augmentation of GS activity may be due to an increase per astroglial cell, since it has been reported that the presence of nerve cells inhibits proliferation of astrocytes (Hatten, 1985; Nagata et al., 1986). Furthermore, the activity of GS in forebrain astrocytes was about 2.5-fold greater than in the cerebellar astrocytes (Table 1). This is consistent with our previous findings that the biological properties of forebrain astrocytes differ markedly from those of cerebellar astrocytes (Patel & Hunt, 1985; Patel, 1986). When the astroglial cells were grown in the conditioned medium obtained from forebrain nerve cell cultures, the activity of GS was markedly increased in both forebrain and cerebellar astrocytes (Table 1). However, the magnitude of the effect was lower in the former than in the latter, indicating marked differences in the quantitative responses to the trophic factor of astrocytes derived from either the forebrain or the cerebellum. Similar regional differences were also observed in the induction of GS by glucocorticoids, both in vivo and in vitro (Patel et al., 1983b; Patel & Hunt, 1985).

Our findings that neuronal cells induce astrocytes to become stellate-shaped and express GS activity in vitro may suggest that during brain development neurons play a role in the differentiation of astrocytes. The results would also indicate that the marked regional variations in the properties of astrocytes may relate to the differences in the cholinergic regions of embryonic brain and grown in the astrocyte-conditioned medium, the activity of ChAT was not divide in vitro, the observed augmentation of ChAT activity would represent the increase per cholinergic cell. Furthermore, in the cultures of dissociated cells derived from the cholinergic regions of embryonic brain and grown in the astrocyte-conditioned medium, the activity of ChAT was 100% greater than in the cell cultures grown in the control medium specific to the cell type. In co-culture experiments, the medium of 7-day-old astrocyte cultures was changed to a chemically defined medium, and three days later the dissociated subcortical cells, derived from the 17-day-old rat embryos, were plated on top of this sheet of astroglial cells. The cholinergic cells, cultured for ten days in vitro under different experimental conditions, were washed and homogenized in 50 mm-sodium phosphate buffer, pH 7.4. Whole homogenate was used for the estimation of ChAT activity and protein (Patel et al., 1987). Enzyme activity was expressed as amount of product formed/h per 60 mm dish or per mg of protein. The characterization of various cell types present in the culture was made by immunocytochemistry or by histochemistry (see Hayashi & Patel, 1987).

The co-culturing of subcortical cholinergic neurons with astrocytes enhanced the expression of ChAT activity, and in comparison with the cells cultured in the absence of astrocytes the increase was about threefold (Table 1). As the astroglial cells were devoid of ChAT activity and neurons do not divide in vitro, the observed augmentation of ChAT activity would represent the increase per cholinergic cell. Furthermore, in the cultures of dissociated cells derived from the cholinergic regions of embryonic brain and grown in the astrocyte-conditioned medium, the activity of ChAT was 100% greater than in the cell cultures grown in the control medium specific to the cell type.

Production of astrocytes of a trophic factor for cholinergic neurons

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In the central nervous system, astrocytes have been implicated in the control of migration and clonage of neurons during development in vivo, and in the survival and emission of neuritic outgrowths of nerve cells in vitro (Rakic, 1972; Lindsay, 1979; Rudge et al., 1985; Hatten & Mason, 1986; Lindner et al., 1986). However, rather little is known about the influence of astrocytes on the development of a defined population of nerve cells. Recently, we have reported (Hayashi & Patel, 1987) the procedures for obtaining, from the septal-diagonal band region of embryonic rat brain, neuronal cultures relatively enriched in cholinergic cells (see also, Hefti et al., 1985). In initial experiments during the development of this culture system, when the dissociated cells were plated at low density in a medium containing 10% (v/v) fetal calf serum (Patel et al., 1982), a close association was detected between astrocytes and nerve cells, including cholinergic neurons (Sensenbrenner & Mandel, 1974; Denis-Donini et al., 1984). Furthermore, we attempted to remove contaminating non-neuronal cells from these cultures grown in serum-containing medium, by treatment with different doses of cytosine arabinoside for various lengths of time. In some of these experiments, the metabolic status of astrocytes and cholinergic neurons were monitored in terms of the activity of glutamine synthetase and of choline acetyltransferase (ChAT), respectively. In our hands, the concentration of 10 μM-cytosine arabinoside normally used (Hefti et al., 1985) killed both glial and neuronal cells. However, under the conditions which removed astroglial cells partially but in varying proportions, a significant correlation was observed

Abbreviations used: ChAT, choline acetyltransferase; NGF, nerve growth factor.
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medium (Table 1). Similar effects were not detected when cerebellar granule cell-conditioned medium replaced the astrocyte-conditioned medium (Table 1). These observations indicated that astrocytes produce a trophic factor which is involved in the regulation of cholinergic cells. However, a separate direct effect mediated by cell-surface molecules of astrocytes in the control of cholinergic cell differentiation cannot be ruled out. In addition, using peripheral neurons, it has been shown that the neurotrophic factor produced by astrocytes contains a functionally-active nerve growth factor (NGF)-like molecule whose activity can be curtailed by anti-NGF serum (Eбедал & Jacobson, 1975; Lindsay, 1979).

Recent observations have shown significant increases in ChAT activity influencing cholinergic cell differentiation does not appear to be related to an NGF-like compound.


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Development of an enzyme assay for the measurement of calmodulin-dependent phosphatase in brain tissue

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Abbreviations used: CaM, calmodulin; TFP, trifluoperazine; BPase, basal phosphatase; BPase + CaM, basal phosphatase plus CaM; DIECA, diethylthiocarbamic acid.

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