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ENZYME ACTIVITIES OF MONOAMINE OXIDASE, CATHECHOL-*O*-METHYLTRANSFERASE AND γ-AMINOUBUTYRIC ACID TRANSAMINASE IN PRIMARY ASTROGLIAL CULTURES AND ADULT RAT BRAIN FROM DIFFERENT BRAIN REGIONS

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The activities of monoamine oxidase (MAO), cathechol-*O*-methyltransferase (COMT) and γ-aminobutyric acid transaminase (GABA-T) were measured in primary cultures from newborn rat cultivated from 6 different brain regions. These primary cultures contained mostly astroglial cells, evaluated by the presence of the glial fibrillary acidic protein (GFAp, α-albumin) and the S-100 protein. The enzyme activities in the corresponding brain areas from adult rat were also quantified. MAO activities were on the same level in 14-day old cultures and in adult rat brain homogenates, with significantly lower values in brain stem as compared to the other brain regions examined. COMT activities were on a higher level in the cultures than in adult rat brain homogenates. Astroglial cells from hippocampus were found to have the highest and those from brain stem the lowest COMT-activities. GABA-T activities were lower in the cultures than in adult rat homogenates. No significant differences were seen in the various astroglial cultures. Accumulation of [^{3}H]dopamine and [^{3}H]γ-aminobutyric acid (GABA) visualized by autoradiography showed only a slight uptake of dopamine in comparison with the uptake of GABA. It is concluded that astroglial cells in culture have enzymatic properties similar to those of astroglial cells in different brain regions of adult rat brain. Studies are in progress to evaluate if the regional heterogeneity observed

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among cultivated astroglial cells is affected by in vivo differentiation until cultivation and/or time in culture.

INTRODUCTION

There is a growing evidence that astroglial cells in different brain areas have distinct biochemical and physiological properties (19, 37). One model suitable for such studies is primary cultures from the nervous system, as such cultures provide the option of choosing tissue sources of the desired species, region and developmental age. The source material will have acquired specific properties, depending on its in vivo differentiation until cultivation. Several approaches have been attempted to cultivate nervous tissue material. Until now primary cultures containing astroglial cells from one large brain area (mostly hemispheres), or in some cases from whole brain, have been made (2, 9, 31, 32, 35, 39) even if cultures from anatomically different regions of the nervous system have appeared (cerebellum: 4, 27, 38; brain stem and spinal cord: 25).

It would be important to cultivate astroglial cells from different brain regions to evaluate the presumably different characteristics of the cells. Such attempts have in fact already been initiated (6) even if little attention has been paid to the cellular composition of the different cultures including growth-rate and degree of differentiation of the cells.

We cultivated primary cultures from cerebral cortex, striatum, hippocampus, brain stem and cerebellum. The cellular characterization is as ever important when working with cultures and from our earlier studies concerning primary cultures from newborn rat cerebral hemispheres (13, 14) the same methods are under consideration.

This report concerns the biochemical characterization—enzyme activity of MAO, COMT and GABA-T in the different cultures, compared with homogenates from the corresponding brain areas of adult rat, and the uptake of [^{3}H]dopamine and [^{3}H]GABA, visualized by autoradiography. The immunohistochemical localization of the astroglial enriched proteins glial fibrillar acidic protein (GFAp; α-albumin) and S-100 was also made.

EXPERIMENTAL PROCEDURE

Tissue Culturing. The primary cultures were made from newborn rat (Sprague-Dawley strain, Anticimex, Sweden) cerebral cortex, striatum, hippocampus, brain stem (under the IVth ventricle) and cerebellum (11) or from cerebral hemispheres (2, 13, 14, 39). They were cultivated in Eagle's minimum essential medium (MEM, Flow laboratories Ltd., Scotland), supplied with extra substances to make up the following final composition: double concen-

trations of amino acids, 2 mM glutamine, 7 mM glucose, and quadruple concentrations of vitamins. Penicillin (250.000 IU/liter), 0.5 per cent streptomycin and 20 per cent (v/v) fetal calf-serum (GIBCO Bio Cult. Lab Ltd, UK) were added. The pH was set at 7.3. They were cultivated in plastic Petri dishes (NUNC A/S, Denmark) for 14 days in a humidified atmosphere with medium change three times a week.

Animals. Adult rats of the Sprague-Dawley strain were decapitated and the cerebral hemispheres, cerebral cortex, striatum, hippocampus, brain stem (under the IVth ventricle), and cerebellum were removed, immediately frozen on dry ice and stored at −80°C until used.

Protein Determinations. The cultures were scraped off in 500 µl PBS (0.15 M NaCl and 0.01 M KH_2PO_4, pH 7.8), frozen (−20°C), thawed and homogenized (glass/glass). Aliquotes of the homogenates were left for 24 h in 1 M NaOH at room-temperature. The rest of the material was centrifuged at 85,000 x*g* for 60 min. Protein determinations were done on the supernatants and on the homogenates, according to Lowry et al. (29).

Conjugation of Antisera and Preparation of Cultures for Immunofluorescence Microscopy for GFAp (α-albumin) and S-100 was performed as described in Hansson et al. (13, 14).

Uptake of Indian Ink for studying phagocytotic activity was done according to Romeis (36) as described by Hansson et al. (13).

Histochemical Method for Alkaline Phosphatase was done according to Burstone (3) as described by Hansson et al. (13).

Enzyme Determinations. The procedures of the enzyme determinations of MAO, COMT and GABA-T have been described in an earlier paper (16). The MAO determination is based on the method of Wurtman and Axelrod (45), the COMT determination on the method of Silberstein et al. (42) and Goldstein et al (7) and the GABA-T determination on the method of Hall and Kravitz (8) and Schousboe et al. (40). Protein content was determined by the method of Lowry et al. (29).

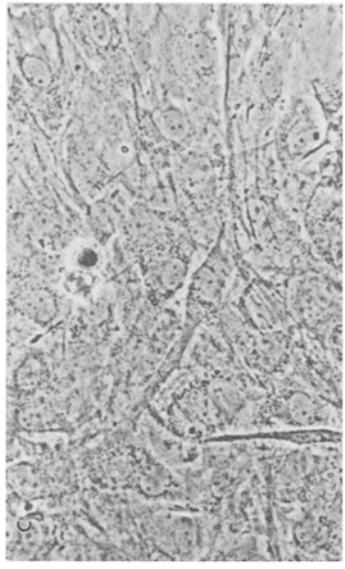
Uptake of GABA and Dopamine. The cultures were incubated in [^{3}H]GABA (NEN, Boston, Mass. USA) (35.1 Ci/mmol) or [^{3}H]dopamine (NEN) (25.8 Ci/mmol) in final concentrations of 10^{-6} M for 10 min according to Hansson et al. (12, 13).

RESULTS

Protein Amount in the Different Cultures. The amount of seeding material was chosen so that the six different cultures all became monolayers after 6–7 days. The amount of seeding material and the protein amount in 14 day old cultures are shown in Table I.

Cell Composition. Most cells in the different cultures were polygonal or spindle-shaped with an average diameter of ~16 µm. They stained with fluorescein isothiocyanate (FITC)-conjugated antibodies against S-100 and tetramethylrhodamine isothiocyanate (TRITC)-labelled antibodies against GFAp (α-albumin). These cells were considered to be astroglial cells (Figure 1 a,b) and constituted approximately 70 per cent of the cells in the different cultures.

Morphologically some macrophages (~10 per cent) were seen visualized by Indian ink uptake (Figure 1 c). These cells had a diameter of 6–8 µm and were phase bright. Cells containing alkaline phosphatases were considered endothelial-like (Figure 1 d) (2 per cent of the total cell number



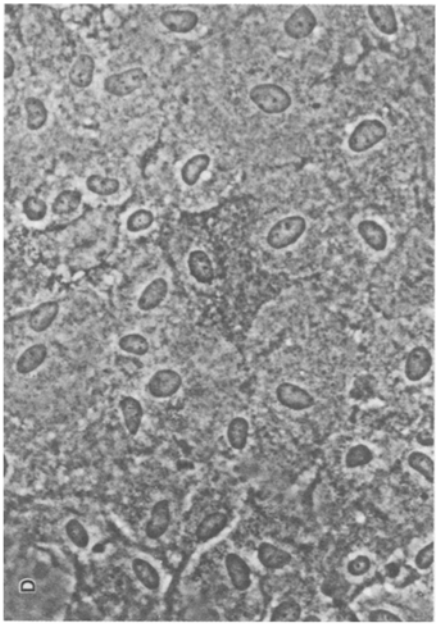


TABLE I

AMOUNT OF MATERIAL SEEDED INTO EACH PETRI DISH AND TOTAL AND SOLUBLE PROTEIN IN 14-DAY OLD PRIMARY CULTURES FROM CEREBRAL HEMISPHERES, CORTEX, STRIATUM, HIPPOCAMPUS, BRAIN-STEM, AND CEREBELLUM

Culture	mg material seeded into each Petri dish	mg total protein per Petri dish[a]	mg water-soluble protein per Petri dish[b]
Hemisphere	30.8 ± 3.6	0.56 ± 0.03	0.32 ± 0.01
Cortex	16.4 ± 0.4	0.56 ± 0.02	0.40 ± 0.01
Striatum	11.6 ± 1.3	0.49 ± 0.03	0.33 ± 0.04
Hippocampus	14.2 ± 0.6	0.39 ± 0.02	0.28 ± 0.02
Brain-stem	20.9 ± 1.5	0.39 ± 0.02	0.28 ± 0.02
Cerebellum	8.4 ± 0.9	0.35 ± 0.04	0.23 ± 0.01

Values are mean ± SEM from five experiments.

[a] Cultures were scraped off in 500 μl of PBS, homogenized (glass/glass), frozen (−20°C) and thawed. Aliquots of the homogenates were left for 24 hrs in 1 M NaOH at room temperature. Protein determinations were made according to Lowry et al. (1951).

[b] After homogenization and thawing (see *a*) the material was centrifuged at 105,000 *g* for 60 min. Protein determinations were made on the supernatants.

in the different cultures). Large flat cells (~30–40 μm in diameter) formed a partial monolayer upon which the other cells grew and were regarded mesenchymal-like. Neuronal-like cells could not be seen in the cultures.

MAO Activities. The enzyme activities of MAO (Figure 2) in the different 14-day old primary cultures and adult rat brain homogenates showed a level of about 1.0 nmol indoleacetic acid produced per mg protein per min. The activity in brain stem cultures was, however, at a lower level, as was the activity in brain stem homogenate of adult rat. The higher activity in striatum from the adult rat was not seen in the striatum cultures.

COMT Activities. Figure 3 shows the COMT-activity expressed as moles 4-hydroxy-3-methoxybenzoic acid (vanillic acid) per mg protein/min. Higher COMT levels were found in the cultures than in rat brain

FIG. 1. 14-day old rat cerebral cortical culture incubated using the direct method with both FITC-conjugated antiserum against S-100 (a) (X980) and TRITC-conjugated antiserum against GFAp (α-albumin) (b) (X980). Most cells are positively stained with both labelled antisera. c. 14-day old rat cerebral cortical culture incubated with Indian ink for 1 hr. Round or oval cells, 6–8 μm in diameter, with one central nucleus and a few processes accumulated the Indian Ink. (X650). d. Similar culture as above prepared for demonstrating alkaline phosphatase activity. A positive reaction (red reaction product) is seen in some polygonal flat cells. (X700).

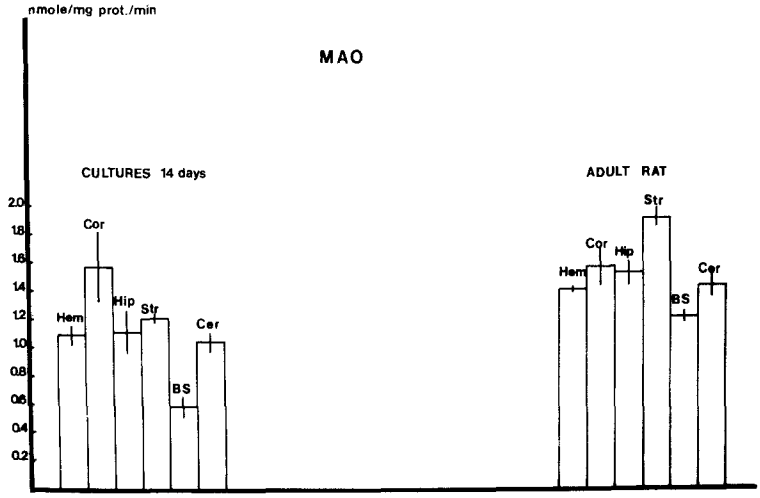


FIG. 2. Monoamine oxidase (MAO) activity expressed as nmol [^{14}C] indoleacetic acid/mg protein/min in primary astroglial cultures and adult rat brain from different regions. Values are mean +SEM of six experiments. Student's *t*-test.
$P < 0.05$ hippocampus vs. brain stem cultures; striatum vs. cerebellum cultures; hemisphere vs. striatum of adult rat; cortex vs. striatum of adult rat; cortex vs. brain stem of adult rat; brain stem vs. cerebellum of adult rat.
$P < 0.01$ cortex vs. brain stem cultures; brain stem vs. cerebellum cultures; hippocampus vs. striatum of adult rat; hippocampus vs. brain stem of adult rat; striatum vs. cerebellum of adult rat.
$P < 0.001$ hemisphere vs. brain stem cultures; striatum vs. brain stem cultures; striatum vs. brain stem of adult rat.

homogenates. Cultures from cerebral hemispheres, cerebral cortex and cerebellum expressed the same enzyme activity, while the hippocampal cultures had a much higher activity and striatum and brain stem cultures a lower enzyme activity.

In the adult rat whole hemispheres, hippocampus and cerebellum, showed a higher enzyme activity, compared to cortex, striatum and brain stem.

GABA-T Activities. The GABA-T activity expressed as nmole succinate formed per mg protein per min was on a similar level (3–4 nmol/mg protein/min) in the different primary cultures. The enzyme activity in the adult rat showed a three times higher activity than in the cultures. Homogenates from the different brain areas showed the same GABA-T activity except a somewhat higher activity in brain stem compared to cortex and hippocampus (Figure 4).

Uptake of GABA and Dopamine. A moderate accumulation of GABA was seen in the different cultures. The localization of grains was seen in

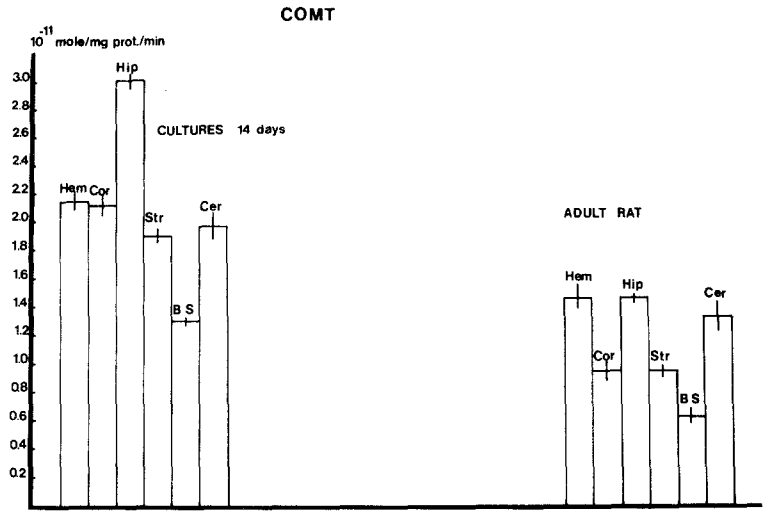


FIG. 3. Catechol-*O*-methyltransferase (COMT) activity expressed as 10^{-11} mol [^{14}C]4-hydroxy-3-methoxybensoic acid/mg protein/min in primary astroglial cultures and adult rat brain from different regions. Values are mean ± SEM of six experiments. Student's *t*-test.
$P < 0.05$ hemisphere vs. striatum cultures; cortex vs. striatum cultures; cortex vs. cerebellum of adult rat.
$P < 0.01$ cortex vs. brain stem of adult rat; striatum vs. cerebellum of adult rat.
$P < 0.001$ hemisphere vs. hippocampus cultures; hemisphere vs. brain stem cultures; cortex vs. hippocampus cultures; cortex vs. brain stem cultures; hippocampus vs. striatum culture; hippocampus vs. brain stem cultures; hippocampus vs. cerebellum cultures; striatum vs. brain stem cultures; brain stem vs. cerebellum cultures; hemisphere vs. cortex of adult rat; hemisphere vs. striatum of adult rat; hemisphere vs. brain stem of adult rat; cortex vs. hippocampus of adult rat; hippocampus vs. striatum of adult rat; hippocampus vs. brain stem of adult rat; striatum vs. brain stem of adult rat; brain stem vs. cerebellum of adult rat.

the astroglial-like cells and not in macrophages and mesenchymal-like cells (Figure 5).

The uptake of dopamine was very weak or none at all in the different cultures examined (Figure 6).

DISCUSSION

The cerebral hemisphere culture from newborn rat contains predominantly astroglial-like cells (1, 2, 13, 14, 39). Subpopulations of the hemisphere culture, i.e. cultures from the cerebral cortex, the striatum and the hippocampus, as well as cultures from brain stem and cerebellum,

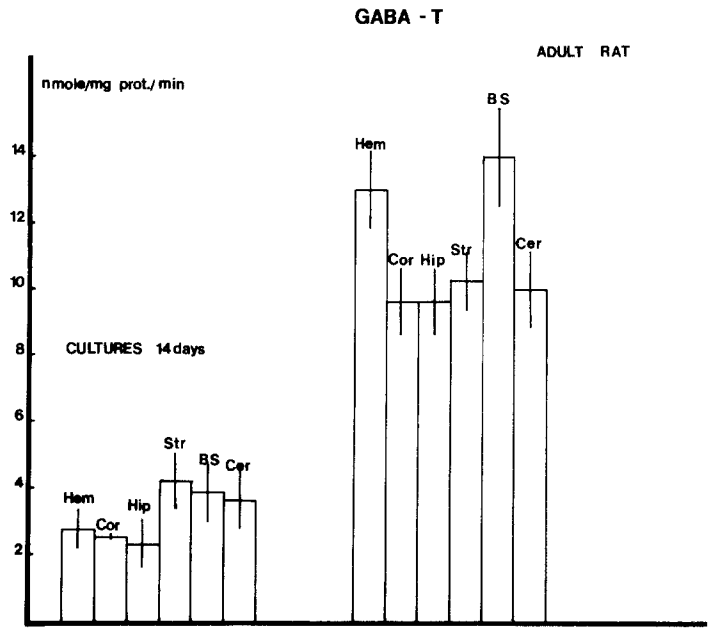


FIG. 4. γ-Aminobutyric acid transaminase (GABA-T) activity expressed as nmol [^{14}C]succinate/mg protein/min in primary astroglial cultures and adult rat brain from different regions. Values are mean ± SEM of six experiments. Student's *t*-test.
$P < 0.05$ cortex vs. brain stem of adult rat; hippocampus vs. brain stem of adult rat.

contained 60–70 per cent S-100 and GFAp (α-albumin) positive cells, using immunohistochemistry. The different cultures also contained some macrophages, endothelial-like cells, mesenchymal-like cells and oligoblasts, but no neurons or oligodendroglia could be detected (15).

Regional variations in biochemical parameters are found in brain, which may be related to the presence of different neuronal pathways utilizing different neurotransmitters (33, 34). The astroglial cells which appear to be of importance for control of neuronal activity, may also show regional heterogeneity (6, 12, 19, 21, 28, 35). The amount of seeding material was chosen so that the different cultures reached confluence 6–7 days after cultivation. The protein amount was on a similar level in 14-day old cultures. Assuming that all cells in the different cultures are multiplying at a constant rate, these data might indicate that the number of cell divisions in the cultures are similar. Further studies have to enlighten to what degree tissue maturation at seeding might affect the survival and multiplication of cells in culture.

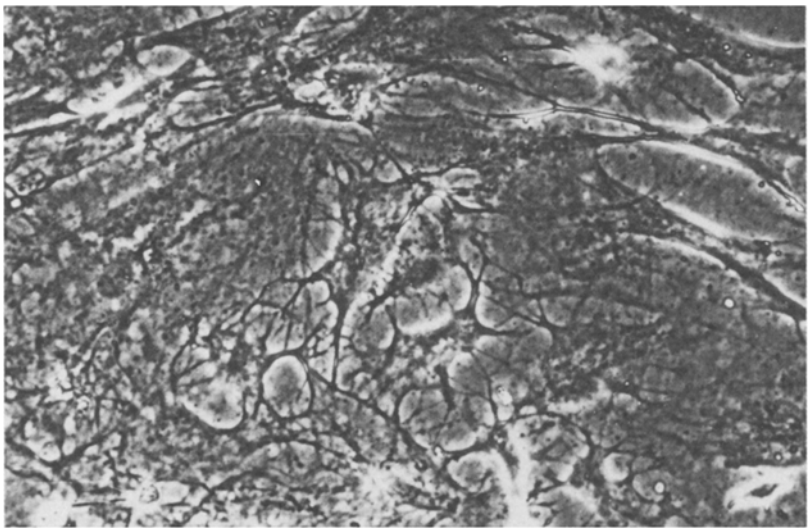


FIG. 5. Accumulation of [^{3}H]GABA in primary astroglial culture from cerebral cortex visualized by autoradiography. There is a moderate accumulation of grains in astroglial-like cells.

Concerning MAO, Hazama et al. (18) found no regional difference in enzyme activity of bulk-isolated glial cells from different brain areas. However, in the neuronal fractions higher MAO activities were found in the amygdala, the hypothalamus and the hippocampus. In the present study, homogenates from the striatum expressed a higher MAO-activity, while in brain stem a lower enzyme activity was found compared to other brain areas studied. Even in the primary cultures from brain stem a lower enzyme activity was found than in astroglial cell cultures obtained from other brain regions.

There is a close correlation in COMT-activities in the different cultures and in the corresponding areas of the adult rat brain, although higher COMT activities were found in the cultures than in brain homogenates, in accordance with a previous study (16). The lowest activity was found in brain stem, with higher levels in cortex, striatum, and cerebellum, and the highest enzyme activity found in hippocampus. This might be in correlation to the breakdown of monoamines which appear in high concentration in these regions (34).

The GABA-T activity was similarly distributed in the various astroglial cultures and in the adult brain preparations, although at a lower level in the cultures in accordance with Hansson and Sellström (16). GABA is a

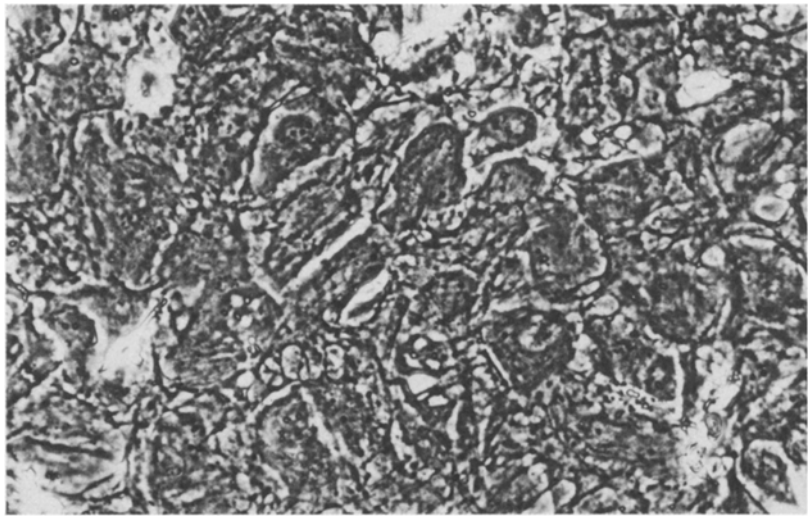


FIG. 6. Accumulation of [^{3}H]dopamine in primary astroglial culture from cerebral cortex visualized by autoradiography. There is a weak accumulation of grains in astroglial-like cells.

transmitter well distributed in brain (33), which might be an explanation for the uniform GABA-T distribution. Similar results on the regional distribution were obtained by Van den Berg et al. (43) and by Waksman et al. (44), although they found a higher GABA-T activity in brain stem than in the other regions measured. Hyde and Robinson (23) measured the GABA-T activity by a histochemical technique and found small variations in enzyme intensity in the different regions studied. GABA-T activity, measured in homogenates from different regions, gave the same results by De Boer and Bruinvels (5) and Maitre et al. (30).

As GABA-T is the metabolising enzyme to GABA, the accumulation of this neurotransmitter was investigated autoradiographically. It is well known that GABA is taken up by glial cells and the uptake has characteristics of a high-affinity sodium-dependent transport system (10, 17, 20, 21, 22, 24, 41). The GABA accumulation was similar in the different cultures examined, which is also in good correlation with the GABA-T distribution.

Monoamines, on the other hand, were not accumulated by glial cells in cultures of rat brain-stem and cerebellum (26, 27). Those results were confirmed in this report and extended for dopamine also in the other cultures studied. In another study, we have not found an active uptake with a high-affinity sodium-dependent transport, but just a diffusion (17).

This diffusion uptake can explain the enzyme-amount found in the cultures, respectively.

In conclusion, there are many correlations in enzyme activities in the different cultures and in homogenates from various regions of adult rat brain. This might indicate that astroglial cells in culture show at least some characteristics similar to those in adult rat brain (16). It might also indicate that astroglial cells obtained from different brain regions express variations in enzymatic activities which reflect different functional requirements. Studies are in progress in our laboratory to evaluate if the regional heterogeneity observed among cultivated astroglial cells is affected by in vivo differentiation until cultivation and/or time in culture.

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