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γ-AMINOBUTYRIC ACID DIRECTLY DEPOLARIZES CULTURED OLIGODENDROCYTES[1]

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Abstract

γ-Aminobutyric acid (GABA) depolarizes in a dose-dependent manner approximately one-third of all immunologically identified oligodendrocytes in cultures of mouse spinal cord. Measurements of $[K^+]_o$ indicate that the response to GABA is not due to K^+ released from active neurons. The depolarization is not accompanied by a change in cell input resistance. Replacement of sodium in the bathing solution abolishes the entire response, whereas ouabain only inhibits the repolarization phase. Current clamp experiments with two separate intracellular electrodes show that the depolarization increases at more positive potentials while the repolarization increases at more negative potentials. Bicuculline and picrotoxin but not nipecotic acid reduce the GABA effect. Pentobarbital and chlordiazepoxid also reduce the GABA-induced depolarization. Muscimol produces a depolarization similar to that of GABA. Heterogeneity in the oligodendrocyte population is indicated by the observation that some cells respond to both GABA and glutamate, while others respond only to one and some are not responsive to either.

Neuron-glia interactions play an important yet largely unknown role in the maintenance of nervous system functions (Sears, 1982). Since glial cells are electrically inexcitable cellular elements, their role in the regulation of the neuron's ionic environment has been assumed to be a largely passive one. They have been shown to respond to elevation in extracellular K^+ following the Nernst equilibrium potential for this ion (Kuffler et al., 1966; Kettenmann et al., 1983) and are presumed to act as spatial buffers to K^+ released by the electrically active neuron (Orkand et al., 1966). Another function of glial cells is the uptake and accumulation of neurotransmitters, such as γ-aminobutyric acid (GABA) and glutamate (for review see Hertz, 1979). Uptake of neurotransmitters has been suggested to represent a clearance function for transmitters released presynaptically by the neuron.

Glial cells in the cortex of the cat respond to neurotransmitters by a change in membrane potential (Krnjevic and Schwartz, 1967). This observation was later thought to be an indirect effect on the glial cell mediated by K^+ release from stimulated neurons (Somjen, 1975). In the intact tissue, however, an intrinsic reaction of glial cells to transmitters could not be excluded entirely, since changes in ion concentrations in the extracellular space surrounding reacting cells are difficult to determine with the necessary accuracy. Cell cultures offer the advantage of a more controlled extracellular environment. Using this system, Hösli et al. (Hösli and Andres, 1978; Hösli et al., 1981) could demonstrate that the depolarization of astrocytes cultured in explants to applied GABA is a result of K^+ release from adjacent neurons. A direct depolarizing effect of neurotransmitters on pure glial preparations has been shown in the denervated *Necturus* optic nerve and with a cultured glioma cell line (Reiser and Hamprecht, 1982; Tang and Orkand, 1982).

The present study was undertaken to investigate the effects of neurotransmitters in a mammalian cell culture system on an unequivocally identified glial subpopulation, the oligodendrocyte.

Materials and Methods

Cell cultures. Explant cultures of spinal cord were prepared from 13- and 14-day-old embryos of NMRI mice as described previously (Kettenmann et al., 1983). Explants were plated on collagen-coated glass coverslips and maintained in culture at 35.5°C and 3.5% CO_2 for 3 to 6 weeks. The culture medium, Eagle's Basal Medium with Earle's salts supplemented with 10% calf serum,

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was changed weekly. No antibiotics were used. For experiments in which the action of glutamate was tested, cultures were maintained in freshly prepared glutamate-free culture medium supplemented with 10% dialyzed calf serum one week prior to recording.

Electrophysiological procedures. For intracellular recordings and current and dye injection, two types of electrodes were used as described by Gilbert et al. (1982). Single-barreled electrodes filled with 3 M K^+-acetate (resistance 50 megohms) were used to record membrane potential or to inject current. To record membrane potential and then inject fluorescent dyes, double-barreled theta glass electrodes were used. One barrel was filled with 3 M potassium acetate, the other with 4% Lucifer Yellow (LY) or SITS (4-acetamido-4′-isothiocyanato-stilbene-2,2′-disulfonic acid disodium salt) in water. Extracellular K^+ concentration was measured by a double-barreled K^+-sensitive microelectrode (exchanger: model 477317, Corning, New Haven, CT) as described by Sonnhof et al. (1977). In some cases, GABA was pressure-ejected from a blunt-tipped electrode (tip diameter approximately 3 μm) filled with 1 mM GABA in bath solution (see below). For ejection, a pressure of 0.2 to 4 bar was applied on the sealed end of the capillary. The amount of pressure and the duration of application were controlled by a pressure ejection unit.

For recording, the culture dish was mounted on the stage of a Zeiss inverted microscope and maintained at 30 to 35°C. The pH of the bathing fluid was controlled by a 3.5% CO_2 atmosphere or by buffering with HEPES (10 mM). The reference electrode consisted of a silver/silver chloride pellet connected to the bathing fluid via a strip of filter paper. The bathing fluid contained the following salts: NaCl, 116 mM; KCl, 5.3 mM; $CaCl_2$, 1.8 mM; $NaHCO_3$, 26 mM; NaH_2PO_4, 1 mM; $MgSO_4$, 0.8 mM; glucose, 5.5 mM. Bathing fluid buffered with HEPES contained additionally 10 mM HEPES, 21.5 mM glucose (final concentration) but no $NaHCO_3$. The bathing fluid could be exchanged during experiments within 1 min. For bath application, substances to be tested were added to the salt solution. No perfusion artifacts were observed as described by MacDonald and Wojtowicz (1982). Neurotransmitters and other agents were obtained from Sigma (Muenchen, FRG) and were used in the following concentrations: GABA, 0.001 mM, 0.005 mM, 0.01 mM, 0.05 mM, 1 mM; sodium glutamate, 0.01 mM, 0.05 mM, 1 mM; sodium aspartate, 1 mM; adrenaline, 1 mM, acetylcholine, 1 mM; bradykinin, 1 mM; nipecotic acid, 1 mM; β-alanine, 1 mM; muscimol, 1 mM; picrotoxin, 1 mM. Bicuculline was used as methobromide in a concentration of 0.5 mM. It was prepared as described by Collins and Cryer (1977).

The microscope image was displayed on a TV screen at a final magnification of × 250 to allow precise determination of cell and electrode positions during experiments. The TV screen images were photographed by an oscilloscope camera. Cells were penetrated with the aid of two independent step motor-driven micromanipulators (Sonnhof et al., 1982). Positioning of extracellular electrodes was accomplished by Leitz micromanipulators. Intracellular recording and dye injection electrodes were connected to WPI-707 microprobe systems, and the K^+-sensitive electrode was connected to a high resistance amplifier (Oertel, Muenchen, FRG). Some cells were stained after recording by injection of LY (Stewart, 1981) or SITS (Gilbert et al., 1982) by passing hyperpolarizing current of 5 to 10 nA for 0.5 to 5 min.

Immunological methods. After electrophysiological recordings, cultures with LY-stained cells were labeled with cell type-specific antibodies by indirect immunofluorescence procedures as described previously by Schnitzer and Schachner (1981). Antibodies to O4 antigen were applied to live cells to label the cell surface of oligodendrocytes (Sommer and Schachner, 1981). Tetanus toxin was used to label neurons (Dimpfel et al., 1977). After application of O4 antibody or tetanus toxin, cells were fixed with 4% paraformaldehyde and treated with fluorochrome-conjugated anti-immunoglobulin antibodies. Rabbit anti-mouse Ig coupled with fluorescein isothiocyanate (from Paesel, Frankfurt, FRG) or tetramethylrhodamine isothiocyanate (from Dynatech DmbH, Plochingen, FRG) served to distinguish labeling of antigens from staining with LY and SITS (Gilbert et al., 1982; Kettenmann et al., 1983). Cells were photographed using a Zeiss fluorescence photomicroscope equipped with epi-illumination and the appropriate filters.

Results

Effect of GABA on membrane potential of oligodendrocytes. Cells were chosen for study by morphological appearance and, in some cases, subsequently were identified immunologically as oligodendrocytes (see below). With a trained eye it is possible to identify oligodendrocytes unequivocally in our culture system by morphological criteria only, as this can be verified by use of an oligodendrocyte-specific antibody.

GABA was applied to the cell cultures either by bath perfusion or pressure ejection from a blunt-tipped pipette while recording membrane potential. Sixty-seven of 197 cells tested (34%) were depolarized by GABA. The percentage of responsive cells was the same for both bath application and pressure ejection. Figure 1 shows the depolarizing action of GABA and muscimol on an oligodendrocyte. GABA (1 mM) had a maximal effect and depolarized cells by 4 mV (range, 2 to 8 mV; SD = 1.8

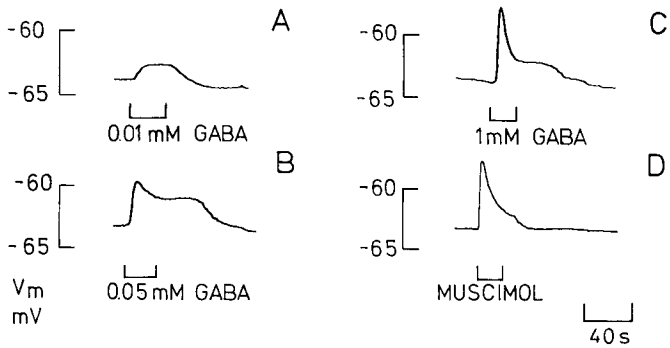

Figure 1. Effect of GABA and muscimol on membrane potential. GABA was applied to oligodendrocytes at three concentrations. *A* shows changes in membrane potential (V_m) to pulses of GABA (duration displayed by *bar*) of 0.01 mM, *B*, 0.05 mM, and *C*, 1 mM. *D* demonstrates the effect of 1 mM muscimol on the membrane potential.

mV). During continuous application of 1 mM GABA the depolarization was followed by a partial repolarization to a stable plateau (Fig. 1C). The time to peak of the depolarizing response averaged 0.6 sec (N = 12; SD = 0.16 sec) when GABA was applied by pressure ejection. During bath application the time to peak of the depolarizing response averaged 2.5 sec (N = 9; SD = 0.4 sec). The time period of repolarization from the peak was independent of the duration of GABA application and lasted for 10.8 sec (SD = 1.8 sec, for pressure ejection) or for 14.9 sec (SD = 1.9 sec, for bath application). After replacement of the GABA-containing solution by normal bathing solution, cells repolarized to the original membrane potential within 10.7 sec (SD = 1.1 sec, for pressure ejection) or 16 sec (SD = 3.1 sec, for bath application). In some cases, cells showed a small hyperpolarization.

The change in membrane potential was dependent on GABA concentration. Applying GABA in concentrations smaller than 0.005 mM showed no effect on the membrane potential. An increasing depolarization was observed from 0.01 mM to a maximum at 1 mM GABA (Fig. 1).

Immunological identification of oligodendrocytes. After recording, some cells (N = 6) were stained by injecting LY or SITS. The culture was then immunolabeled with monoclonal antibody O4 to identify oligodendrocytes (Sommer and Schachner, 1981; Gilbert et al., 1982; Kettenmann et al., 1983). All dye-injected cells were identified as oligodendrocytes. Figure 2 shows examples of immunologically identified oligodendrocytes. One reacted to GABA, the other did not.

GABA-induced depolarization and extracellular potassium. Since the GABA-induced depolarization of oligodendrocytes could result from a potassium release from adjacent neurons as described for cultured astrocytes (Hösli et al., 1981), extracellular K^+ concentrations were measured at several locations close to the oligodendrocyte surface during GABA application. Figure 3 shows recordings of membrane potential and extracellular K^+ concentrations in response to application of GABA and K^+ pulses in combination with the positions of recording and pressure ejection electrodes along an oligodendrocyte stained with LY. Pressure ejection of GABA resulted in a depolarization of the impaled cell, which subsequently showed a depolarization in response to an elevation in the extracellular K^+ concentration by pressure ejection. The positions of electrodes along the oligodendrocytes were photographed with an oscilloscope camera adapted to a TV screen. In order to define the vertical position the ion-sensitive electrode was slowly lowered until ground contact was seen by the resulting bending potential. Subsequently the electrode was pulled back for 20 μm. After recording, the cell was stained with LY. The positions of the K^+-sensitive electrodes were matched with the geometry of the cell body and cellular extensions (Fig. 3). Five cells were investigated in this manner. In no case was K^+ increased during or after GABA application.

To further exclude the hypothesis that the depolarizing response of oligodendrocytes to GABA was mediated by neurons, tetanus toxin was used to identify neurons by indirect immunofluorescence in a culture with GABA-reactive and LY-injected cells. Very few tetanus toxin-positive cells were present in our cultures and these were never in the vicinity of the investigated oligodendrocytes. This observation further indicates that the membrane reaction of oligodendrocytes is not a result of K^+ release from adjacent neurons or other cells, but is an intrinsic reaction of the oligodendrocyte membrane.

Cell input resistance during the action of GABA. In neurons the GABA-induced polarization results from a change of ionic conductances (for reviev see Krnjevic, 1974). Cell input resistance was therefore determined during the action of GABA as shown in Figure 4 and as described by H. Kettenmann, U. Sonnhof, H. Camerer, S. Kuhlmann, R. K. Orkand, and M. Schachner (submitted for publication). Oligodendrocytes were impaled with two separate electrodes filled with 3 M potassium acetate. Current was passed through one electrode, while the other served to record the membrane potential. Current was continuously altered in a ramp form with a frequency of 1 to 5 Hz (Fig. 4). The resulting changes in membrane potential were compared before, during, and after application of GABA. These measurements showed no change in cellular input resistance for five cells tested in response to GABA.

Action of GABA in sodium-free solution and with addition of ouabain. Figure 5 shows that when the Na^+ in the bathing fluid was exchanged for an equimolar concentration of choline, the depolarizing response to a GABA pulse was completely abolished (Fig. 5). After replacement of the sodium-free bathing fluid with normal medium, a GABA-triggered depolarization of the same size could again be recorded (Fig. 5A). These experiments (5 cells) indicate that Na^+ is essential for the GABA-induced depolarization of oligodendrocytes.

Addition of ouabain (0.5 mM) to the bath shortly before application of GABA still results in a depolarization of the five cells tested. The repolarization, however, was significantly prolonged or, in some cases, totally abolished. Replacement of the bathing fluid with normal medium showed that the effect of ouabain is reversible (Fig. 5B). Within the short time of ouabain application the cell showed no significant change in membrane potential. It seems, therefore, that the action of the Na/K-ATPase is required for the repolarization phase of the GABA action.

The GABA-induced response at current clamped depolarized and hyperpolarized potentials. Three cells were depolarized or hyperpolarized with constant current using two separate electrodes for voltage recording and current injection. At the resting membrane potential the cell showed the described de- and repolarization responses to GABA. When the membrane potential was set to approximately 0 mV the size of depolarization was significantly increased while the repolarization was almost abolished (Fig. 6). At hyperpolarizing membrane potentials up to 150 mV the GABA-induced depolarization was reduced in size while the repolarization phase became more pronounced and even exceeded the value of the original membrane potential (Fig. 6). The depolarization triggered by GABA therefore increases when the membrane is polarized closer to the sodium equilibrium potential (Buehrle and Sonnhof, 1983). These observations indicate that the GABA-induced depolarization is not caused by an increase in Na^+ conductance.

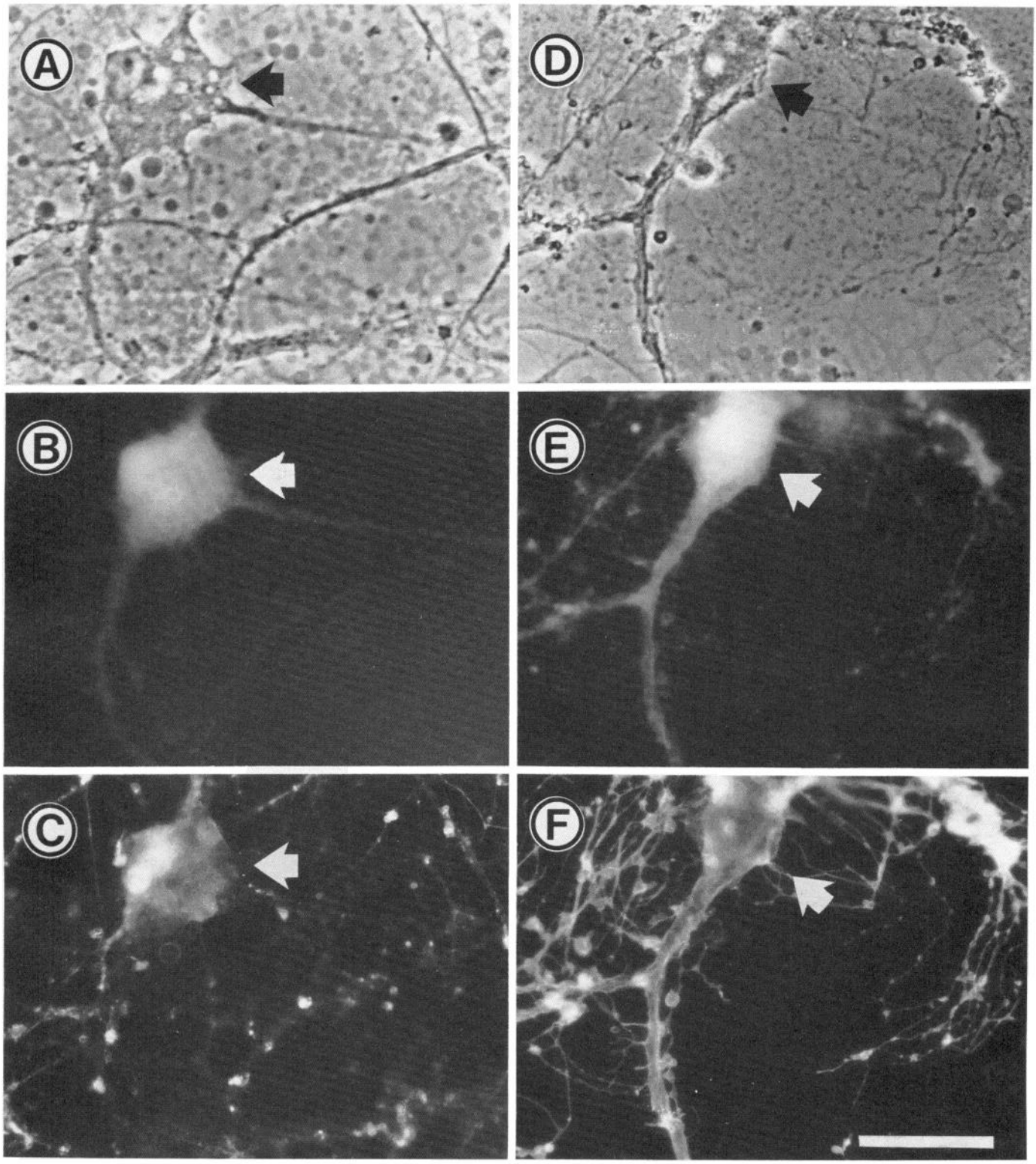

Figure 2. Immunological identification of oligodendrocytes. *A* to *C* and, respectively, *D* to *F* are identical microscopical fields. A cell which showed no response to GABA (*A* to *C*) and one depolarized by GABA (*D* to *F*) were shown to be O4-positive oligodendrocytes by indirect immunofluorescence. *A* and *D*, Phase contrast micrograph. *B* and *E*, Lucifer Yellow-injected cells. *C* and *F* demonstrate cell surface staining with the oligodendrocyte-specific antibody to O4 using tetramethylrhodamine as fluorochrome. *Bar* = 50 μm.

Effect of muscimol, bicuculline, picrotoxin, and nipecotic acid on the GABA-induced membrane response. The GABA agonist muscimol (Krogsgaard-Larsen et al., 1975; Enna et al., 1979) was applied by pressure ejection to oligodendrocytes at a concentration of 1 mM. A similar depolarization and hyperpolarization as produced by GABA was observed (Fig. 1).

When the GABA antagonists bicuculline and picro-

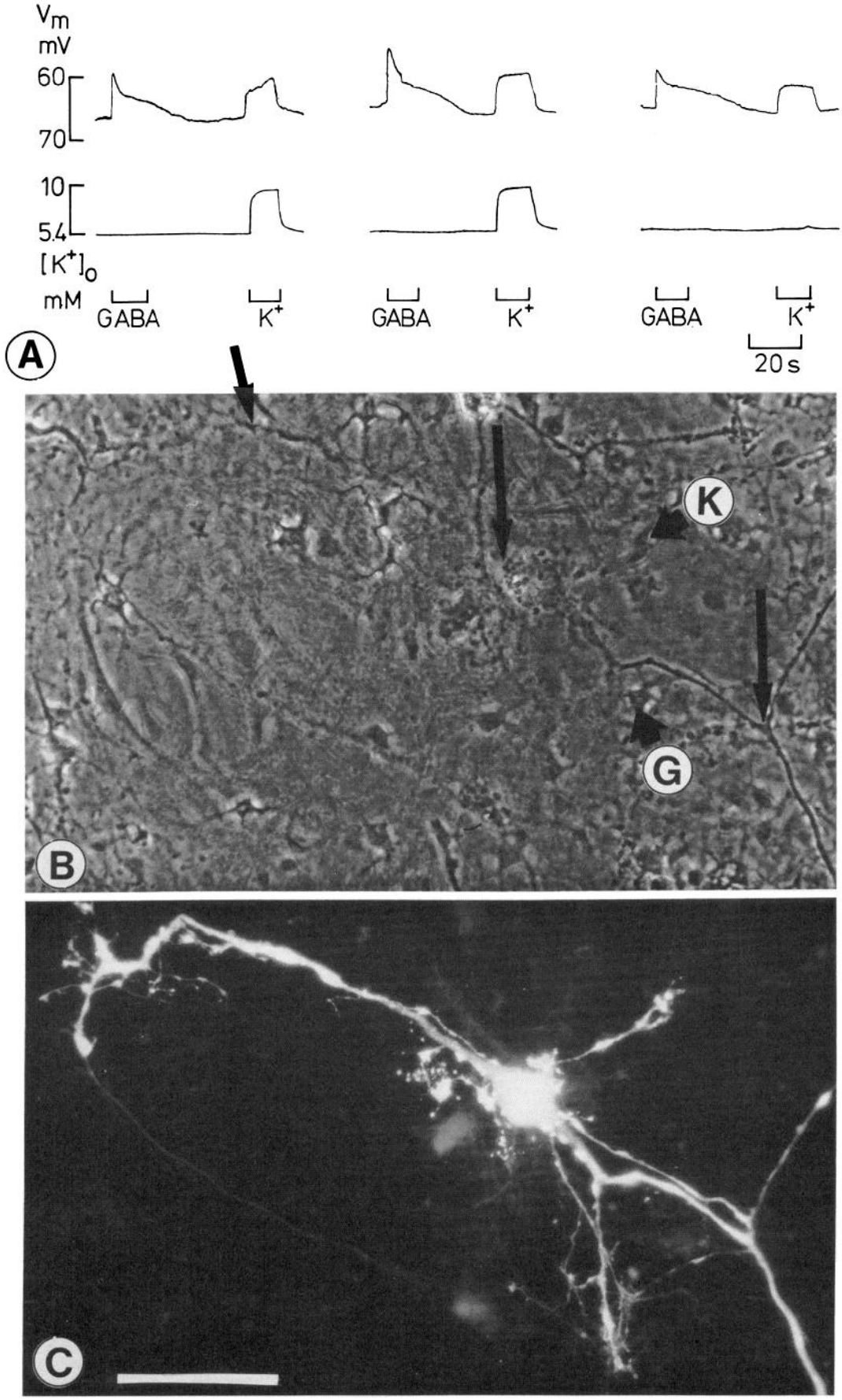

Figure 3

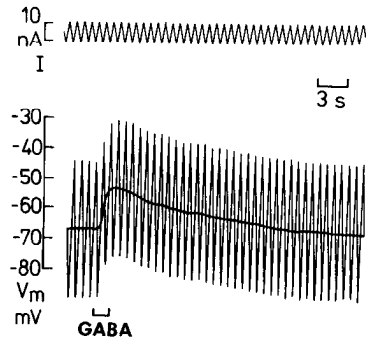

Figure 4. Measurement of input resistance during GABA-induced membrane depolarization. Two separate electrodes served for injection of current (*I, upper trace*) and for recording the membrane potential (V_m, *lower trace*) during application of 1 mM GABA by pressure ejection (marked with a *bar*). Peak values of continuously altered current and resulting voltage changes are constant during GABA-induced depolarization and indicating no change in cellular input resistance. The midpoints of membrane polarizations due to injected current are connected by a *line* resembling membrane potential during GABA effect without current injection.

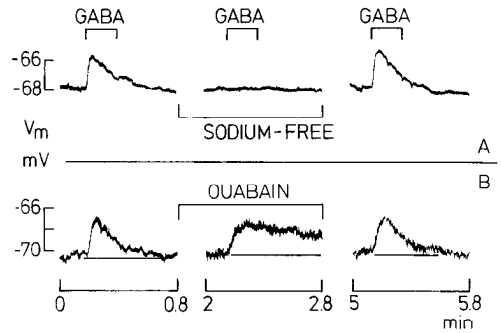

Figure 5. Response of membrane potential to application of GABA in Na^+-free solution and in the presence of ouabain. During continuous recording of membrane potential (V_m) cells were perfused with Na^+-free bath solution (*A*) and with 0.5 mM ouabain (*B*) (*large bars*). Pulses of pressure-ejected GABA (1 mM) are denoted by *small bars* (for *A* and *B*).

toxin (Curtis et al., 1971; Olsen, 1981) were added to the bath while short pulses of GABA were given at intervals of 2 min, 0.5 mM bicuculline completely blocked the effect of GABA. The effect was reversible after about 10 min (Fig. 7*A*). Picrotoxin at a concentration of 1 mM reduced the depolarization in response to GABA to about 15%. In contrast to bicuculline and similar to its action on neurons (Aickin et al., 1981), this toxin did not completely abolish the GABA depolarization. The effect was also reversible after 10 min (Fig. 7*B*). Nipecotic acid and β-alanine, inhibitors of GABA transport (Iversen and Kelly, 1975; Krogsgaard-Larsen and Johnston, 1975; Brown and Galvan, 1977), did not effect the GABA-induced depolarization at concentrations of 1 mM (Fig. 7*C*; not shown for β-alanine).

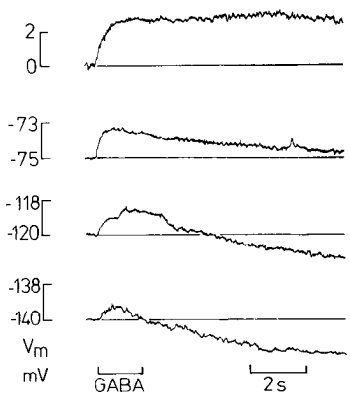

Figure 6. Effect of GABA on membrane potential under current clamp conditions. Oligodendrocytes were current-clamped at different membrane potentials with two intracellular electrodes for constant current injection and recording of membrane potential (V_m). Pulses of GABA are indicated by the *bar*.

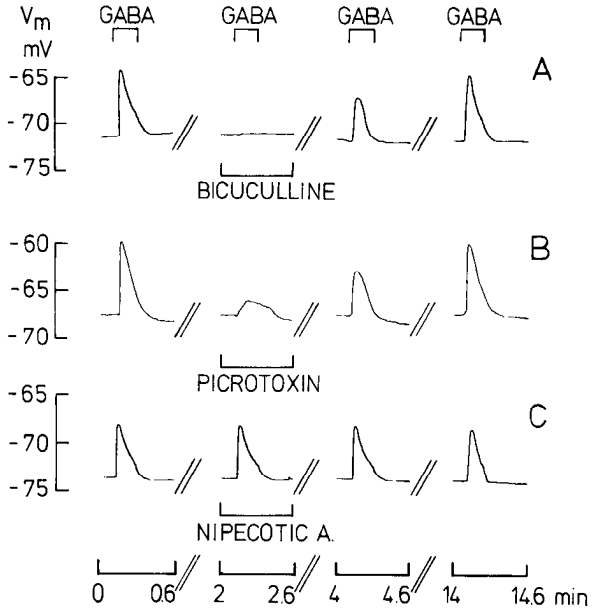

Figure 7. Effects of bicuculline, picrotoxin, and nipecotic acid on GABA-induced depolarization of membrane potential. During continuous recording of membrane potential (V_m) pulses of 1 mM GABA were given to an oligodendrocyte at intervals of 2 min (indicated by *small bars*). Bicuculline (0.5 mM) (*A*), 1 mM picrotoxin (*B*), and 1 mM nipecotic acid (*C*) were added by perfusion (indicated by *large bars*) to monitor their effect on the GABA-induced membrane depolarization.

Effect of chlordiazepoxid and pentobarbital on the GABA-induced response. Chlordiazepoxid and pentobarbital were applied by bath perfusion to oligodendrocytes at concentrations of 0.5 mM (Fig. 8). The effect of short GABA pulses on the membrane potential was recorded continuously during the exchange of bathing fluid. Both

Figure 3. Simultaneous measurements of membrane potential and extracellular K^+ concentration during GABA application. During recording of membrane potential (V_m) pulses of GABA and K^+ were pressure ejected from electrodes marked as *G* and *K*, respectively, while extracellular potassium concentration $[K^+]_o$ was measured at three locations close to the recorded cell (indicated by *long arrows* in *B*. *A* displays membrane potential and extracellular potassium concentration. Note that in the *third trace* the K^+-sensitive electrode does not respond to the ejected stream of elevated K^+ since the solution flows in the direction of the arrow *G*. *B* is a phase contrast micrograph showing the sites where K^+ was measured (marked by *long arrows*). *C* is a corresponding LY fluorescence micrograph for subsequent identification of the cell.

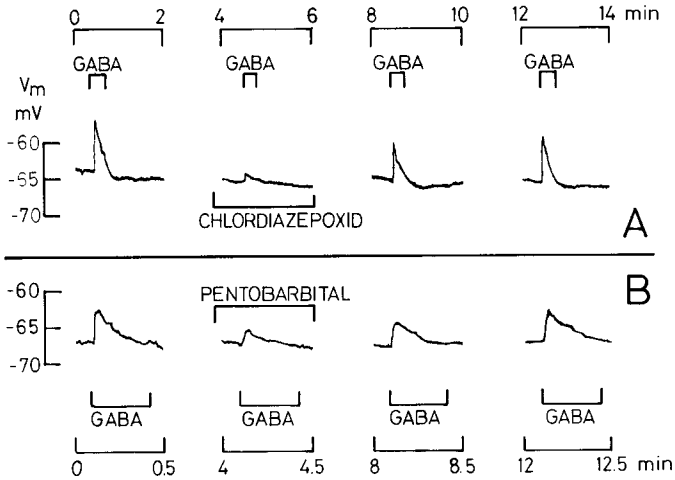

Figure 8. Effect of chlordiazepoxid and pentobarbital on GABA-induced depolarization of membrane potential. During continuous recording of membrane potential (V_m) pulses of 1 mM GABA were applied at intervals of 4 min (indicated by *small bars*). Chlordiazepoxid (0.5 mM (*A*) and 0.5 mM pentobarbital (*B*) were added by perfusion to monitor their effect on the GABA-induced membrane depolarization.

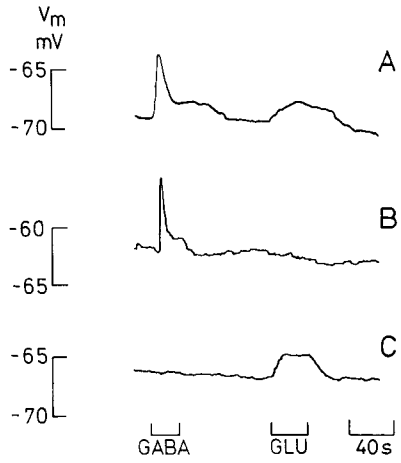

Figure 9. Effects of GABA and glutamate on membrane potential of oligodendrocytes. Three different oligodendrocytes (*A*, *B*, and *C*) received pulse ejections of 1 mM GABA and 1 mM glutamate (*GLU*) as indicated by *bars*. One cell (*A*) responded by depolarization of membrane potential (V_m) to application of both GABA and glutamate. Another cell (*B*) responded only to GABA, while the third (*C*) depolarized only to glutamate.

substances significantly reduced the response of the membrane potential to GABA, for chlordiazepoxid to 16% ($N = 6$; SD = 4.6%) and for pentobarbital to 41% ($N = 5$; SD, 5.3%). The action of these drugs was reversible after 10 min in normal medium (Fig. 8).

Effect of other transmitters on oligodendrocytes. The putative transmitters glutamate, aspartate ($N = 49$), glycine ($N = 45$), serotonin ($N = 38$), adrenaline ($N = 37$), noradrenaline ($N = 36$), acetylcholine ($N = 41$), and bradykinin ($N = 43$) were tested for their ability to affect the membrane potential of oligodendrocytes at concentrations of 1 mM. Bath perfusion with glutamate resulted in a dose-dependent depolarization of the membrane potential in 38% of all tested cells ($N = 54$). Fifteen of 20 glutamate-positive cells also responded to GABA (Fig. 9). Glutamate-positive and -negative cells were immunologically identified as oligodendrocytes as described above. All other substances tested showed no effect on the membrane potential of oligodendrocytes (Fig. 10).

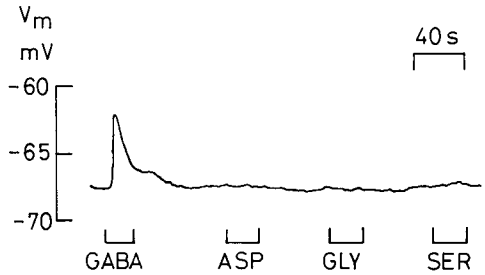

Figure 10. Effects of aspartate, glycine, and serotonin on membrane potential of oligodendrocytes. GABA (1 mM), 1 mM aspartate (*ASP*), 1 mM glycine (*GLY*), and 1 mM serotonin (*SER*) were added by perfusion during recording of membrane potential (V_m).

Discussion

In the present study we have shown that some cultured oligodendrocytes from the mouse spinal cord are depolarized directly by the neurotransmitter GABA. This contrasts with results of cultured astrocytes in which it appears that GABA depolarization is the result of K^+ released from neurons (Hösli et al., 1979). By ion-sensitive measurements we could exclude that the GABA-induced depolarization of oligodendrocytes is mediated by elevated extracellular K^+. Furthermore, no neurons could be detected in the vicinity of the GABA-reactive cells by immunological methods using tetanus toxin as neuronal marker. A depolarizing membrane response of some oligodendrocytes to glutamate but not to other neurotransmitters was also found in our culture system, but was not investigated in detail. These observations support the notion that ionic mechanisms of glial cell membranes can be directly and selectively influenced by some neurotransmitters as it has been suggested recently for a depolarizing effect of glutamate in the amphibian optic nerve (Tang and Orkand, 1982). Walz (1982) showed a small hyperpolarizing influence of serotonin on glial cells of the leech, while Reiser and Hamprecht (1982) found bradykinin to hyperpolarize cells of a clone derived from a rat glial tumor. Taken together, these findings suggest that there is a considerable diversity among glial cells in different species with respect to their response to neurotransmitters.

A heterogeneity of oligodendrocytes in response to neurotransmitters has been found in our cell culture system. Some oligodendrocytes respond neither to GABA nor to glutamate, some to either GABA or glutamate, and some to both. We have not been able to find morphological criteria that distiguish between these different types of oligodendrocytes. It remains to be seen whether this apparent diversity in the oligodendrocyte population reflects different stages in development which cannot be

distinguished by using O4 antibody (Schachner et al., 1981). Oligodendrocytes at a particular developmental stage could also express intrinsically different membrane properties with regard to some neurotransmitters. It is conceivable, although highly conjectural, that oligodendrocytes specialize in their response to neurotransmitters depending on their location within the nervous tissue and relative to particular neuronal cell types and their particular neurotransmitter as it has been suggeted for astrocytes (Hamberger et al., 1977). Whether our culture system indeed reflects the cellular properties of oligodendrocytes *in vivo* remains an open question. It is interesting to note, however, that oligodendrocytes in our culture system respond at GABA concentrations which are in the physiological range of GABA in the extracellular space and of susceptibility of the neuronal GBA receptor (Roberts et al., 1978). Therefore, it might be necessary to consider that extracellular recorded DC potentials in brain which were found to be affected by GABA (Krnjevic, 1974) can be influenced directly by oligodendrocytes.

The pharmacological properties of the oligodendrocyte response to GABA are to some extent similar to those of the neuronal GABA receptor (Krnjevic, 1974; De Feudis, 1977; Olsen, 1981) and should be taken into consideration in the attempt to purify the neuronal GABA receptor from whole brain tissue. The depolarizing response in oligodendrocytes is affected by the GABA antagonists bicuculline and picrotoxin, but not by the inhibitors of GABA uptake, nipecotic acid and β-alanine. In addition, chlordiazepoxid and pentobarbital, which have been shown to affect the neuronal GABA receptor (Barker and Ransom, 1978; Choi et al., 1981; Olsen, 1981), were also found to modify the oligodendrocyte's response to GABA. These observations could suggest that a GABA-induced depolarization of the oligodendrocyte membrane is mediated by a binding site or receptor similar to the neuronal one. Binding studies with radioactively labeled GABA could not demonstrate GABA receptors on cultured astrocytes (Hösli and Andres, 1978). On the other hand, Möhler et al. (1981) discussed the possibility that glial structures adjacent to synapses might contain benzodiazepine receptors. The oligodendroglial response to GABA differs, however, in an important aspect from the neuronal one, namely that it is not associated with membrane conductance changes large enough to be detected by our methods. An increased permeability to Na^+ and K^+ ions can be excluded from polarization experiments and measurements of membrane conductance.

Previous investigations have shown that not only Na^+ but also Cl^- contributes minimally to the membrane potential of cultured oligodendrocytes (Kettenmann et al., 1983) and that the equilibrum potential of Cl^- of glial cells from the frog spinal cord is close to the membrane potential (Buehrle and Sonnhof, 1983). Therefore, an increased Cl^- conductance too small to be detected by our resistance measurements cannot be responsible for the depolarizing action of GABA.

The GABA-induced ionic mechanisms which lead to changes in the polarization of the oligodendrocyte's membrane are likely to involve an active process or processes. The repolarization phase in response to GABA depends on the action of the Na/K-ATPase. It is possible that the depolarization phase in GABA action described in this study could be related to an active GABA uptake. An active transport system for GABA has been postulated for glial cells (Iversen and Kelly, 1975; Sellstroem and Hamberger, 1975; Hertz, 1979). An involvement of Na^+ ions in glial uptake of GABA has been demonstrated by East et al. (1980). Furthermore, an effect of bicuculline on the Na^+-dependent uptake of GABA (East et al., 1980) is also in concordance with our observations. In contrast to the observations of these authors, however, an inhibition by β-alanine or nipecotic acid of the direct influence of GABA action on the membrane potential of oligodendrocytes has not been found in our studies. It remains to be seen whether GABA is indeed taken up by oligodendrocytes and whether this uptake is associated with a change in membrane potential.

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