Neurochemical Research, Vol. 14, No. 7, 1989, pp. 693-700

Effect of Histamine on the Development of Astroglial Cells in Culture

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(Accepted May 15, 1989)

The effect of histamine on different aspects of the growth of astrocytes was studied using primary cultures derived either from forebrain or from cerebellum of the rat. The influence on general growth and differentiation was monitored in terms of the activities of ornithine decarboxylase and glutamine synthetase enzymes, whereas [^{3}H]thymidine incorporation into DNA was used as a specific index of cell proliferation. Treatment with 500 nM histamine of cells grown for 6 days in vitro, caused a time-dependent significant increase in ornithine decarboxylase activity of astrocytes from both sources. The maximum increase was observed at 4 h after histamine treatment, at that time the elevation in ornithine decarboxylase activity being about 80% and 300% over control values in the forebrain and the cerebellar astrocytes, respectively. Under similar experimental conditions, addition of histamine (500 nM) to medium resulted in a significant increase in [^{3}H]thymidine incorporation into DNA in both types of cultures: in comparison with control, the elevation was about 45% at 48 h in forebrain astrocytes and at 24 h in cerebellar astrocytes. On the other hand, the specific activity of glutamine synthetase in cerebellar astrocytes was markedly enhanced (about 100%) by treatment with histamine (500 nM) for 4 days, but forebrain astrocytes were little affected. Addition of histamine to the culture medium produced no significant alteration in the activity of lactate dehydrogenase and protein content of either type of astroglial cells. The present findings, which support our earlier proposal that the biochemical properties of astrocytes differ between various brain regions, provide direct evidence for the involvement of histamine in the regulation of growth and development of astrocytes.

KEY WORDS: Astrocyte cultures; development; histamine; ornithine decarboxylase; glutamine synthetase; astrocyte proliferation.

INTRODUCTION

A growing body of evidence suggest that biogenic amines and other substances, which in the adult brain function as neurotransmitters, may also act as neurohumoural agents, involved in the regulation of the early development and of cell proliferation (see 1). In this respect it is not surprising that in recent years the physiological role played by histamine in the maturation of brain has attracted considerable interest. Involvement of histamine in the regulation of cell division and other developmental processes has been well documented in non-neural tissues (2-5). In the rat brain, histamine is present in two different cell types, namely neurons and mast cells (6-9). The neuronal histamine is widely ac-

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0364-3190/89/0700-0693$06.00/0

cepted to play a neurotransmitter role in the mammalian central nervous system (10,11). On the other hand, our knowledge about the specific functions carried out by histamine present in brain mast cells is very limited. The fact that brain histamine levels, including those of mast cells, are transiently very high in the neonate (6-9) when many critical developmental changes are occurring, suggests a role of this amine in brain development. Consistent with this proposal, it has been shown that intracerebroventricular administration of histamine or of compounds that release mast cell histamine increase ornithine decarboxylase (ODC) activity in the brain of the neonatal rats (12,13). ODC is an important enzyme in the biosynthesis of polyamines, which play a role in nucleic acid and protein synthesis, and appears to be closely associated with both cellular growth and differentiation (14-16). However, the ODC enzyme is localized in all cells of the brain and this precludes the assignment of the effect of histamine to a specific cell type.

It is now possible to obtain a relatively pure culture of astroglial cells from neonatal rats (see 17). Furthermore, glutamine synthetase (GS), which catalyzes the formation of glutamine from glutamate and ammonia in an ATP-dependent reaction, is mainly localized to astrocytes in the brain (18-20). The enzyme is involved in the detoxification of ammonia and in the processing of amino acid neurotransmitters (20,21). During development the major increase in GS activity is associated with differentiation rather than proliferation of astroglial cells (22,23). The differentiation of astrocytes measured in terms of GS activity and other biochemical properties are very similar in vitro and in vivo (see 24). Also, unlike nerve cells, astrocytes are able to multiply in vitro (17). Thus more precise information on the overall growth of a specific brain cell type can be derived using a primary culture of astrocytes.

In the present study, we have examined the effects of histamine on the growth and differentiation of two types of cultured astrocytes, derived from newborn forebrain or from either newborn or 8-day old cerebellum, in terms of activities of ODC and GS, while [^{3}H]thymidine incorporation into DNA was used as a direct measure of cell division.

EXPERIMENTAL PROCEDURE

Materials. Culture media and heat-inactivated foetal calf serum were purchased from Flow Laboratories, Irvine or Imperial Laboratories, Salisbury. L-[1-^{14}C]glutamic acid (specific radioactivity 58 mCi/mmol), [6-^{3}H]thymidine (specific radioactivity 2 Ci/mmol) and DL-[1-^{14}C]ornithine hydrochloride (specific radioactivity 61 mCi/mmol) were obtained from Amersham International, Amersham. Other materials and chemicals used were of the highest quality and purchased from the sources mentioned in previous publications (16, 21, 24).

Astrocyte Cultures. Primary cultures enriched in astroglial cells were obtained by plating dissociated cells, isolated from newborn forebrain or from either newborn or 8-day old cerebellum of rats, at a density of either 2×10^6 cells per 35 mm or 6×10^6 cells per 60 mm diameter plastic dishes as described by Patel and Hunt (17). The culture medium contained basal Eagle's medium supplemented with 10% heat-inactivated foetal calf serum, 50 U/ml penicillin and 50 μg/ml streptomycin. The culture dishes were incubated at 37° C in 5% CO_2 in air saturated with water and the medium was changed every 3-4 days. Both forebrain and cerebellar astroglial cultures used in the present study contained more than 95% of cells positive for glial fibrillary acidic protein (17).

Biochemical Procedures. In the experimental groups, the cultures grown for 6 days in vitro (DIV) were treated with different concentrations of histamine (between 1 nM and 250 μM) for various lengths of time (1 h to 4 days) as indicated in the text or on the Tables or Figures. At the end of the experimental period, the cells were washed three times with prewarmed phosphate-buffered saline (PBS) and once with saline at 37° C. Finally, the cells were scraped into 0.01 M imidazole buffer, pH 7.4 at 0° C, with a plastic scraper and homogenized using an all glass homogenizer. Whole homogenate was used for the estimation of ODC (EC 4.1.1.17), GS (EC 6.3.1.2) and lactate dehydrogenase (LDH; EC 1.1.1.27) activities and protein concentration as described previously (25). The results are expressed as enzyme activity per mg protein or per culture dish.

Incorporation of [^{3}H]thymidine into DNA. The astrocyte cultures, grown for 6 DIV in 35 mm diameter dishes were used for the estimation of DNA labelling as described by Patel and Hunt (17). Briefly, the cells treated with different concentrations of histamine were exposed to 0.5 μCi of [6-^{3}H]thymidine (2 nmol) per ml medium. At different times, the medium was removed and used for radioactivity measurement. The cells were washed three times with prewarmed PBS, harvested in 1 ml of the same solution and homogenized at 0° C. To an aliquot of cell homogenate, an equal volume of 0.4 M perchloric acid (PCA) was added. The resultant solution was filtered onto a Whatman GF/C disk using a Millipore filtering system. The disks were each washed twice with 5 ml of 0.2 M PCA, and transferred to glass scintillation vials containing 0.5 ml of 1 M PCA. The vials were then incubated at 80° C for 20 min to elute the [^{3}H]DNA from the disks. After cooling the vials to room temperature, 0.5 ml of water and 10 ml of Triton-based scintillation fluid were added. The vials were mixed and the radioactivity was determined with a Nuclear Chicago Scintillation Spectrometer Mark III. The [^{3}H]thymidine remaining in the medium was estimated by adding 50 μl of the medium to a counting vial with 10 ml of scintillant. The [^{3}H]thymidine incorporation results are expressed as d.p.m. per mg of protein divided by d.p.m. per μl in extracellular medium.

Statistical Analysis. The results were analyzed either by Student's *t* test or by analysis of variance (ANOVA) followed by Duncan's multiple comparison. The significance of the differences between the mean values of different groups was calculated in the appropriate way ($P < 0.05$).

RESULTS

In primary cultures of astroglial cells, derived from newborn forebrain or from either newborn or 8-day old cerebellum, the number of cells increases more or less

linearly during the second week in vitro and as expected this is accompanied by a large amount of [^{3}H]thymidine incorporation into DNA (17). Therefore, in the present study we have used cells grown for 6 DIV to investigate the effect of histamine on the growth and differentiation of astrocytes.

Effect of Histamine on Morphological Features of Astrocytes. The morphological appearance of both forebrain and cerebellar astrocytes in primary cultures have already been described in detail (see 17). The present study confirms these observations by showing that the cultures contained mainly polygonal epitheloid cells (Figure 1). At an early age, a small number of cells had fibrous branched processes, but their proportion decreased dramatically during development (17, 26). In comparison with controls, treatment with histamine for 4 days had no visible effect on the morphological appearance of cerebellar astrocytes (Figure 1). Neither did treatment with histamine have any significant effect on the appearance of cultured forebrain astroglial cells (not shown).

Effect of Histamine on Ornithine Decarboxylase Activity in Primary Cultures of Astrocytes. Preliminary experiments carried out to scan the influence of treatment with different concentrations of histamine for various lengths of time indicated that the amine may have a regulatory effect on the activity of ODC in astroglial cells. The dose which produced the largest effect appeared to be 500 nM histamine, and a dose 20 times greater than this failed to increase ODC activity appreciably further. Moreover, as the astrocyte cultures derived from either newborn or 8-day old cerebellum showed a similar response to histamine (see also 17), the more detailed experiments were carried out on cultured astroglial cells derived from 8-day old cerebellum only (data not shown).

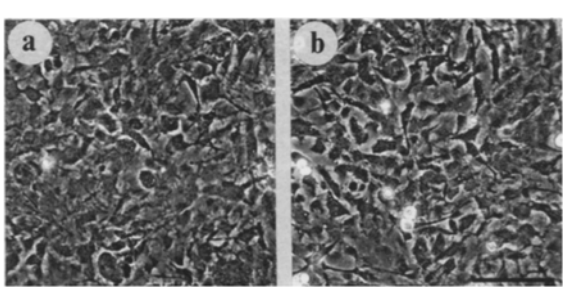

Fig. 1. Appearance in phase contrast of primary cultures of astrocytes derived from 8-day old cerebellum. The 10 DIV astrocytes (a) controls and (b) treated for the last 4 days with 500 nM histamine. Bar = 100 μm.

The 500 nM concentration of histamine was selected to study the time-course of increase in the activity of ODC in astrocytes grown for 6 DIV (Figures 2 and 3). The results showed that the effect of histamine on ODC activity in both forebrain and cerebellar astrocytes was time-dependent. In forebrain astrocytes, after an apparent reduction at 1 h, treatment with histamine resulted in a significant increase in ODC activity starting from 2 h onward (Figure 2). The maximal effect, about 80% over control values, was observed at 4 h after histamine treatment. The activity of ODC remained at a statistically significant elevated level up to 8 h and decreased thereafter, reaching more or less the control level by 24 h (Figure 2). Like forebrain astrocytes, treatment of cerebellar astrocytes with histamine also elicited a time-dependent marked increase in the activity of ODC (Fig-

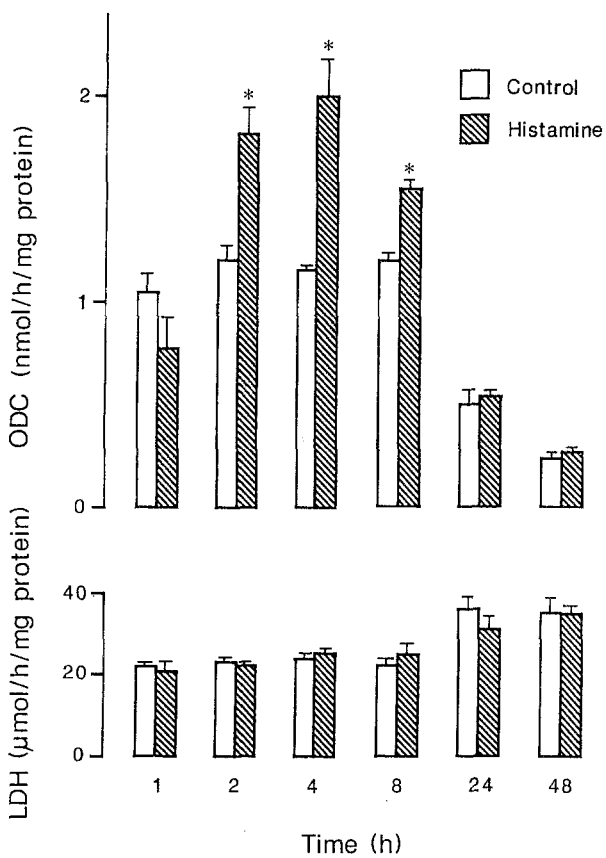

Fig. 2. Time-course of effect of histamine on ornithine decarboxylase (ODC) and lactate dehydrogenase (LDH) activities of primary cultures of forebrain astrocytes. The cells grown for 6 DIV were treated with 500 nM histamine and at the indicated times the activities of ODC and LDH were estimated using whole homogenate. Each column is the mean of 2-3 experiments with SEM indicated by the bar. Significant differences ($P<0.05$) between mean values of the control and experimental groups are indicated by asterisks.

ure 3). Moreover, once again the maximum effect was observed at 4 h after histamine treatment. However, in comparison with forebrain astrocytes, the effect on cerebellar astrocytes was much greater (about 300%) at 4 h, and decreased more rapidly reaching the control level by 8 h after histamine treatment (Figure 3). In contrast to ODC activity, in both types of astroglial cell cultures the treatment with histamine had no significant effect either on LDH activity (Figures 2 and 3) or on protein concentration (data not shown).

Effect of Histamine on [^{3}H]Thymidine Incorporation into DNA. In order to determine whether the increase in ODC activity caused by histamine is associated with cell division, we have measured the effect of different concentrations of the amine on [^{3}H]thymidine incorporation into DNA (17). In both types of cultured astrocytes, derived from either forebrain or cerebellum, treatment with histamine increased the DNA labelling (Tables I and II). However, like ODC activity, the time-course of histamine effects differed in forebrain astrocytes from cerebellar astroglial cells. In astrocytes derived from forebrain, the augmentation in [^{3}H]thymidine incorporation into DNA was first seen at 24 h and increased further reaching between 45 and 70% over control values at 48 h, depending on the concentration of histamine used (Table I). The effect of histamine on the cerebellar astrocytes was similar but observed at earlier time points than on the forebrain astrocytes (Table II). In cerebellar cells, the first significant increase in DNA labelling was observed at 8 h after treatment with 10 μM histamine and the maximum effect (about 40% over con-

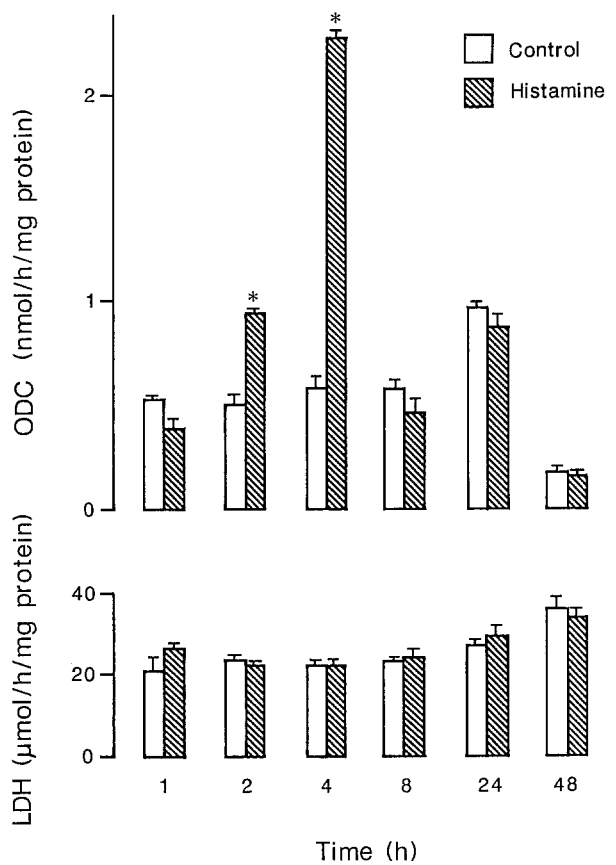

Fig. 3. Time-course of effect of histamine on ornithine decarboxylase (ODC) and lactate dehydrogenase (LDH) activities of primary cultures of cerebellar astrocytes. The cells grown for 6 DIV were treated with 500 nM histamine and at the indicated times the activities of ODC and LDH were estimated using whole homogenate. Each column is the mean of 2-3 experiments with SEM indicated by the bar. Significant differences ($P<0.05$) between mean values of the control and experimental groups are indicated by asterisks.

Table I. Effect of Histamine on [^{3}H]Thymidine Incorporation into DNA in Primary cultures of Forebrain Astrocytes

Histamine concentration	Thymidine incorporation			
	4 h	8 h	24 h	48 h
0 (Control)	54± 3(3)	209± 8(3)	522±10 (3)	581±55(2)
10 nM	67±10(3)	206±14(3)	642±33(3)*	856±38(3)*
500 nM	76± 2(3)	238±24(3)	504±22(2)	843±40(2)*
10 μM	75± 6(3)	174±12(3)	600±11(3)	990±14(3)*

The primary cultures of astrocytes derived from the forebrain of newborn rats were exposed to different concentrations of histamine and [6-^{3}H]thymidine (0.5 μCi and 2 nmol per ml medium) at 6 DIV. At indicated times the cells were harvested and the whole homogenate was used for the estimation of [^{3}H]thymidine incorporation into DNA as described in Experimental Procedure. The results are expressed as dpm per mg protein/dpm per μl of medium. The values are means ± SEM. The number of observations in each group is given in parentheses. Significant differences between mean values of the control and experimental groups ($P < 0.01$) are indicated by asterisks.

Table II. Effect of Histamine on [^{3}H]Thymidine Incorporation into DNA in Primary Cultures of Cerebellar Astrocytes

Histamine concentration	Thymidine incorporation			
	4 h	8 h	24 h	48 h
0 (Control)	21 ± 1(3)	37 ± 1(3)	257 ± 17(3)	410 ± 3(3)
10 nM	18 ± 1(3)	40 ± 2(3)	283 ± 19(3)	345 ± 23(3)
500 nM	17 ± 1(3)	40 ± 1(2)	359 ± 14(3)*	403 ± 16(2)
10 μM	18 ± 1(3)	44 ± 1(3)*	339 ± 8(3)*	335 ± 25(3)

The primary cultures of astrocytes derived from the cerebellum of 8-day old rats were exposed to different concentrations of histamine and [6-^{3}H]thymidine (0.5 μCi and 2 nmol per ml medium) at 6 DIV. At indicated times [^{3}H]thymidine incorporation into DNA was estimated as described in Experimental Procedure and Table 1. The results are expressed as dpm per mg protein/dpm per μl medium. The values are means ± SEM. The number of observations in each group is given in parentheses. Significant differences between mean values of the control and experimental groups ($P<0.05$) are indicated by asterisks.

trol value) was found at 24 h. The results would indicate that the cerebellar astrocytes may be more receptive to histamine than forebrain astrocytes, and in both types of cells a close association may exist between the changes in the activity of ODC on the one hand and DNA labelling on the other.

Effect of Histamine on Glutamine Synthetase Activity. The activity of GS was used as a marker to examine the effect of histamine on the differentiation of astroglial cells (17). In these experiments the cultured astrocytes, derived from either forebrain or cerebellum, were treated with different concentrations of histamine at 6 DIV and the activity of GS was measured at 10 DIV (Tables III and IV). In control cultures, the specific activity of GS in astrocytes derived from cerebellum was much lower than those derived from forebrain (17). The effect of histamine was selective: only in the cerebellar astrocytes did treatment with this amine result in a dose-dependent increase in the specific activity of GS (Table III). In comparison with control values, the elevation in GS activity was about 65% at 500 nM and 130% at 250 μM histamine, while the increases at lower than 500 nM concentrations of histamine failed to reach statistically significant levels (Table III). In forebrain astrocytes, by comparison with cerebellar astrocytes, the activity of GS was not significantly altered after treatment with histamine (Table IV). Moreover, treatment with higher concentrations of histamine, for a longer period than used in the ODC experiments, also had little effects on the LDH activity in both types of cultured astrocytes (Tables III and IV). The results would once again indicate marked differences in the biochemical characteristics between the forebrain astrocytes and the cerebellar astroglial cells (see also 24).

Table III. Effect of Histamine on Activity of Glutamine Synthetase (GS) and Lactate Dehydrogenase (LDH) in Cultured Cerebellar Astrocytes

Histamine concentration	Number of observation	LDH (μmol/h/60 mm dish)	GS (nmol/h/mg protein)
0 (Control)	5	43 ± 4	132 ± 19
1 nM	6	45 ± 5	170 ± 23
10 nM	6	41 ± 4	183 ± 20
100 nM	3	49 ± 2	220 ± 35
500 nM	6	43 ± 5	262 ± 22*
1 μM	3	45 ± 5	276 ± 23*
250 μM	3	31 ± 4	301 ± 51*

Primary cultures of cerebellar astrocytes were exposed to the indicated concentrations of histamine at 6 DIV. At 10 DIV the activity of LDH and GS were estimated using whole homogenate. The values are means ± SEM. Significant differences between mean values of the control and experimental groups ($P<0.01$) are indicated by asterisks.

Table IV. Effect of Histamine on the Activity of Glutamine Synthetase (GS) and Lactate Dehydrogenase (LDH) in Cultured Forebrain Astrocytes

Histamine concentration	LDH (μmol/h/60 mm dish)	GS (nmol/h/mg protein)
0 (Control)	39 ± 4	3337 ± 200
10 nM	37 ± 4	3350 ± 124
50 nM	47 ± 3	3156 ± 37
200 nM	44 ± 1	2546 ± 282
500 nM	36 ± 6	3230 ± 33
1 μM	39 ± 3	3089 ± 50
100 μM	37 ± 3	3198 ± 143

Primary cultures of forebrain astrocytes were treated with the indicated concentrations of histamine at 6 DIV. At 10 DIV the activities of LDH and GS were estimated using whole homogenate. The results are mean of two observations ± variation between two values.

DISCUSSION

The heterogeneity of brain tissue presents major difficulties in the evaluation of experimental findings at the cellular level. One way to overcome this problem is to develop a culture system containing predominantly a single class of neural cells. In recent years we have successfully used the tissue culture technique to investigate the effects of metabolic, hormonal and neurotrophic factors on the development and biochemical characteristics of astroglial cells (17, 20, 25, 27). Of course the tissue culture technique, like any other in vitro method, has considerable drawbacks, but valuable information can be obtained as long as one is aware of the methodological limitations.

The present results are consistent with the previous in vivo studies showing a marked increase in the activity of ODC after intraventricular administration of histamine to neonatal rats (12, 13). ODC catalyzes the formation of putrescine from ornithine, the initial and important reaction in the polyamine biosynthetic pathway (14-16). The activity of ODC is high during periods of normal, neoplastic or regenerative growth and decreases when these processes cease (15, 16). In the developing rat brain, ODC activity reaches a maximal level around birth and then falls gradually to low adult values after the second postnatal week (28-30). However, the changes in ODC activity are not uniform throughout the brain, but each part of the brain has its own characteristic profile for enzyme activity which appears to follow closely the region specific pattern of cell proliferation. For example, in brain parts such as brainstem and midbrain, which mature early, the maximal activity of ODC is

observed before birth, whereas in late maturing regions such as cerebellum, the peak for ODC activity is observed after birth (28, 30). The present findings would suggest that the previously observed effect of histamine on whole brain in vivo (12, 13) may relate to the effect of the amine on astroglial cells (Figures 1 and 2). The histamine mediated elevation in the activity of ODC was closely followed by a significant rise in astroglial cell proliferation, measured in terms of [^{3}H]thymidine incorporation into DNA (Tables I and II). In the forebrain astrocytes, the ODC activity remained induced for at least 6 h (from 2 to 8 h) followed by a maximal [^{3}H]thymidine incorporation into DNA at 48 h (Figure 1; Table I). On the other hand, in the cerebellar astrocytes both the decay in histamine mediated increase in ODC activity and the subsequent rise in DNA labeling were earlier than in the forebrain astrocytes (compare Figure 1 with 2 and Table I with II).

In comparison with forebrain astrocytes or with DNA labeling, a relatively large increase in the activity of ODC was observed after histamine treatment in cerebellar astrocytes (Figures 1 and 2; Tables I and II). This may relate to the fact that the activity of ODC is associated with not only mitotic but also with non-mitotic growth, such as axonal regeneration and survival and differentiation of neural cells (31-35). After histamine treatment a selective elevation in the activity of GS, a reasonable marker for astrocyte differentiation (17, 22, 23), in cerebellar astrocytes (Table III), is consistent with the above proposal. It has been reported that histamine increases the level of cyclic AMP and induces the breakdown of glycogen in astrocyte-rich cultures, derived from perinatal mouse or rat brain (36-38). However, no significant alterations in the morphological appearance (Figure 1), the increase in DNA labeling (Tables I and II) and a selective elevation in GS activity (Table III) would preclude the possibility that the present effects of histamine are mediated by an increase in cyclic AMP. Moreover, our more recent preliminary results would indicate that the effect of histamine may be relatively specific to astrocytes, since the amine failed to induce the activity of ODC, of choline acetyltransferase or of glutamate decarboxylase in the neuronal cultures containing subcortical cholinergic and GABAergic nerve cells (39; J. Rodriguez, A. Hunt, I. Blanco and A. J. Patel, unpublished observations). In the more recent years, the presence of H_1 and H_2 histamine receptors has been demonstrated on the cultured astrocytes (40). It would be, therefore, interesting to derive further information on the effects of histamine receptor agonists and antagonists on the biochemical development of astroglial cells.

There is a growing mass of information suggesting that astrocytes in different parts of the brain have distinct morphological (17, 26, 41) and biochemical properties (17, 23-26, 42-46), which include differences in the concentration of free amino acids, uptake of neurotransmitter compounds, amount of glial fibrillary acidic protein and activities of GS and ODC (also see Figures 1 and 2; Tables III and IV). Recently Hayashi et al (27) proposed that one of the factors contributing to the differences in the properties of astrocytes may relate to varying requirements of astroglial cells during differentiation for trophic substances produced by nerve cells. In this context, our observation that histamine induced the activity of GS in cerebellar astrocytes (Table III) and failed to do so in forebrain astrocytes (Table IV), provides further support for the existence of astrocytes with different biochemical properties. Moreover, since the major increase in GS activity is mainly associated with the differentiation of astroglial cells, the marked elevation in the activity of this enzyme in cerebellar astrocytes after treatment with histamine (Table III) would suggest that, under present experimental conditions, histamine has a role in the maturation of at least some of the astrocytes, though precise mechanisms remain to be determined.

In conclusion, the present findings provide direct evidence for the involvement of histamine in the regulation of certain processes related to growth and differentiation of astroglial cells. Moreover, the observed marked variations in the effects of histamine between forebrain and cerebellar astrocytes give further support to our proposal of differing biochemical properties of astrocytes in the brain.

ACKNOWLEDGMENTS

J.R. and J.M. gratefully acknowledge the award of the University Scholarship and of the British Council Scholarship, respectively. We thank Dr. M.S.G. Clark for helpful discussions. A part of this work was supported by grant DGICYT PB86-0355 and by Beechams Pharmaceuticals.

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