Sodium Channels in Axons and Glial Cells of the Optic Nerve of *Necturus maculosa*

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**ABSTRACT** Experiments investigating both the binding of radioactively labeled saxitoxin (STX) and the electrophysiological response to drugs that increase the sodium permeability of excitable membranes were conducted in an effort to detect sodium channels in glial cells of the optic nerve of *Necturus maculosa*, the mudpuppy. Glial cells in nerves from chronically enucleated animals, which lack optic nerve axons, show no saturable uptake of STX whereas a saturable uptake is clearly present in normal optic nerves. The normal nerve is depolarized by aconitine, batrachotoxin, and veratridine (10^{-6}-10^{-5} M), whereas the all-glial preparation is only depolarized by veratridine and at concentrations greater than 10^{-3} M. Unlike the depolarization caused by veratridine in normal nerves, the response in the all-glial tissue is not blocked by tetrodotoxin nor enhanced by scorpion venom (*Leiurus quinquestriatus*). In glial cells of the normal nerve, where axons are also present, the addition of 10^{-3} M veratridine does lead to a transient depolarization; however, it is much briefer than the axonal response to veratridine in this same tissue. This glial response to veratridine could be caused by the efflux of K⁺ from the drug-depolarized axons, and is similar to the glial response to extracellular K⁺ accumulation resulting from action potentials in the axon.

**INTRODUCTION**

It is generally accepted that glial cells provide mechanical and, perhaps, metabolic support for the neuronal cells that they surround but do not participate directly in the events of excitability (Nakai, 1963; Kuffler and Nicholls, 1966). However, in 1976 Villegas et al. (1976) reported electrophysiological evidence for the existence of sodium channels in Schwann cells of squid giant axons. The membrane of these Schwann cells was depolarized by drugs that were known to activate the voltage-dependent sodium channels in resting excitable membranes, and such depolarizations were reversed by tetrodotoxin (TTX), a drug that specifically blocks these sodium channels (Kao, 1966). This provocative result, if true in other excitable tissues, demands...
modification of the current concepts about the limited role of glia in neuronal tissues. One particular consequence would be to qualify seriously most of the values for densities of sodium channels estimated from tetrodotoxin binding studies, inasmuch as almost all the tissues in which toxin binding has been measured contain significant glial cell membranes (Ritchie and Rogart, 1977 a).

The aim of the research reported here was, therefore, to detect sodium channels in a tissue containing only glial cells and no neuronal membranes. We have taken two experimental approaches, measuring the binding of radioactively labelled saxitoxin (STX), a drug analogous to TTX, and measuring the electrophysiological response of the glial membrane potential to drugs that activate neuronal sodium channels. The optic nerve from the mudpuppy, Necturus maculosa, was chosen for these experiments because the glial cells (astrocytes) can be studied electrophysiologically (Kuffler et al., 1966) and because the non-myelinated axons in the nerve degenerate and disappear 2 mo after surgical removal of the eye, leaving a preparation consisting exclusively of glia (Orkand et al., 1973).

**METHODS**

**Histology**

Under ether anesthesia, the optic nerves of Necturus maculosa were exposed from the ventral side through the upper palate. In most cases the leptomeninges, with its blood vessels, were left intact. Histological treatment followed that of Bracho et al., 1975. Fixative containing 1% glutaraldehyde and 4% paraformaldehyde in a phosphate buffer at pH 7.4 was poured on the nerves and allowed to remain for about 10-15 min. The partially fixed nerves were dissected out and placed in fresh fixative for about 16 h at room temperature. They were then rinsed in phosphate buffer, sometimes containing 6% sucrose and postfixed in 1% OsO4 buffered with either cacodylate or phosphate for 2 h. They were then stained en bloc in saturated aqueous uranyl acetate before being dehydrated and embedded in Araldite or Epon 812 (Shell Chemical Co., New York). Thick (~1 μm) sections were stained with toluidine blue for light microscopic study. Thin sections were cut with glass or diamond knives on a Sorvall MT2 ultramicrotome (DuPont Instruments-Sorvall, Newtown, Conn.), stained with lead acetate and examined in a Hitachi 11B (Hitachi, Ltd., Tokyo) or a JEOL 7T electron microscope (JEOL USA, Medford, Mass.).

Axon and glial cell membrane areas were measured from electron micrographs using the stereological method of Weibel and Bolender (1973). Test grid spacing was 0.5 μm, a distance which produced rapid convergence of the values of standard deviations of membrane densities from several different micrographs. The total nerve cross-sectional areas were taken as 6,500 and 4,750 μm² for normal and eyeless animals, respectively.

**Electrophysiology**

The animal was decapitated and the intracranial portion of the optic nerve removed (5–6 mm length). The sheath was dissected away and the nerve was mounted in a lucite chamber separated into three compartments by vaseline seals compressed under a glass cover slip. Glial cell potentials and neuronal DC potentials were measured by using the sucrose-gap method (Stämpfli, 1954; Kuffler et al., 1966): flowing isotonic
sucrose through a central compartment and measuring the potential difference between the two ends of the “nerve,” in contact with Ringer solution in the two side compartments. Measurements of changes in the membrane potential of glial cells are possible in this chamber because the cells are electrically coupled and the potential changes at one end are conducted through the sucrose gap by low resistance intercellular syncytia (Kuffler and Nicholls, 1966). After a nerve was mounted in the chamber, it was “calibrated” by measuring the potential changes produced by raising $K_\text{a}$ to 30 mM and then lowering it to 0.3 mM. Such a calibration provides a measure of the relative impedance coupling through the sucrose gap for the comparison of data among preparations of the same type. The calibration signals differed by $< 10\%$ for both normal and all-glial nerves, and the values of membrane potential presented here are the actual measured values.

Extracellular potentials were measured by chloride-coated silver wires coupled through a FET input preamplifier to the differential inputs of a Tektronix 565 oscilloscope (Tektronix, Inc., Beaverton, Ore.). Action potentials were measured in a similar chamber configuration, but the sucrose in the central compartment was replaced by Ringer, and the nerve was stimulated between the central and one side compartment, and the potential was recorded between the central and the other side compartment. Intracellular measurements were made with glass microelectrodes filled with 3 M KCl and which had resistances of 30-50 MΩ. The electrodes were coupled to a WPI electrode follower, model M4 (W-P Instruments, Inc., New Haven, Conn.). DC potential changes measured in the sucrose gap or with intracellular microelectrodes were recorded on a strip chart recorder.

Ringer solution contained (in mM) NaCl 110, KCl 3.0, CaCl₂ 2.0, glucose 11, HEPES buffer 5, pH = 7.4. Saxitoxin was obtained from the NIH, Bethesda, Maryland, tetrodotoxin from Cal Biochem., LaJolla, Ca., veratridine and scorpion venom from Sigma Chemical, St. Louis, Mo., aconitine from K and K Laboratories, Plainview, N.Y., and batrachotoxin was generously supplied by Dr. John W. Daly, NIH.

**Binding Studies**

Tritiated saxitoxin (*STX) was prepared by the method of Ritchie et al., 1976. The solution was bioassayed against a standard preparation of paralytic shellfish poison (FDA Lot 7, Serial 508) and the radiochemical purity, determined by the method of Ritchie and Rogart (1977), was 70%. The final specific radioactivity of *STX was $8.1 \times 10^3$ mCi/mmol, which is equivalent to about one tritium per four STX molecules. Dissected optic nerves of measured length were incubated for 2-3 h at 8-10°C in Ringer solutions containing *STX and $[^{14}\text{C}]$mannitol, as an extracellular space marker. The nerves were blotted dry and then digested in 0.5 ml of tissue solubilizer (Protosol, New England Nuclear, Boston, Mass.) and 0.1 ml H₂O at 60°C for 30 min. Radioactivity was determined by double-label scintillation counting, and the bound *STX calculated by subtracting the extracellular component scaled to the $[^{14}\text{C}]$mannitol uptake.

**RESULTS**

**STX Binding**

The presence of sodium channels in the normal optic nerve is evidenced by both *STX binding studies and by electrophysiological measurements. Table 1 lists the uptake of *STX by normal nerves at two STX concentrations.
There is a saturable component, which is abolished in the presence of very high concentrations (10 μM) of unlabelled TTX. Since the all-glia preparation shows no saturable binding (see below), the saturable uptake is probably due to STX binding to axon membranes.

**Table I**

<table>
<thead>
<tr>
<th></th>
<th>(a) Total uptake</th>
<th>(b) Nonsaturable uptake</th>
<th>(a − b) Saturable uptake</th>
<th>Membrane area</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>μmol/mm length</td>
<td>μmol/mm length</td>
<td>μmol/mm length</td>
<td>μm²/mm length</td>
</tr>
<tr>
<td>Normal nerve</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>20 nM *STX</td>
<td>2.16 ± 0.045 (4)</td>
<td>0.284 ± 0.055 (3)</td>
<td>1.88</td>
<td></td>
</tr>
<tr>
<td>20 nM *STX</td>
<td>2.00 ± 0.40 (3)</td>
<td>--</td>
<td>--</td>
<td></td>
</tr>
<tr>
<td>20 nM *STX</td>
<td>2.16 ± 0.31 (3)</td>
<td>0.311 ± 0.445 (3)</td>
<td>1.63</td>
<td></td>
</tr>
<tr>
<td>20 nM *STX</td>
<td>2.52 ± 0.47 (4)</td>
<td>0.316 ± 0.070 (4)</td>
<td>2.20</td>
<td></td>
</tr>
<tr>
<td>5 nM *STX</td>
<td>2.38 ± 0.32 (3)</td>
<td>0.301 ± 0.270 (3)</td>
<td>1.98</td>
<td></td>
</tr>
<tr>
<td>Average normal nerve</td>
<td>2.16 ± 0.30 (14)</td>
<td>0.356 ± 0.334 (10)</td>
<td>1.88</td>
<td>4.70 ± 0.91 × 10⁷ axons</td>
</tr>
<tr>
<td>All-glia “nerve”</td>
<td></td>
<td></td>
<td></td>
<td>1.81 ± 0.30 × 10⁷ glia</td>
</tr>
<tr>
<td>20 nM *STX</td>
<td>0.531 ± 0.166 (3)</td>
<td>--</td>
<td>--</td>
<td></td>
</tr>
<tr>
<td>20 nM *STX</td>
<td>1.13 ± 0.423 (3)</td>
<td>1.45 ± 0.453 (3)</td>
<td>0.52</td>
<td></td>
</tr>
<tr>
<td>10 nM *STX</td>
<td>0.492 ± 0.165 (3)</td>
<td>0.323 ± 0.075 (5)</td>
<td>0.171</td>
<td></td>
</tr>
<tr>
<td>Average glial binding</td>
<td>0.820 ± 0.177 (11)</td>
<td>0.822 ± 0.307 (8)</td>
<td>-0.002</td>
<td>4.21 ± 0.91 × 10⁷ glia</td>
</tr>
</tbody>
</table>

Values in mean ± SE (n).

1 Uptake in the presence of 10 μM unlabelled TTX.
2 Glial areas calculated assuming cylindrical geometry; for spherical geometry multiply listed areas by 4/π to calculate area.

The density of this saturable component can be calculated by dividing the uptake by the axonal area; the latter value, determined from a morphometric analysis of electron micrographs of normal nerves, is equal to 4.7 × 10⁷ μm² mm⁻¹ nerve length. The calculated density of saturable sites is then about 24 μm⁻², which is close to the density of STX binding sites in the small nonmyelinated axons of the garfish olfactory nerve, 30 μm⁻² (Ritchie et al., 1976). A dissociation constant for this saturable binding can be calculated assuming Langmuir binding of *STX (T) to a single class of receptors: T + R ⇌ T · R. In every excitable tissue studied to date the saturable uptake has this concentration dependence. The uptake at any one toxin concentration (T₁) is given by:

\[ U₁ = \frac{MT₁}{K_D + T₁}, \]  

where \( M \) is the total number of binding sites and \( K_D \) is the dissociation constant for the equilibrium binding reaction. If \( α = U₁/U₂ \), then from Eq. 1:

\[ K_D = \frac{T₁(1 - α)}{α - T₁/T₂}. \]

From the saturable uptake data at 5 and 20 nM STX, listed in Table I, the dissociation constant calculated from Eq. 2 equals 0.8 nM, at 8°C. This value
is similar to that measured from *STX binding to homogenized rabbit brain, 1.5 nM (Ritchie and Rogart, 1977 b), and rat synaptosomes, 0.8-1.0 nM (Weigele and Barchi, 1978), and also agrees with KD values determined electrophysiologically in voltage-clamped frog muscle, 0.88 nM (Campbell and Hille, 1976), and frog node of Ranvier 1.3-1.4 nM (Wagner and Ulbricht, 1975).

In contrast to the normal optic nerve, the all-glial nerve shows no significant saturable *STX binding. Table I shows that the mean values of total uptake and linear, nonsaturable uptake are indistinguishable; nonparametric statistical analysis comparing all the binding values to individual nerves for total and linear uptake shows that they are not statistically different: P < 0.1 (Mann-Whitney U test). If a normal distribution of the binding capacities is assumed (there are too few values to determine unequivocally that this distribution holds), then more powerful parametric statistics can be applied. Under these assumptions, Student's t test shows that the all-glial preparation has the same number of STX binding sites in the presence or absence of unlabelled TTX (P < 0.001). Thus, there is no positive evidence for saturable STX binding to glial cells. However, the scatter in the experimental data is large and within this scatter some small saturable binding could exist.

Electrophysiological Studies

Action potentials in axons of the normal optic nerve are reversibly blocked by both saxitoxin and tetrodotoxin. Fig. 1 shows the dose-response relationship for the inhibition of the height of the compound action potential (AP) by

![Figure 1. Dose-response function for the reversible reduction of the compound action potential (AP) by STX. Symbols identify results of three separate preparations. The inset shows the action potential before (solid line), during (dashed line), and after (dotted line) addition of 10 nM STX. T = 20-22°C.](image-url)
STX. The concentration to reduce the AP by half is 6-7 nM. 50 and 100 nM concentrations of STX and TTX completely abolish the AP. For comparison, in frog sciatic nerve the TTX concentration required to halve the AP is 8 nM (Strong et al., 1973), whereas the dissociation constant calculated from the reduction of sodium currents in voltage-clamp studies is about 1.5 nM (Schwarz et al., 1973). The effects of STX on APs in \textit{Necturus} optic nerve axons are thus consistent with both the measured STX affinity from binding studies to this same nerve and with the effects of STX and TTX on other amphibian excitable membranes.

The normal nerve in the sucrose gap undergoes a constant depolarization in response to drugs which open sodium channels at rest. We investigated the effects of three of these pharmacological activators, veratridine, aconitine, and batrachotoxin. All three drugs have been shown to produce sodium-dependent membrane depolarizations and to increase the population of open sodium channels under voltage-clamp at normal resting potentials (Ulbricht, 1969; Schmidt and Schmitt, 1974; Albuquerque and Daly, 1976). Fig. 2 A-E shows the depolarizing response of normal optic nerve to increasing concentrations of veratridine. The depolarization is reversed by high concentrations of TTX and STX (not shown) leading to membrane hyperpolarization (Fig. 2 C-E, Fig. 3 A). This hyperpolarization is probably due to the activity of an electrogenic pump in the axons, activated by a rise in intracellular sodium, because it is absent in $10^{-4}$ M strophanthidin. Addition of 10 µM TTX alone hyperpolarizes the membrane by only 1-3 mV. The effects of both veratridine and TTX (STX) are reversed by washing with normal Ringer.

The effects of activator drugs on normal optic nerves are potentiated by certain scorpion venoms, as they are in other excitable cells (Catterall, 1975).
Fig. 4 shows depolarizations produced by veratridine in the absence and presence of venom from the scorpion *Leiurus quinquestriatus* (10 μg/ml). Both the rate of depolarization and its steady-state value are enhanced by the scorpion venom although the maximum depolarization is about the same (Table II). Similar depolarizing effects were observed with aconitine and

**Figure 3.** (A) Peak change in membrane potential (open symbols) and maximum rate of change of membrane potential (solid symbols) in response to veratridine addition for three normal (○, □, Δ) and one all-glia (▽) optic nerve. Slashed symbols identify the membrane potential change after the addition of 10^{-6} M TTX relative to the potential before veratridine addition. (B) Peak change in glial membrane potential produced by veratridine. Slashed symbols pointing downward show change upon adding 10^{-6} M TTX relative to initial base line, before veratridine addition.
batrachotoxin, although the rates of depolarization they produced were markedly slower than those with veratridine, and their effects could not be reversed by washing the nerve with Ringer solution. The results from studies of the activator drugs are collected in Table II.

There is no electrophysiological evidence for normal sodium channels in the all-glia preparation. Activator drugs are almost completely ineffective in depolarizing the glial membrane; only at $10^{-3}$ M veratridine could a slow, small depolarization be produced (Figs. 2 F, 3 B). Unlike the axonal response these veratridine-induced depolarizations of glia are not potentiated by scorpion venoms (Fig. 4 E-F) nor inhibited by TTX. However, they are reduced when the sodium concentration of the bathing Ringer is lowered, and the response of the veratridine-depolarized glial membrane to elevated external $K^+$ is smaller than that of the drug-free glia. Apparently concentrations of veratridine above $10^{-3}$ M increase the ratio of sodium to potassium permeability, but not by activating sodium channels such as those in nerve membranes. Aconitine at $10^{-5}$ to $\sim 5 \times 10^{-4}$ M concentrations, and batrachotoxin at concentrations up to $10^{-4}$ M produce no changes in the glial membrane potential. Scorpion venom (10 $\mu$g/ml) had no effect by itself nor did its presence promote any effect by aconitine or batrachotoxin. Glial membranes in the axon-free optic nerve from enucleated mudpuppies show none of the specific responses which are evidence for the type of sodium channels found in excitable cells.

From the preceding experiments we cannot determine whether sodium channels might be present in glial membranes in the normal nerves, when axonal processes are present. It is difficult to demonstrate glial sodium channels in the whole nerve because any biochemical or electrophysiological measure will collect results averaged over and influenced by all the cell types
contained therein. However, the membrane potential of individual glial cells in the normal nerve can be measured using intracellular microelectrodes with no direct contribution from the axons (Kuffler et al., 1966). Fig. 5 A shows the results of such an intracellular measurement when $10^{-5}$ M veratridine is applied to the normal nerve. The glial cell undergoes a transient depolarization, but with a different time-course than the axonal response to veratridine. (Fig. 5 B). The axonal depolarization, measured in the sucrose gap, occurs more rapidly than that recorded in the glia, and the axon remains depolarized for almost 20 min (Fig. 5 B), while the glial response has almost completely reversed in about 3 min.

### Table II

<table>
<thead>
<tr>
<th>Drug</th>
<th>Concentration</th>
<th>Scorpion venom</th>
<th>$\Delta V_{m}$</th>
<th>$\Delta V/\Delta t$ max$^|$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Veratridine</td>
<td>$10^{-7}$</td>
<td>-</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>$10^{-6}$</td>
<td>+</td>
<td>34</td>
<td>145</td>
</tr>
<tr>
<td></td>
<td>$10^{-5}$</td>
<td>+</td>
<td>10</td>
<td>850</td>
</tr>
<tr>
<td></td>
<td>$10^{-4}$</td>
<td>-</td>
<td>46</td>
<td>175</td>
</tr>
<tr>
<td>BTX*</td>
<td>$10^{-7}$</td>
<td>-</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>$10^{-6}$</td>
<td>-</td>
<td>Incomplete</td>
<td>2</td>
</tr>
<tr>
<td>Aconitine$^\dagger$</td>
<td>$10^{-7}$</td>
<td>-</td>
<td>-2</td>
<td></td>
</tr>
<tr>
<td></td>
<td>$10^{-6}$</td>
<td>-</td>
<td>8</td>
<td>16</td>
</tr>
</tbody>
</table>

$^\*$ Maximum depolarizations were not measured against the concentration of aconitine or BTX because of their irreversibility and aconitine’s limited solubility in aqueous solution.

$^\dagger$ A small, transient hyperpolarization precedes the slow, steady depolarization produced by aconitine. $\Delta V_{m}$ values listed are the maximum change in membrane potential on adding $10^{-7}$ M BTX following aconitine-induced depolarizations.

$^\|$ $\Delta V/\Delta t$ max is the tangent to the trace of membrane potential vs. time which has the maximum slope (e.g., see Fig. 2).

The differences in the onset of depolarization between axons and glia are probably real, even though mixing conditions differ between the two chambers used for these recordings. When the depolarizations produced by raising external $K^+$ to 30 mM are compared, the intracellular glial response lags behind the axonal response measured in the sucrose gap by only 10 s, for the time from start of perfusion to 50% of peak response. This lag probably reflects the larger volume and greater dead-space of the intracellular recording chamber. In comparison, the response to veratridine addition, measured at 50% of peak response, occurs 30 s sooner in axons than in the glia. The slower response of the glia to veratridine addition cannot be explained entirely by differences in mixing and must arise from the actual drug-tissue interaction.

The veratridine-induced depolarization reverses relatively rapidly in the glia. Even when the Na-K pump is blocked by $10^{-4}$ M strophanthidin, the...
glial response to veratridine remains transient (Fig. 5 C). Therefore, the rapid repolarization of the glial membrane potential is not due to a high density of electrogenic pumps in this cell which are not present in axons.

The veratridine-induced glial depolarization can be explained by either of two mechanisms. One mechanism proposes that glial membranes in normal nerves do contain sodium channels which are activated directly by veratridine, albeit transiently. The alternative mechanism is that the glia are depolarized by extracellular potassium ions which flow out of the axons when they are depolarized by veratridine. Axonal depolarization during action potentials has been shown to release potassium which accumulates in the diffusion-restricted extracellular space during impulse activity and produces a slowly decaying depolarization as it diffuses out of the nerve bundle through narrow extracellular clefts (Orkand et al., 1966). The transient kinetics of the veratridine-induced change could also be due to an initial large potassium efflux which then declines to a lower rate producing a rapid, transient increase in extracellular K⁺. The exact kinetics of axonal potassium efflux are impossible to predict, however, because both the axonal potassium conductance, and the extracellular potassium concentration are changing in time, and even if the axon membrane potential remained at a constant depolarization, slow inactivation mechanisms could reduce the potassium conductance (Schwarz and Vogel, 1971). When veratridine is added to normal nerves that are bathed in elevated extracellular K⁺, the absolute size of the drug-induced glial depolarization is reduced (Fig. 6). However, since the glial potential is a logarithmic function of the extracellular K⁺ (Kuffler et al., 1966), the size of the drug-induced increase in extracellular K⁺ is calculated to be the same in normal and elevated K⁺, about 3-4 mM. The change in the axonal membrane

![Figure 5](image-url)
potential due to veratridine is smaller (18 mV) in 12 mM K⁺ Ringer than in normal Ringer (34 mV), but this reduced depolarization starts from a resting potential which is already 20 mV more depolarized due to the elevated K⁺ and actually reaches a greater absolute level of depolarization. Therefore, it is impossible to know the real change in extracellular potassium which affects the glial cells, and the results in elevated K⁺ are consistent with either mechanism.

![Diagram](A) V. Ringer

![Diagram](B) 12 mM K⁺ Ringer

![Diagram](C) 20 mM K⁺ Ringer

**Figure 6.** Veratridine-induced changes in glial membrane potential in normal and elevated extracellular potassium. Intracellular measurements in normal optic nerve; resting membrane potentials in normal Ringer (3 mM K⁺) were -85 to -90 mV. Veratridine (V.), added at arrows, equals 10⁻³ M final concentration.

**DISCUSSION**

The electrophysiological and pharmacological properties of nonmyelinated axons in the mudpuppy optic nerve are very similar to those from nonmyelinated nerves in other central and peripheral nervous systems. The conduction velocity in *Necturus* optic nerves ranges from 0.2–0.5 m·s⁻¹ (Orkand et al., 1966), compared to the same values in frog optic nerve (Orkand et al., 1966), 0.1–0.2 m·s⁻¹ in the gar olfactory nerve (Easton, 1965), and ~ 0.5 m·s⁻¹ in the rabbit vagus nerve (Howarth et al., 1968), all at 20°C. The density of sodium channels in mudpuppy axons is 24 μm⁻², equal to the density in gar olfactory axons, but less than the density in the lobster walking leg nerve or the rabbit vagus nerve (Ritchie et al., 1976). These last two nerves are composed of nonmyelinated axons having larger average fiber diameters (0.6–0.75 μm; Keynes and Ritchie, 1965; Moore et al., 1967) than the gar or mudpuppy axons (0.1–0.3 μm; Easton, 1965; Kuffler et al., 1966), so their higher conduction velocity is accompanied by both a larger diameter and a higher density of sodium channels.

Our experiments reveal no evidence for this type of sodium channel in the glial cells of the mudpuppy optic nerve. In the all-glial "nerve" no saturable
component of STX binding can be detected, and none of the normal pharmacological responses are present. In contrast, glia in the normal nerve are depolarized when veratridine is added to this preparation. There are two simple explanations for this glial depolarization. First, glial cells in the intact nerve may have sodium channels that are activated by veratridine. However, previously published electrophysiological results testify against sodium channels in glia of normal optic nerve. Kuffler et al. (1966) showed that the current-voltage relationship in the normal mudpuppy glia displayed neither rectifying nor negative conductance characteristics, and that the glial membrane potential responded in a purely Nernstian manner to changes of external K\(^+\) from 1.5 to 150 mM. These properties differ markedly from those of an excitable membrane such as the squid giant axon, where the steady-state response of the membrane potential to increased external potassium is not a simple logarithmic function and is less steep than 58 mV per 10-fold change in K\(^+\) for all potassium concentrations less than 130 mM (Hodgkin and Katz, 1949), and where current-voltage relationships are characteristically nonlinear.

The electrical responses and the non-Nernstian behavior of the squid axon are the result of voltage-dependent potassium and sodium channels, which are not manifested in the glial cells. However, a low density of sodium channels could be present in the glia and not be detected by electrical measurements. As an example, the resting potential and action potentials of cultured chick embryonic cardiac cells are not affected by high concentrations of tetrodotoxin (60 \(\mu\)M), but the cells can be depolarized by veratridine, and this depolarization is reversed by TTX (Sperelakis and Pappano, 1969). Evidently there are sodium channels in the cultured heart cells that are not responsive to membrane potential yet can be activated pharmacologically. Similar channels might also be present in glia. The second explanation for the veratridine-induced response is that glial depolarization follows the accumulation of extracellular potassium that is released from axons, the only processes directly affected by veratridine. It is known that potassium efflux from electrically stimulated axons leads to a transient glial depolarization, and that extracellular K\(^+\) is probably removed by diffusion through narrow clefts out of the nerve (Orkand et al., 1966). According to the second mechanism the axonal membrane depolarization from veratridine precedes the glial depolarization because of the obligatory mediating process of axonal K\(^+\) efflux. Axonal depolarization reverses much more slowly than glial depolarization because the primary effect of veratridine is to increase the sodium permeability of the axons, and changes in extracellular K\(^+\) have only modest effects on the drug-activated axonal membrane. The slow glial repolarization in veratridine (half-decay time of \(~40\) s) takes much longer than glial repolarization from an equally large depolarization resulting from tetanic nerve stimulation (half-decay time \(~6\) s; Orkand et al., 1966), because the axons, which are the K\(^+\) source, remain depolarized in veratridine. The potassium accumulation hypothesis explains the results of veratridine on glia in normal nerves with no requirement for postulating additional or modified channels or pumps in glial membranes.
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