. In the physiological state, Ba⁺⁺ is absent and the cells are much more hyperpolarized. Under these conditions, the steady state polarity of the cotransporter may not be inward (Lopes et al., 1987). Unfortunately, the changes in membrane potential produced by the cotransporter are too small to study in the physiological state, when the K⁺ conductance is present. One solution to this problem would be to investigate the cotransporter with ion-selective microelectrodes.

Electrogenic Na⁺/HCO₃⁻ cotransport has not been described in neurons or vertebrate glia. In invertebrate glia, Deitmer and Schlue (1987) have described a Na⁺-dependent, SITS-insensitive, hyperpolarizing effect of HCO₃⁻ that might be due to the cotransporter. It is tempting to speculate that electrogenic Na⁺/HCO₃⁻ cotransport is a unique glial contribution to ionic regulation in the brain.

The Effect of Cl⁻ Removal

After at least 1 h in Cl⁻-free solutions, when the [Cl⁻]ᵢ should be near zero, the Ba⁺⁺-induced depolarization increases (Fig. 6). This result is consistent with the hypothesis that the membrane has a finite permeability to Cl⁻ and Eₘ is more negative than the membrane potential in the presence of Ba⁺⁺. Thus, the removal of Cl⁻ would remove a shunt that tends to hold the membrane potential near Eₘ. When the shunt is eliminated, the Ba⁺⁺-induced depolarization increases.

Why Does Ba⁺⁺ Cause a Depolarization?

When Ba⁺⁺ is added to reduce the K⁺ conductance, the new membrane potential must be a function of the remaining K⁺ permeability, other ionic gradients and permeabilities, and steady-state electrogenic fluxes (e.g., the Na⁺/K⁺ pump). Because these factors vary significantly from cell type to cell type, the addition of Ba⁺⁺ leads to a depolarization of some cells (Werman et al., 1961; Burkhardt et al., 1984; MacVicar, 1984) and hyperpolarization of others (Werman and Grundfest, 1961; Nishi and Soeda, 1964).

In the glial cells of Necturus optic nerve, Ba⁺⁺ (2–5 mM) produced a depolarization of 25–50 mV (Fig. 1). The mechanism of the depolarization is not clear. The Na⁺/HCO₃⁻ cotransporter and the Na⁺/K⁺ pump, both of which appear to be pres-
ent in the presence of Ba\(^{++}\), are hyperpolarizing. The addition of Cd\(^{++}\) or Ca\(^{+}\) had no effect on the membrane potential in the presence of Ba\(^{++}\), suggesting that the membrane has a relatively low conductance to Ca\(^{++}\), and that it is impermeable to Ba\(^{++}\) through Ca\(^{++}\) channels. As discussed above, there might be a small permeability to Cl\(^{-}\). However, if this is true it is most likely that \(E_{\text{Cl}}\) is negative to the membrane potential that is observed in Ba\(^{++}\)-containing Ringer's solution. A remaining possibility is that the Ba\(^{++}\)-induced depolarization is due to a small permeability to Ba\(^{++}\) through K\(^{+}\) channels.

**Optical Recording of Changes in Glial Membrane Potential**

Changes in absorbance and fluorescence of voltage-sensitive dyes have been used to study changes in membrane potential in excitable tissues (for reviews see Cohen and Salzberg, 1978; Salzberg, 1983; Grinvald, 1985). The optical signals from the nervous system reported so far have monitored the electrical activity of neuronal populations, or of a combination of neural and glial elements (Salzberg, 1983; Konnerth and Orkand, 1986; Lev-Ram and Grinvald, 1986; Konnerth et al., 1987). The optical signals illustrated in Figs. 7 and 8, unlike the previously reported signals, are purely glial in origin, as the stimulating electrode is in a glial cell and only the glial cells are coupled (Cohen, 1970). This is the first report of optical signals from glial cells without an additional neural component. Further development of this technique might permit the study of glial preparations which presently cannot be impaled with microelectrodes.

After Ba\(^{++}\) was added, the input resistance increased and the potential decayed less rapidly as a function of distance (Figs. 7 and 8). From a qualitative point of view, these results are those expected for a syncytium in which Ba\(^{++}\) increases the membrane resistance more than the resistance of the intercellular junctions (Kettenmann and Ransom, 1988). If Ba\(^{++}\) increased the junctional resistance more than the membrane resistance, the input resistance of the cells would increase but the potential would decay more rapidly with distance. This is seen in *Necturus* optic nerve when the syncytium is uncoupled after the addition of CO\(_2\) (Tang et al., 1985).

From a quantitative point of view, the question arises as to the utility of performing, with these data, a rigorous analysis of glial membrane properties. We recognize a number of limitations to the optical technique that make such an analysis nugatory (Cohen and Salzberg, 1978). A detailed analysis of the distribution of amplitudes is not feasible because of uncertainties as to the uniformity of staining and variations in the amount of membrane imaged on each photodiode. This is compounded by dye bleaching that occurs during the experiment. A cable analysis based on the time-course of the electrotonic potentials might be possible (Jack et al., 1975), but it would require noise reductions as well as adjustments in computer software that are incompatible with our present experimental arrangement.

MacVicar (1984) has shown action potentials evoked by depolarizing current pulses in cultured glial cells in the presence of high concentrations of external Ba\(^{++}\). The optical techniques that were used in our experiments are ideal to record such fast signals anywhere in the glial syncytium. In optical experiments on five nerves as well as experiments on three nerves using standard two intracellular electrode techniques, no action potentials were elicited by current injection in the presence of Ba\(^{++}\).
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