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Neuronal modulation of calcium channel activity in cultured rat astrocytes

(neuron–glia interaction/oligodendrocytes/patch clamp)

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ABSTRACT **The patch-clamp technique was used to study whether cocultivation of neurons and astrocytes modulates the expression of calcium channel activity in astrocytes. Whole-cell patch-clamp recordings from rat brain astrocytes cocultured with rat embryonic neurons revealed two types of voltage-dependent inward currents carried by Ca^{2+} and blocked by either Cd^{2+} or Co^{2+} that otherwise were not detected in purified astrocytes. This expression of calcium channel activity in astrocytes was neuron dependent and was not observed when astrocytes were cocultured with purified oligodendrocytes.**

Cell–cell interaction in the nervous system has long been considered a major determinant of cell growth and differentiation. Evidence that glial cells play an essential role in neuronal function has supported the concept of neuron–glia interaction as an important element in understanding the dynamics of the nervous system. Glial cells are no longer considered as only buffers of extracellular K^+ and neurotransmitters, but also as part of an active molecular interaction with neighboring neurons (1, 2), which ranges from cell growth and differentiation (1, 2) to exchange of molecules and nutrients (1–4).

Coculture systems have provided a useful tool to study neuron–glia interactions (2, 5–10). We have used the patch-clamp technique to study whether the interactions between neurons and astrocytes established in coculture modulate calcium channel activity in astrocytes. Neuronal modulation of the expression of calcium channel activity would have a profound significance on astrocyte physiology because of the widespread role of calcium in excitability and intracellular signal transduction (11, 12).

In culture, purified type 1 astrocytes display only K^+ outward currents (13). However, calcium currents can be induced when astrocytes are treated with substances that increase the intracellular levels of cAMP (14, 15). In this study, we report that in purified astrocytes, calcium current activity can be expressed by cocultivating neurons and astrocytes. It is also shown that this effect is specifically dependent on neuron–astrocyte interactions, since calcium currents are not detected from astrocytes cocultured with oligodendrocytes.

MATERIAL AND METHODS

Astrocyte and Oligodendrocyte Culture. Astrocytes and oligodendrocytes were purified from primary cultures of neonatal rat brain cortex by a modification (17) of the method of McCarthy and de Vellis (16). Briefly, brain cortices from 1- to 2-day-old rats were mechanically dissociated and cell suspensions were plated in 75-cm^2 flasks at densities of $\approx 2 \times 10^5$ cells per cm^2 in serum-supplemented medium [Dulbecco's modified Eagle's medium/F-12 (D/F) + 10% fetal calf serum]. In the early phase of the culture, neurons began to degenerate and were eliminated by the second medium change at day 4–5. After 7–8 days, the culture consisted of small, phase-dark, process-bearing cells, corresponding to immature oligodendrocytes (18, 19), residing on top of a phase-light, confluent bedlayer of cells, corresponding to type 1 astrocytes. Cells were subsequently separated by the "shaking off" procedure (16, 17) that selectively detached the phase-dark oligodendrocytes from the phase-light astrocytes. Detached cells, consisting primarily of oligodendrocytes, were filtered (pore size, 25 μm) and replated in 75-cm^2 flasks. This procedure was repeated at least two times to obtain further purifications of astrocytes and oligodendrocytes. At 21 days in culture, immunocytochemical assays indicated that >98% of the cells in the astrocyte cultures were glial fibrillary acidic protein positive, corresponding to protoplasmatic or type 1 astrocytes (20). On the other hand, 85% of the cells in the oligodendrocyte cultures tested galactocerebroside positive, and <5% of the cells were glial fibrillary acidic protein positive.

Neuron–Astrocyte Coculture. Enriched neuronal cultures were prepared from 16-day embryonic rat cerebral cortices according to the method described by Syapin *et al.* (21). Cell suspensions were plated on polylysine-coated or glow-discharged flasks (22, 23) at densities of $\approx 1 \times 10^5$ cells per cm^2. Medium containing cytosine arabinoside (10 μM) was added to the cell cultures for 2–4 days, depending on the appearance of the dividing cells. Immunocytochemical assays indicated that in these enriched neuronal cultures <10% of the cells were glial fibrillary acidic protein positive. Purified astrocytes (21 days) were trypsin treated and added to the neuronal culture (2–4 days) in a 3:1 ratio. Cocultures were kept for 7–10 days, in D/F + 10% fetal calf serum without cytosine arabinoside before patch-clamp experiments were performed.

Oligodendrocyte–Astrocyte Coculture. Purified oligodendrocytes (21 days) were trypsin treated and plated over purified astrocytes (21 days) at densities of $\approx 1 \times 10^5$ cells per cm^2 in D/F medium supplemented with 10% fetal calf serum. Cocultures were kept for the same period of time as the neuron–astrocyte coculture before patch-clamp experiments were carried out.

Solutions. The composition of the external solution was as follows: 140 mM NaCl/5 mM KCl/2.5 mM $CaCl_2$/1.5 mM $MgCl_2$/10 mM Hepes, pH 7.4, adjusted with NaOH. The composition of the pipette solution was as follows: 130 mM KCl/10 mM Hepes/10 mM EGTA/2.0 mM $CaCl_2$/1.6 mM $MgCl_2$/0.5 mM ATP, pH 7.4, adjusted with KOH. Pipette solutions containing Cs and tetraethylammonium (TEA)

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Abbreviation: TEA, tetraethylammonium.

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were made by replacing KCl with 125 mM CsCl and by adding 10 mM TEA; pH was adjusted with CsOH.

Electrophysiology. Confluent astrocyte cultures, neuron–astrocyte cocultures, and oligodendrocyte–astrocyte cocultures were trypsin treated and replated at lower densities on glass coverslips. Patch-clamp experiments were conducted within 48 hr. Whole-cell patch-clamp currents were recorded with a List L/M-EPC7 patch-clamp amplifier (List Electronics, Darmstadt, F.R.G.). Patch pipettes were fabricated from borosilicate glass capillaries. Pipettes had resistances of 3–5 MΩ when filled with pipette solution. Experiments were carried out at room temperature (20°C–22°C).

Data Acquisition and Pulse Generation. Data acquisition and pulse generation were performed by using a system based on that described by Stimers *et al.* (24). Current signals were filtered by using an eight-pole Bessel filter, set at corner frequencies of 2 kHz or 450 Hz, depending on the pulse duration and sampling rate. Each current trace corresponded to a collection of 1250 samples, taken at intervals of 60 or 450 μs per point. Linear leak and capacitive transient currents were digitally subtracted by using a p/n procedure (25), with pulses of the same polarity as the test pulse, applied from the holding potential. Membrane capacitance was measured by integrating the capacitive transient current elicited by a 10-mV pulse applied from the holding potential.

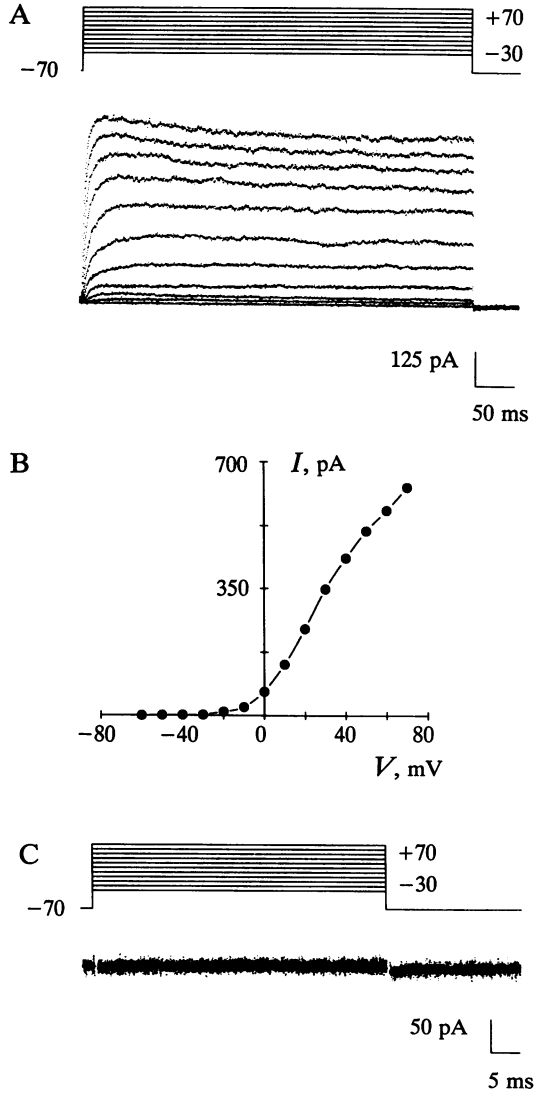

FIG. 1. Whole-cell patch-clamp recordings from purified astrocytes. (*A*) Voltage-dependent outward currents elicited by depolarizing pulses of 500 ms duration applied from a holding potential of −70 mV. Current records shown correspond to voltage steps ranging from −30 to +70 mV in 10-mV increments. (*B*) Current–voltage (*I–V*) relationship. Outward current amplitude measured at the end of the pulse plotted versus the pipette potential. (*C*) Superimposed current traces corresponding to voltage steps indicated in *A*, recorded from a different cell, using a patch pipette containing Cs^+ and TEA to block the outward currents and with 10 mM Ca^{2+} in the external solution. Holding potential, −70 mV. Pulse duration, 50 ms.

RESULTS

Whole-cell patch-clamp recordings from type 1 astrocytes purified by a modification (17) of the "shaking off" method of McCarthy and de Vellis (16) are shown in Fig. 1. Voltage-dependent outward currents, as shown in Fig. 1*A*, were recorded with KCl in the patch pipette and with NaCl in the external solution as main charge carriers. These outward K^+ currents were elicited by depolarizing pulses of 40 mV or higher (see Fig. 1 *A* and *B*), applied from a holding potential of −70 mV. No inward currents were detected in any of the cells examined ($n = 28$), even under conditions in which the outward currents were blocked by replacing K^+ with Cs^+ and adding TEA to the pipette solution, and the Ca^{2+} concentration in the external solution was elevated from 2.5 to 10 mM (see Fig. 1*C*).

When astrocytes were cocultured with neurons, in addition to the outward currents, voltage-dependent inward currents were also recorded (see Fig. 2). To detect inward currents, it was necessary to coculture astrocytes with neurons for 7–10 days. We were unable to detect calcium currents from astrocytes cocultured for shorter periods of time. The fraction of cells displaying inward currents varied within the different cocultures, and they were detected in 37 of the 69 cells examined. This variation could be attributed to the sampling limitations imposed by the patch-clamp technique, the mechanisms of calcium channel induction, or heterogeneities in the neuronal and astrocyte (26) populations.

Control experiments were carried out with astrocytes derived from the same primary culture used for cocultivation. These secondary cultured astrocytes maintained in the absence of neurons, for the same period of time and in the same medium conditions as the cocultured astrocytes, exhibited only voltage-dependent outward currents similar to those shown in Fig. 1.

Fig. 3*A* illustrates a family of current traces recorded from a cocultured astrocyte, with 10 mM Ca^{2+} in the external solution and with Cs^+ and TEA in the pipette solution. These

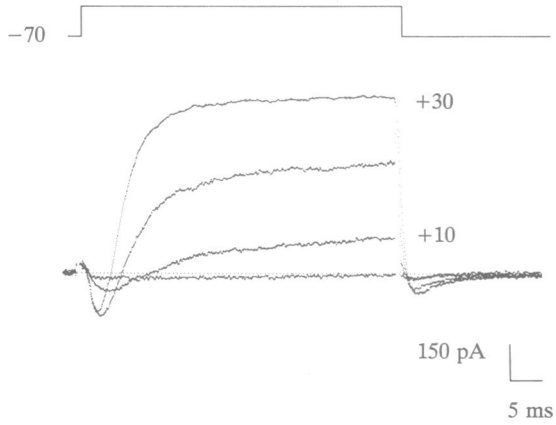

FIG. 2. Voltage-dependent currents recorded from a cocultured astrocyte. Currents recorded in the presence of 10 mM Ca^{2+} in the external solution. Shown are the current traces in response to voltage steps to 0, 10, 20, and 30 mV from a holding potential of −70 mV. Pulse duration, 50 ms.

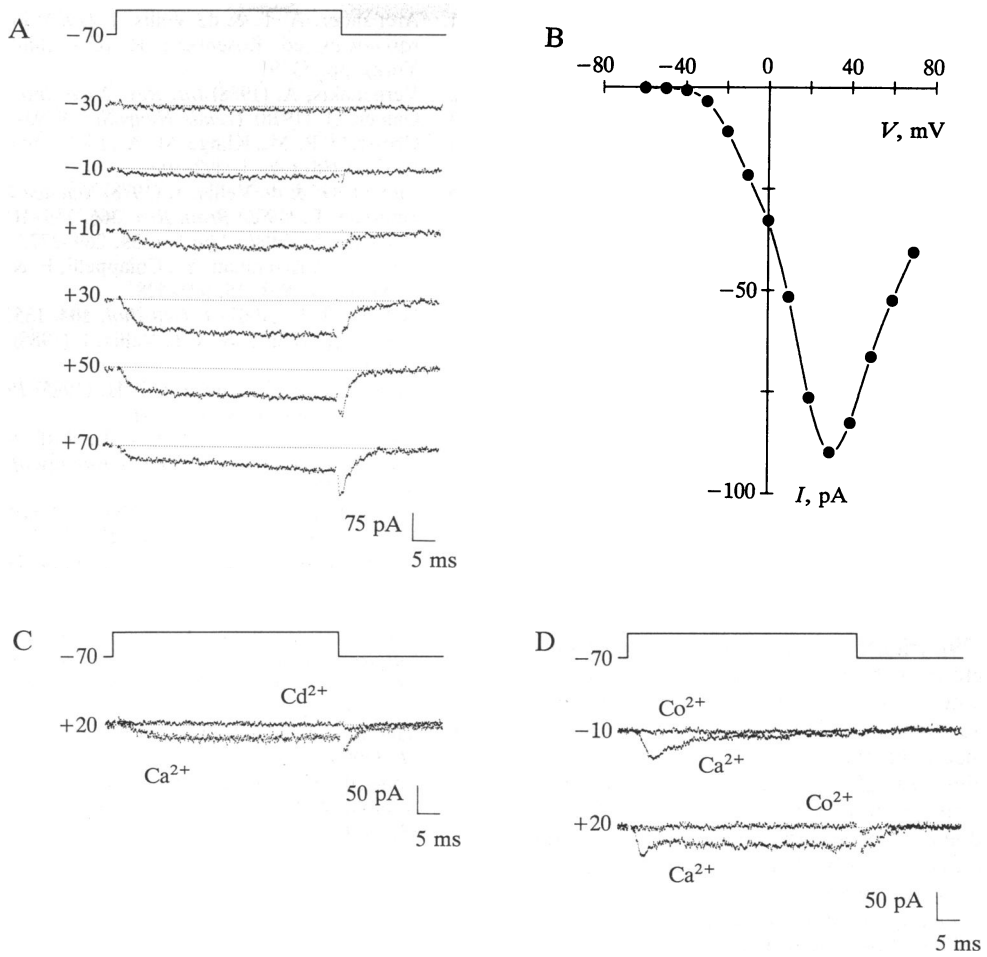

FIG. 3. Inward currents recorded from cocultured astrocytes. Depolarizing pulses of 50 ms duration were applied from a holding potential of −70 mV to the pipette potential indicated next to each record. (*A*) Long-lasting inward currents recorded with a patch pipette containing Cs^+ and TEA to block the outward currents and with 10 mM Ca^{2+} in the external solution. (*B*) Current–voltage (*I–V*) relationship. Maximum amplitude of the inward current plotted versus the pipette potential. (*C*) Blocking effect of Cd^{2+} on the long-lasting inward current. Current traces were recorded from an astrocyte different from that in *A* by using a patch pipette containing Cs^+ and TEA in the presence of 10 mM Ca^{2+} or with 10 mM Ca^{2+} and 0.5 mM Cd^{2+} in the external solution. (*D*) Transient and long-lasting inward currents recorded from a cocultured astrocyte with a patch pipette containing Cs^+ and TEA. Each pair of current traces corresponds to recordings in the presence of 10 mM Ca^{2+} or 10 mM Co^{2+} in the external solution.

current recordings were characterized by a slow long-lasting inward current, activated by depolarizing pulses of 40 mV or higher, applied from a holding potential of −70 mV (see Fig. 3 *A* and *B*). This inward current was carried by Ca^{2+}, since it was blocked by either 0.5 mM Cd^{2+} (Fig. 3*C*) or 10 mM Co^{2+} (Fig. 3*D*). The amplitude of this current varied largely from cell to cell, and this variation was not correlated with the cell surface area, expressed as cell membrane capacitance. The maximum amplitude of the inward current standardized by the membrane capacitance ranged from 0.41 to 3.84 pA/pF, with a mean value of 1.39 ± 0.72 pA/pF (±SD; n = 31). This inward current recorded from cocultured astrocytes exhibited properties that resembled the L-type calcium current observed in neurons (27).

A second type of voltage-dependent inward current was recorded from cocultured astrocytes. This transient type, observed in six cells, showed a faster activation and quick inactivation (see Fig. 3*D*). It was always detected in conjunction with the long-lasting type (see current record corresponding to voltage step to −20 mV in Fig. 3*D*) and was also carried by Ca^{2+}, since it was blocked by 0.5 mM Cd^{2+} or by 10 mM Co^{2+} (Fig. 3*D*).

Calcium currents were recorded only from astrocytes cocultured with neurons. We did not detect inward currents from astrocytes cocultured with purified oligodendrocytes (n = 18). These cocultures were maintained in the same medium and for the same period of time as the neuron–astrocyte coculture. Recordings from these astrocytes were similar to those obtained from purified astrocytes shown in Fig. 1.

DISCUSSION

The results obtained in this study indicate that cocultivation of neurons and astrocytes modulates the expression of two types of calcium channel activity in astrocytes. These long-lasting and transient types of calcium currents are not detected in purified astrocytes, which only display K^+ outward currents (13).

The expression of calcium channel activity induced in astrocytes was a neuron-dependent effect. It was observed when astrocytes were cocultured with neurons but was not detected in astrocytes cocultured with purified oligodendrocytes, which constitute the other major glial cell type found in the brain. In similar coculture systems, neuronal cell type dependence has also been found for the induction of glutamine synthetase activity, a specific marker of astrocytes (10), and for myelin basic protein synthesis by oligodendrocytes (8).

Barres *et al.* (15) have reported that calcium currents similar to the neuronal L-type can be detected only when type

1 astrocytes are treated with substances known to increase intracellular levels of cAMP. This induction is dependent on the batch of fetal calf serum used to culture the astrocytes. These authors have also reported a transient type of calcium current, which seems to be dependent only on the batch and source of fetal calf serum. In our experiments, the transient type calcium current, observed in a fraction of the cells displaying inward currents, seemed only dependent on the presence of neurons in the culture, since this current was always recorded from astrocytes cocultured with neurons and not from astrocytes cocultured with oligodendrocytes or from purified astrocytes.

The mechanisms by which neurons modulate calcium channel activity in astrocytes are unknown. The expression of calcium currents in astrocytes might be mediated by cell–cell contact (9, 10) or by a factor secreted by the neurons (1, 8). The intracellular cascade of events following the membrane transduction process is also unclear. The fact that calcium channel activity is induced by substances that increase the intracellular levels of cAMP does not provide a clear clue about the intracellular pathway leading to the expression of calcium currents. These substances, which include neurotransmitters such as the β-adrenergic agonist isoproterenol (28, 29), trigger a series of intracellular events that result in a state of high cellular activity. In addition to induction (14, 15) and enhancement (30) of calcium currents in astrocytes, these substances also increase enzymatic activity (10) and induce gene expression (31, 32) and morphological differentiation (14, 15, 30, 33, 34). Furthermore, it might be possible that the induction of calcium currents by these substances does not necessarily share the same pathway of intracellular events involved in the neuronal induction of calcium currents, as has been suggested for the induction of glutamine synthetase activity in astrocytes (10).

It has not yet been shown whether type 1 astrocytes express calcium channel activity *in vivo* (15, 35). The lack of specific markers imposes technical limitations on the identification of type 1 astrocytes, making difficult the study of freshly dissociated cells. Nevertheless, if type 1 astrocytes are capable of expressing calcium channel activity as observed in cocultured conditions, it is plausible to postulate that this activity is manifested *in vivo*, and its expression is modified during the isolation and long-term culture procedures that lead to purified astrocytes, probably due to the absence of neurons. Although the mechanisms that modulate the expression, as well as the role of the calcium channels, in astrocytes remain to be elucidated, our results suggest that the expression of calcium channel activity in astrocytes represents a specific interaction between neurons and glial cells.

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1. Arenander, A. T. & de Vellis, J. (1983) in *The Clinical Neurosciences*, ed. Rosenberg, R. (Churchill Livingstone, New York), pp. 53–91.
2. Vernadakis, A. (1988) *Int. Rev. Neurobiol.* **30,** 149–224.
3. Gainer, H. (1978) *Trends NeuroSci.* **1,** 93–96.
4. Grossfeld, R. M., Klinge, M. A., Lieberman, E. M. & Stewart, L. C. (1988) *Glia* **1,** 292–300.
5. Ciment, G. & de Vellis, J. (1978) *Science* **202,** 765–768.
6. Hansson, E. (1986) *Brain Res.* **366,** 159–168.
7. Hansson, E. (1986) *Life Sci.* **39,** 269–277.
8. Bologa, L., Aizenman, Y., Chiappelli, F. & de Vellis, J. (1986) *J. Neurosci. Res.* **15,** 521–528.
9. Hatten, M. E. (1987) *J. Cell Biol.* **104,** 1353–1360.
10. Wu, D. K., Scully, S. & de Vellis, J. (1988) *J. Neurochem.* **50,** 929–935.
11. Kandel, E. R. & Schwartz, J. H. (1985) *Principles of Neural Science* (Elsevier, New York).
12. Alberts, B., Bray, D., Lewis, J., Raff, M., Roberts, K. & Watson, J. D. (1989) *Molecular Biology of the Cell* (Garland, New York).
13. Bevan, S. & Raff, M. (1985) *Nature (London)* **315,** 229–232.
14. McVicar, B. A. (1984) *Science* **226,** 1345–1347.
15. Barres, B. A., Chun, L. L. Y. & Corey, D. P. (1989) *J. Neurosci.* **9,** 3169–3175.
16. McCarthy, K. D. & de Vellis, J. (1980) *J. Cell Biol.* **85,** 890–902.
17. Cole, R. & de Vellis, J. (1989) in *A Dissection and Tissue Culture Manual of the Nervous System*, eds. Shahar, A., de Vellis, J., Vernadakis, A. & Haber, B. (Liss, New York), pp. 121–133.
18. Saneto, R. P. & de Vellis, J. (1985) *Proc. Natl. Acad. Sci. USA* **82,** 3509–3513.
19. Rome, L. H., Bullock, P. N., Chiapelli, F., Cardwell, M. C., Adinolfi, A. M. & Swanson-Hayes, D. (1986) *J. Neurosci. Res.* **15,** 49–65.
20. Raff, M. C., Abney, E. R., Cohen, J., Lindsay, R. & Noble, M. (1983) *Neuroscience* **19,** 29–41.
21. Syapin, P. J., Cole, R., de Vellis, J. & Noble, E. P. (1985) *J. Neurochem.* **45,** 1797–1801.
22. Llewellyn-Jones, F. (1966) *The Glow Discharge*, Methuen's Monographs on Physical Sciences (Wiley, New York).
23. Amstein, C. F. & Hartman, P. A. (1975) *J. Clin. Microbiol.* **2,** 46–54.
24. Stimers, J. R., Bezanilla, F. & Taylor, R. E. (1987) *J. Gen. Physiol.* **89,** 521–540.
25. Bezanilla, F. & Armstrong, C. (1977) *J. Gen. Physiol.* **70,** 549–566.
26. Lerea, L. S. & McCarthy, K. (1989) *Glia* **2,** 135–147.
27. Fox, A. P., Nowycky, M. C. & Tsien, R. W. (1987) *J. Physiol. (London)* **394,** 149–172.
28. McCarthy, K. D. & de Vellis, J. (1978) *J. Cyclic Nucleotide Res.* **4,** 15–26.
29. Wu, D. K., Morrison, R. S. & de Vellis, J. (1985) *J. Cell. Physiol.* **122,** 73–80.
30. MacVicar, B. A. & Tse, F. W. Y. (1988) *Glia* **1,** 359–365.
31. Arenander, A. T., de Vellis, J. & Herschman, H. R. (1989) *J. Neurosci. Res.* **24,** 107–114.
32. Arenander, A. T., Lim, R. W., Varnum, B. C., Cole, R., de Vellis, J. & Herschman, H. R. (1989) *J. Neurosci. Res.* **23,** 247–256.
33. Shapiro, D. L. (1973) *Nature (London)* **241,** 203–204.
34. Pollenz, R. S. & McCarthy, K. D. (1986) *J. Neurochem.* **47,** 9–17.
35. Waltz, W. & McVicar, B. (1988) *Brain Res.* **443,** 321–324.