On the Role of Glial Cells in the Mammalian Nervous System

UPTAKE, EXCRETION, AND METABOLISM OF PUTATIVE NEUROTRANSMITTERS BY CULTURED GLIAL TUMOR CELLS

BRUCE K. SCHRIER AND EDWARD J. THOMPSON*

From the Behavioral Biology Branch, National Institute of Child Health and Human Development, and the Laboratory of Biochemical Genetics, National Heart and Lung Institute, National Institutes of Health, Bethesda, Maryland 20014

SUMMARY

The synthesis, uptake, and efflux of some putative neurotransmitters by cultured rat glial tumor cells were studied. Three glial cell clones were capable of pyridoxal-dependent synthesis of γ-aminobutyrate both in cell-free homogenates and in intact cells in monolayers. Substantial catabolism of γ-aminobutyrate was not found. Synthesis of taurine and isethionic acid, but not β-alanine, by one of the glioma lines was also shown. Glioma lines were found to take up glutamate and exhibited Na+-dependent uptake of γ-aminobutyrate. The uptake of γ-aminobutyrate consisted of a slow saturable component (K<sub>T</sub> ~ 13 to 30 μM) and a rapid nonsaturable component. Both of these were antagonized by some structural analogs of γ-aminobutyrate as well as by taurine, β-alanine, bicuculline, and low temperature. Similar kinetic parameters were found for three different glioma lines. Fibroblast-like cells obtained from rat brain cell cultures had only the nonsaturable component of γ-aminobutyrate uptake. Taurine uptake also consisted of two Na+-dependent and temperature-dependent components: a rapid saturable component (K<sub>T</sub> ~ 10 to 17 μM) and a nonsaturable component which varied in magnitude between cell lines. These uptakes were antagonized by β-alanine but not by γ-aminobutyrate. The glioma lines also excreted the concentrated γ-aminobutyrate into the extracellular milieu, but could maintain cell to medium concentration ratios of >80-fold. In taurine efflux experiments, cell to medium concentration ratios in excess of 1500-fold could be maintained by one of the gliomas. The data are consistent with a possible role of central nervous system glial cells in the modulation of neuronal excitability via control of the levels of neuroactive substances in the extracellular milieu of neurons.

Galambos in 1961 (1) suggested that glial cells may have major functional roles in the central nervous system. Several specific suggestions for such functions have been formed, including uptake of K<sup>+</sup> ions (2–4) and neurotransmitters (5–8) from synaptic clefts, as well as guides for neuronal migration (9). An ability of partially purified glial cells to specifically take up γ-aminobutyrate, norepinephrine, and serotonin, albeit with lower affinity than did neurons, was suggested by the work of Henn and Hamberger (10). BLEECKER (11), using electron microscopic autoradiographic techniques, has found that glial cells in brain slices incubated with [3H]taurine, a putative neurotransmitter (12–14), appeared to have concentrated that compound. Thus, some more complex role of glia in brain neurotransmitter control seemed likely.

In previous studies (15–17), we found that some rat glial tumor cell lines contained substantial activity of the enzyme glutamic acid decarboxylase (EC 4.1.1.15), although we were unable to find evidence for the pyridoxal-independent type II (18) activity. In the present work we have investigated the ability of these tumor cells to synthesize some putative neurotransmitters, to concentrate them from the extracellular fluid and to release them. It was found that the glioma cell lines C-6, C-2, and NT-1 behaved similarly in synthesis, uptake, and efflux of γ-aminobutyrate and taurine. In addition, it was found that C-6 could synthesize significant amounts of taurine from cystine in the medium. The three cell lines exhibited active, Na<sup>+</sup>-dependent concentration of γ-aminobutyrate and taurine from the medium; the data suggested that there were separate transport channels for the two putative transmitters, although some overlapping functions were found. It is concluded that glial cells may play an important role in controlling the levels of neurotransmitters in the milieu of neurons. A preliminary report of this work has appeared (16).

MATERIALS AND METHODS

Cell Lines

The C-6 cell line (19, 20) was obtained from the American Type Culture Collection (CCL No. 107). The C-21 cell line was obtained originally from Dr. S. E. Pfeiffer, University of Connecticut, Farmington, Conn. The B-82 clone of L-929 mouse fibroblasts and the CHB glioma (21) were obtained from Dr. Nirenberg, Laboratory of Biochemical Genetics, National Heart and Lung Institute, National Institutes of Health, Bethesda, Md. The NT-1 glioma (22) was obtained through Dr. Alfred Gilman, Department of Pharmacology, University of Virginia School of
cysteine to cysteic acid (27). Portions of this mixture were then subjected to electrophoresis, with samples from \([U-14C]caspase incubations and appropriate standards, in a Shandon L24 apparatus at 3.5 kV (180 mA) for 60 to 90 min in pyridine-glacial acetic acid-water (1.8:6:199.5, pH 3.95). Dried electrophoretograms were sprayed with ninhydrin and 0.05% bromphenol blue in ethanol, and channels were cut into transverse strips for determination of radioactivity by liquid scintillation counting.

Uptake and Excretion

Except as noted, uptake and excretion studies were performed in one of the following solutions (adjusted to pH 7.3 with KOH) after the cells were washed three times with the same solution.

**N-2-Hydroxyethylpiperazine-N’-2-ethanesulfonic Acid Buffered Dulbecco-Vogt Modification of Eagle’s Medium**—This is growth medium without NaHCO₃, serum, or antibiotics, but containing 22 mM N-2-hydroxyethylpiperazine-N’-2-ethanesulfonic acid (Calbiochem) (sodium salt) at a final pH of 7.3 (25). Solution 1—This solution consists of 134 mM NaCl, 6 mM KCl, 2 mM CaCl₂, 0.9 mM MgCl₂, 0.9 mM NaHCO₃, 44.4 mM dextrose, 0.032 mM choline-Cl, and 1.0 mM NaHPO₄, buffered with 10.7 mM N-2-hydroxyethylpiperazine-N’-2-ethanesulfonic acid (Calbiochem) (4.14 mM Na⁺) at pH 7.3 and 332 mosm (final sodium concentration 141.3 meq per liter).

Uptake—This solution is the same as Solution 1 except taurine (Sigma) added to a final concentration of 4 mM.

Solution 1b—This solution is the same as Solution 1 except without NaCl and with choline-Cl increased to 134.3 mM; 328 mosm, final Na⁺ was 6.94 meq per liter.

Solution 2a—This solution is the same as Solution 2 except containing 4 mM taurine.

Solution 2b—This solution is the same as Solution 2 except containing 3 mM taurine. Solution 1 was of similar composition to the salts in the growth medium except that NaHCO₃ was replaced by sodium N’-2-hydroxyethylpiperazine-N’-2-ethanesulfonic acid.

Unless otherwise stated, uptake and excretion assays were performed in Linbro wells by a modification of the method described by Richelson (29). Cells were grown in the 1.5-cm diameter (1.77 cm² surface area) wells of Linbro Dispo-Trays (Catalog No. FB16-241C), obtained from Belco Glass, Vineland, New Jersey. The cells were washed three times with 1 ml of one of the above solutions and then incubated in 0.5 ml of the same solution while floating the tray in a covered 37°C water bath for 15 min before addition of the labeled molecule. Uptake incubations were for 20 min unless otherwise indicated. Labeled molecules used were \(\gamma\)-aminobutyric acid (563 μCi per mmole), \(\gamma\)-amin[1-14C]-butyric acid (8.14 μCi per mmole), and \(\gamma\)-[1-14C]taurine (2.42 μCi per mmole), all obtained from New England Nuclear. Radioactive molecules were diluted with water with or without non-radioactive diluents so that 0.01 volume of these solutions was added to the cells in the uptake incubation medium. After incubation with labeled compound as noted in the individual experiments, the cells were rapidly washed three times with 1.0 ml of one of the incubation solutions and then were allowed to dry, still at 37°C. When all the wells had dried, 300 μl of 0.2 N NaOH were added to each well and the wells were covered; after 15 min at room temperature the well was scraped with a rubber policeman, and the extract was combined with an additional wash of each well with 100 μl of 0.2 N NaOH. One portion (200 μl) of this extract was used for determination of protein (26), the remainder was neutralized with 0.2 ml HCl in a scintillation vial and 10 ml of Triton-toluene scintillation fluid or Aquasol (New England Nuclear) were added and radioactivity was determined in a liquid scintillation spectrometer at efficiencies of about 80% and 57% for \(^{14}C\) and \(^{3}H\), respectively. Counting efficiencies were determined for each sample from quench curves generated by internal standards of \([1,2,6\text{-hexadecanol (1.855 μCi per g)}\) or \([\text{UC}^-]\text{hexadecane (2,μCi per g)}\) obtained from Amersham, Arlington Heights, Ill.

In some experiments cells were incubated in one of the above solutions for measured periods of time before addition of the tracer molecule. When inhibitors or antagonists were employed, these substances were added to the cells 30 min before addition of

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**Enzyme Assays**

For determination of the activity of glutamate dehydrogenase in cell-free extracts, cells were recovered and homogenized by the scrape-wash procedure as described (15) and modified (25). Homogenates were assayed for the enzyme activity as described (17, 24) and Method a of Wilson et al. (15), except that the specific activity of \([U-14C]glutamate (209 mCi per mmole, New England Nuclear) as described (17, 24). Aminooxyacetic acid was obtained from Sigma, St. Louis, Missouri. Background values (no enzyme or boiled enzyme) were 350 cpm (7 pmol min) for CO₂ production, and 328 cpm (1.7 pmol min) in the γ-aminobutyric acid locus after electrophoresis of one-fourth of the acid-methanol extract.

Formation of γ-aminobutyric acid by living cells was evaluated by growing cells to postconfluence in 150-mm diameter (145 cm² surface area) Falcon dishes in medium containing 0.2 mM glutamate acid. To the cells in fresh medium (20 ml per dish) were added 3 μCi of \([U-14C]glutamate (209 μCi per mmole, New England Nuclear) to give a final specific activity of 0.75 μCi per mmole. After 2.5 hours of incubation under the usual growth conditions, medium was removed and saved, cells were washed three times with solution 1 (15), drained, and then treated with 5 ml of 0.1 M acetic acid in methanol. A portion of the medium was mixed with an equal volume of the same acetic acid-methanol solution, and centrifuged at low speed to remove particulate matter. Portions of the acid-methanol extracts of the cells and of the medium were examined by high voltage electrophoresis as described above. Developed electrophoretograms were cut into strips and radioactivity of the strips determined as above.

**Synthesis of α-alanine, taurine, and isethionate by intact cells** was evaluated as described. Briefly, the assays were performed as follows: postconfluent cells in 100-mm diameter (65-cm² surface area) dishes were incubated for 2 hours under the usual growth conditions with fresh medium containing either 07 μCi of \([2-3H]cysteine (Amersham-Searle; final specific activity in the medium was 9.82 μCi per μmole, final concentration was 0.62 μm cysteine) or 25 μCi of \([U-14C]cysteic acid (New England Nuclear, 297 μCi per μmole, final concentration was 15.1 μCi). The cells were washed three times with isosmotic buffered saline (15), then treated with 3% trichloroacetic acid at room temperature to extract acid-soluble materials (25). Precipitated macromolecules were recovered by scraping in 0.2 N NaOH and portions of this solution were used for protein assay (method of Lowry et al. (26)) and quantification of \(^{14}C\) glutamate

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1 B. K. Schrier, submitted for publication.
tracer. In some instances uptake medium was replaced, after washing, with fresh medium and the cells were subjected to a second incubation, after which both cells and medium were assayed for radioactivity as a measure of efflux. \( \gamma \)-Aminobutyrate analogs, \( \delta \)-alanine and sodium azide (all from Sigma Chemical Co.), and L-isopropylphenepinephrine bitartrate (Nutritional Biochemicals) were prepared in water solution such that 0.01 volume of these solutions was added to the cells in the incubation medium. A 5 mm stock solution of bioculine (K & K Chemicals) was prepared by dissolving 7.5 mg of the alkaloid in 6 ml of 2.83 N HCl with heating, diluting to 25 ml with H2O, titrating to pH 3.0 with NaOH, and finally diluting to 41 ml with H2O. A 0.1 ml solution of 2,4-dinitrophenol (Sigma) was prepared in 95% ethanol. Oxnbain (G-Strophantin, Eli Lilly, Indianapolis, Ind.) was obtained in 0.428 mm (0.25 mg per ml) aqueous solution in 0.145 M NaCl, 0.0123 M Na2HPO4 and 0.007 M KH2PO4.

Calculations of internal concentration of radioactive molecules were made using cell volume to protein constants obtained from the following data: C-6 cells in 100-mm diameter dishes, after thorough washing, were recovered to tared centrifuge tubes by scraping with a Teflon-covered plastic spatula in isosmotic buffered saline, and then centrifuged at 1530 \( \times g \) for 10 min at 6°. Supernatants were decanted and saved; tubes were drained for 15 min at 6°, and then weighed. A specific gravity of 1 g per ml for the pellet was assumed in order to obtain packed cell volume from these wet weights. Pellets were then dissolved in 0.1 N NaOH and protein assays (26) were performed on pellets and supernatants, from which total cell protein per dish was determined. From these data, ignoring the effect of extracellular water in the pellets, it was determined that C-6 cells contained 7.1% protein. Subsequent data calculations employed this value for conversion from protein determinations to cell volume.

Uptake data were corrected for counts trapped in incubation solutions and not removed by washes; such occlusion was estimated by incubation of postconfluent C-6 cells with 3.0 \( \mu \)Ci of \( [\text{carboxyl-}^{14}\text{C}] \)ulinin (27.2 mCi per g, New England Nuclear), followed by normal washing, NaOH dissolution and counting as usual. It was found that only 258 cpm (<0.005%) remained with low substrate concentrations, highly spurious results might be obtained from small proportions of radioactive impurities which were taken up or bound to cells. In Method b each dish or well contained \( \gamma \)-aminoo[\( ^{14}\text{C} \)]butyric acid at the same specific activity (3.14 mCi per mmole) and the substrate concentration was varied (through four orders of magnitude) by changing the amount of labeled substrate added to the wells. This approach was necessary with low specific activity (\( ^{14}\text{C} \)-labeled) molecules, although lower count rates at the lowest concentrations made these data less reliable than those obtained at higher substrate concentrations. The lowest \( \gamma \)-aminoo[\( ^{14}\text{C} \)]butyrate concentration at which reliable counts were obtainable was 60 nm for C-6 cells and 200 nm with the other cell lines. Taurine concentration data were also obtained using Method b. Taurine uptake data were reliable at substrate concentrations of 20 nm and above for C-6 and NT-1 and 60 nm and above for C-2; cells.

**RESULTS**

**Cell-free Synthesis of \( \gamma \)-Aminobutyrate**

Our previous data (15-17) had shown the presence of glutamate decarboxylase activity in cultured glial cells. A more extensive investigation showed (Table I) that the three glial lines tested all contained the activity in varying amounts, while L-929 fibroblasts apparently contained little, if any, activity. These activities were completely inhibited by 1 mm aminooxycetic acid, thus establishing the pyridoxal dependencies of these enzymes, similar to the activity normally found in brain. The specific activities for glia cells were \( \frac{1}{2} \times 5 \) or less of those of adult mouse or rat brain.

Examination of glutamate decarboxylase activity in homogenates from various portions of a growth curve of C-6 glialoma cells (Fig. 1) showed that the development of the activity was regulated. The specific activity increased about 10-fold as cultures became crowded, and a nearly parallel increase of \( ^{14}\text{C} \)CO2 formation by homogenates was found. Relationships between the various activities at low cell density were retained: (a) CO2 production exceeded \( \gamma \)-aminobutyrate production by about 10-fold; (b) CO2 production in assays containing aminooxycetic acid...
acid was usually somewhat lower than in those without it; and (c) the cells never developed an ability to form γ-aminobutyrate in the presence of aminooxycetic acid. Other synthetic pathways utilizing glutamic acid were also active during these in vitro assays. When the complete electrophorograms from one assay of a C-6 homogenate was passed through a strip counter, eight distinct peaks of radioactivity were observed. Two of these (in addition to glutamate), amounting to <0.1% each of the total radioactivity, also were present in electrophorograms of the boiled enzyme blank; these exceeded glutamate in their mobility toward the cathode and were not identified. One radioactive peak (~10% as many counts as γ-aminobutyrate) from the C-6 assays was found to co-electrophorese with aspartic acid, and may have been that amino acid, although further identification was not attempted. Two other minor radioactive spots, not in the control, were detected on the cathodic side of the origin. In the anodic direction, in addition to γ-aminobutyrate, only one other radioactive area was found; that substance co-migrated with glutamine and contained 1.2 times as many 14C counts as the γ-aminobutyrate spot. The presence of 3.97 mm glutamine in the growth medium had not apparently repressed the ability of C-6 cells to form glutamine from glutamic acid. A similarly active synthesis of glutamine was found in assays of L-929 cells, in which no γ-aminobutyrate was formed. In these in vitro assays C-6 homogenates formed a total of 5 different labeled products of which 12% was γ-aminobutyrate. This suggested that glutamate might be utilized by these cells in several ways, in addition to synthesis of γ-aminobutyrate.

Synthesis and Excretion of γ-Aminobutyrate by Living Cells

The ability of living cells to take up glutamate for synthesis of γ-aminobutyric acid and glutamine from glutamate added to the medium was examined in cultures of C-6 and L-929 cells (Table II). The glial cells utilized glutamate in the medium at a very rapid rate to form γ-aminobutyrate. Over 70% of the γ-aminobutyrate formed was lost from the cells into the incubation medium, although a cell to medium concentration ratio of >48-fold was present at the end of the experiment. The sum of the intra- and extracellular contents of labeled γ-aminobutyrate indicated that the apparent specific activity of glutamate decarboxylase in intact cells was similar to that found in homogenates. In contrast, L-929 cells formed no detectable intra- or extracellular γ-aminobutyric acid. This indicated that glutamate decarboxylase activity was not present in all established cell lines, in agreement with previous data (15). The electrophorograms from both cell types showed minor peaks of radioactivity associated with stained areas other than glutamate and γ-aminobutyrate. However, L-929 cells contained >90% of their non-glutamate radioactivity as glutamine, for an apparent specific activity (assuming glutamine to be also uniformly labeled) of glutamine synthetase of 0.414 pmoles per mg of cell protein; this, despite the fact that the growth medium contained 3.97 mm glutamine. The ability of L-929 cells to form glutamine from glutamate in the medium showed that this failure to synthesize γ-aminobutyrate was not due to an inability to take up its precursor. Although there was no control in this intact cell assay for rapid catabolism of γ-aminobutyrate by L-929 cells, which would have masked its synthesis, the in vitro assays with cell-free preparations in which were included 1 mm unlabeled γ-aminobutyrate as a trap, strongly supported the conclusion that this was not the case. Similarly, the γ-aminobutyrate trap in cell-free assays with C-6 homogenates might have masked catabolism of that molecule, but the assay with intact cells showed that glioma cells contained little, if any, γ-aminobutyrate transaminase activity.

From these data on glutamate and γ-aminobutyrate metabolism it was clear that glial tumor cells, but not fibroblasts, had the potential to control extracellular concentrations of two putative neurotransmitters: glutamate, a putative excitatory neurotransmitter, by rapid uptake and metabolism to CO₂, glutamine, γ-aminobutyrate, and other products; and γ-aminobutyrate, by synthesis and excretion. These findings prompted investigation of the following: (a) possible extension of this potential for regulation of levels of neurotransactive substances to other putative neurotransmitter molecules; and (b) ability of glial cells to concentrate γ-aminobutyrate and other potential transmitters from the medium.

Synthesis of Other Putative Neurotransmitters

Glioma cells in culture were capable (Table III) of formation of taurine, another putative neurotransmitter, from cystine in the medium; this is consistent with the suggestion (30) that taurine and γ-aminobutyrate may be formed by the same enzyme. The glioma cells also formed 2-hydroxyethanesulfonic acid (isethionic acid), the deamination product of taurine which has been found to exist in squid giant axons (31), and to be synthesized by heart muscle (28) and brain, but not by skeletal muscle or liver (32). Taurine and isethionic acid together accounted for 19% of the acid-soluble 14C compounds in the cells, and isethionic acid was formed from 23% of the taurine synthesized. Taurine formation had an apparent specific activity which was similar to that for γ-aminobutyrate in intact cell assays. The cells did not, however, form β-alanine, also an inhibitory neurotransactive substance and the decarboxylation product of an excitatory amino acid, from aspartate added to the medium. After incubation with [U-14C]aspartic acid, among the nine distinct radioactive spots on the electrophoretogram, greater than 54% of the counts remained in aspartic acid. However, substantial formations of glutamate and glutamine were found. This glutamate, however, unlike the result when glutamic acid itself was present in the medium (at much higher concentration), was not utilized for synthesis of significant amounts of γ-aminobutyrate. This may reflect a priority for the cells under these growth conditions to form glutamate for detoxification of ammonia by synthesis of glutamine; only when glutamate was formed glutamine and glutamate were used for γ-aminobutyrate synthesis.
TABLE III
Putative neurotransmitter synthesis by living C-6 glioma cells

| Precursor          | Product formed | Rate of formation | Acid-soluble radioactivity in cells |
|--------------------|----------------|-------------------|------------------------------------|
| [35S]Cystine       | Taurine        | 20.2              | 14.6                               |
|                    | Ieothionate    | 3.8               | 4.3                                |
| [U,14C]Aspartate   | Glutamate      | 1.67              | 17                                 |
|                    | Glutamine      | 2.47              | 25                                 |
|                    | β-Alanine      | 0.12              | 1.2                                |
|                    | γ-Aminobutyric acid | 0.10            | 1.0                                |

* B. K. Schrier, submitted for publication.

provided in relatively large quantities did secondary reactions, such as γ-aminobutyrate formation, take place. Clearly from these data, formation of β-alanine from aspartate occurred to only a minor extent. In addition, under normal circumstances of growth (i.e. without glutamate or aspartate in the culture medium), it appears unlikely that cells would make, store, or excrete significant quantities of β-alanine or γ-aminobutyrate. Similar distributions of radioactive products were obtained in repeat experiments with C-6 cells at 84 and 101 μg of cell protein per cm² surface area.

These data showed that glioma cells may act to efficiently remove glutamate and aspartate from extracellular spaces and to synthesize γ-aminobutyrate, taurine, and ieothionic acid. All of these substances have effects on neuronal or muscle membranes, or both (12, 32). In light of data showing active uptake of choline by C-6 (33) as well as apparently active uptake of some neurotransmitter candidates, including γ-aminobutyrate, by a purified “glial” cell fraction from brain (lo), it seemed reasonable that glial cells might be able to remove such substances from the milieu of neurons, and therewith modify local neuronal excitability. Synthesis, uptake and excretory ability of glia for taurine and γ-aminobutyrate might provide the capability of control, by satellite cells, of over-all neuronal excitability.

Influx and Efflux of γ-Aminobutyrate and Taurine

Uptake Time Courses—When the glioma tumor cells were incubated with radioactive γ-aminobutyrate or taurine, it was found that they were able to take up these materials against a concentration gradient. The time courses of uptake of γ-aminobutyrate and taurine by two of the glioma cell lines are shown in Fig. 2. Uptake of γ-aminobutyrate (Fig. 2A) by both cell lines showed a very rapid influx to 5 or 6 min followed by a linear uptake out to at least 30 min.

In contrast, taurine uptake time course (Fig. 2B) showed a slight lag followed by a linear increase in internal taurine concentration out to 30 min. The uptake was more rapid with C-2, reaching an apparent cell to medium concentration ratio of 61 at 30 min. The 30-min transmembrane concentration was 30-fold for C-6. Concentrative uptake against a gradient apparently had occurred with both glioma tumor lines. Although many cell lines and tissues have been shown to concentrate taurine (33, 39), concentrative uptake of γ-aminobutyrate has been reported to be peculiar to brain (40). Because glial uptake of either substances from the extracellular milieu of neurons could have important effects on their function, these uptake phenomena were given further evaluation.

Substrate Concentration Effects—The effects of extracellular substrate concentrations on the rates of γ-aminobutyrate and taurine influxes were evaluated (Fig. 3) in the four cell lines of brain origin: C-6, C-2, and NT-1 gliomas, and the uncloned RBF cell line (see “Materials and Methods”). For γ-aminobutyrate, uptake by C-6 and C-2, when Method a was used, were the same as the results obtained with all the glioma lines with Method b. Differences between the glioma cell lines were negligible, and therefore the representative data obtained with NT-1 cells are presented in Fig. 3A. γ-Aminobutyric acid uptake was nearly linear in the range of 0.06 to 150 μM extracellular...
Taurine uptake also apparently had two components in all glioma lines, but not for RBF cells. These findings led us to believe that two components of uptake were involved for the gliomas: a rapid and perhaps passive nonsaturable component and a much slower saturable influx. When the linear component (picomoles of uptake per min of protein per μM change in substrate concentration) from 100 to 150 μM concentration was subtracted from the data points, the lower curve of Fig. 3A was obtained, which described the kinetic behavior of the saturable uptake. These derived data could be used to obtain reasonable approximations of the transport constant (K_T) and V_max values via double reciprocal plots (Fig. 3A, inset). The data for both components of uptake for all the cell lines are given in Table IV where the similarity of all of these parameters for γ-aminobutyrate and taurine uptake by glioma clones and uncloned brain fibroblastic cells.

All assays were performed for 20 min at 37°C in Solution 1 with cells grown in Linbro wells, with three wells at each substrate concentration and eight substrate concentrations (0.02 to 159 μM γ-aminobutyrate and 0.02 to 200 μM taurine) as described for Method b under “Materials and Methods.” Data for both substrates with NT-1 cells and for taurine with C-6 cells are those described in Fig. 3 and its legend. Average and per cent range of values for cell protein concentrations in other sets were as follows: for γ-aminobutyrate (micrograms of cell protein per cm² surface area of well): C-6, 144 ± 12%; C-21, 54.8 ± 41%; and RBF, 53 ± 12%; for taurine (micrograms of cell protein per cm² surface area): C-2, 58.4 ± 56%; and RBF, 52.6 ± 12%.

Some characteristics of saturable and nonsaturable components of γ-aminobutyrate and taurine uptake by glioma clones and uncloned brain fibroblastic cells.

Table IV

| Parameter                              | Cell line |
|----------------------------------------|-----------|
|                                        | C-6       | C-21     | NT-1     | RBF  |
| V_max, saturable componenta            | 1.03      | 1.04     | 1.01     | 0.96 |
| K_T of saturable component (μM)        | 22.8      | 23.8     | 23.8     |      |
| Maximum concentration gradient (fold)c| 4.5       | 4.6      | 3.4      | 2.3  |
| Taurine uptake                         |           |          |          |      |
| Rate of nonsaturable componenta        | 1.98      | 0.83     | 0.70     | 0.73 |
| V_max, saturable componenta            | 3856      | 443      | 420      | 435  |
| K_T of saturable component (μM)        | 10        | 11       | 16.7     | 14.9 |
| Maximum concentration gradient (fold)c| 65        | 48       | 48       | 51   |

a Picomoles of γ-aminobutyrate or taurine taken up per min per mg of protein per μM substrate concentration.

b Picomoles per min per mg of cell protein.

c Ratio of intra- to extracellular concentrations at 0.6 μM extracellular γ-aminobutyrate.

d Ratio of intra- to extracellular concentrations at 0.06 μM extracellular taurine.
reach a maximal effect in C-6, did not affect taurine uptake in C-2, and appeared to inhibit in C-21. Since, at this substrate concentration, in the absence of bicuculline, an estimated 36% of the total uptake was due to the saturable component, these data may indicate complete inhibition of the saturable component of γ-aminobutyrate uptake in C-2, cells by bicuculline. Various aminobutyrate analogs were also found to affect uptake rates; the major findings were substantial inhibition of γ-aminobutyrate influx by α-aminobutyrate and apparent stimulation of γ-aminobutyrate transport in C-2, cells by α- or L-α-aminobutyric acid. L-Isoproterenol, at a concentration above that necessary for maximal stimulation of cyclic adenosine monophosphate levels in these cells (41) had little if any effect on uptake of either amino acid by C-6 cells, but may have stimulated to a small extent the transport of at least taurine in C-2, cells. Uptakes, both saturable and nonsaturable, of γ-aminobutyrate were markedly inhibited by 4 mM taurine, while 3 mM γ-aminobutyrate appeared to have stimulated slightly the uptake of taurine. Thus, several data suggested that the transports for the two amino acids were occurring via different mechanisms. It was interesting that β-alanine caused marked inhibition of transport of both amino acids, apparently interfering with nonsaturable as well as saturable processes.

**Table V**

| Test substance                  | Uptake by C-6 cells | Uptake by C-21 cells |
|--------------------------------|---------------------|----------------------|
|                                | γ-Aminobutyrate      | Taurine              |
|                                | % of control         | % of control         |
| Picrotoxin (100 μM)            | 69 172              | 90 80                |
| Bicuculline (50 μM)            | 73 68               | 68 97                |
| Bicuculline (100 μM)           | 60 97               | 60 96                |
| Bicuculline (150 μM)           | 27 119              | 53 73                |
| α-Aminoisobutyrate (2 mM)      | 57 120              | 96 110               |
| β-Aminoisobutyrate (2 mM)      | 98                  |                      |
| L-α-Amino-n-butyrate (2 mM)    | 77 88               | 208                  |
| L-β-Alanine (4 mM)             | 20 49               | 29 35                |
| Taurine (4 mM)                 | 0                   | 26                   |
| γ-Aminobutyrate (3 mM)         | 116                 | 121                  |

Effects of Blockers, Analogs, and Catecholamines—Evaluation of the specificity of the uptake mechanisms (Table V) showed that, with some minor exceptions, uptakes by the two glioma lines C-6 and C-2, were very similar. Picrotoxin slowed γ-aminobutyrate uptake and stimulated taurine uptake by C-6 cells, while it had little effect on uptake of either substance by C-2, cells. Bicuculline had a modest effect on both transports in C-6, did not affect taurine uptake in C-2, and appeared to reach a maximal effect on γ-aminobutyrate uptake at 40% inhibition in C-21.

Since, at this substrate concentration, in the absence of bicuculline, an estimated 36% of the total uptake was due to the saturable component, these data may indicate complete inhibition of the saturable component of γ-aminobutyrate uptake in C-2, cells by bicuculline. Various aminobutyrate analogs were also found to affect uptake rates; the major findings were substantial inhibition of γ-aminobutyrate influx by α-aminobutyrate and apparent stimulation of γ-aminobutyrate transport in C-2, cells by α- or L-α-aminobutyric acid. L-Isoproterenol, at a concentration above that necessary for maximal stimulation of cyclic adenosine monophosphate levels in these cells (41) had little if any effect on uptake of either amino acid by C-6 cells, but may have stimulated to a small extent the transport of at least taurine in C-2, cells. Uptakes, both saturable and nonsaturable, of γ-aminobutyrate were markedly inhibited by 4 mM taurine, while 3 mM γ-aminobutyrate appeared to have stimulated slightly the uptake of taurine. Thus, several data suggested that the transports for the two amino acids were occurring via different mechanisms. It was interesting that β-alanine caused marked inhibition of transport of both amino acids, apparently interfering with nonsaturable as well as saturable processes.

Tests for Exchange Diffusion—An apparent concentrative uptake of radioactive amino acids might occur by exchange diffusion with a large internal pool of the same compound (i.e., a one-for-one exchange of internal unlabeled molecule with an external labeled molecule). Although previous data showed that under normal growth conditions C-6 cells did not synthesize γ-aminobutyrate, because they were capable of doing so, those
data could not rule out the possibility of an exchangeable pool within the cells. Such a pool was also possible for taurine since synthesis of that compound did occur in the cells under normal growth conditions. One test that has been used for evaluation of exchange diffusion (42-44) is preloading of cells with the unlabeled substrate before addition of labeled molecule to the exterior of the cells. It is reasoned that the size of the exchangeable pool would be thus increased and the rate of exchange diffusion would be enhanced by preloading. When a similar preloading technique was used with C-6 cells (Table VI), it was found that preloading with either y-aminobutyrate or taurine decreased transport of the amino acid. This result was not consistent with exchange diffusion and, in fact, was consistent with the cells having a limited capacity for total content of these compounds.

An additional test of the exchange diffusion mechanism involved use of the fact that the glioma cells were able to excrete both y-aminobutyrate and taurine into the extracellular medium. If the cells contained large exchangeable pools of y-aminobutyrate or taurine, these pools could be depleted by preliminary incubations without substrate prior to assay for uptake rate with the radioactive molecules. For such preloading studies cells were incubated in Solution 1 without either substrate for varying periods before fresh medium and labeled substrate were added for the usual uptake experiment. It was reasoned that, if progressive release of y-aminobutyrate and taurine took place into the medium, this should have decreased the exchangeable pools with time and caused the apparent concentration rate due to exchange diffusion to progressively decrease. That this, in fact, did not happen is shown in Fig. 5. With neither cell line was there a change in the uptake rate of γ-aminobutyrate with increasing time in the first incubation. In contrast, there appeared to be a small, but definite increase in the rate of taurine uptake by both cell lines. These data strongly argue against exchange diffusion as the mechanism of uptake.

**Effect of Metabolic Inhibitors on Taurine and γ-Aminobutyrate Uptake**—The data of Table VII show that there was little effect on uptake of prior incubation of C-21 or C-6 cells with sodium azide, 2,4-dinitrophenol, or ouabain. At these substrate concentrations, the saturable components of uptake (legend to Table VII) were sufficiently large so that, if the inhibitors affected only those components, an effect would be expected to be demonstrable. Using 10 mM sodium azide in C-21 cells, there was no difference in the 20-min uptake of either amino acid when the prior incubation with the inhibitor was 5 to 25 min (data not shown). It is difficult to explain concentrative uptake without an apparent requirement for energy; the limited effects of metabolic inhibitors on the uptake processes may reflect the availability of large stores of high energy compounds in these cells or the functioning of a chemiosmotic uptake mechanism, or both (45, 46). That taurine uptake showed some inhibition by dinitrophenol not found with γ-aminobutyrate is consistent with these explanations (more rapid transport of taurine than γ-aminobutyrate and a higher percentage of that transport via a saturable, concentrative, and therefore probably energy-requiring process). When incubations were carried out in 6.9 meq per liter of sodium ion or at 0.7°, transport of both amino acids was markedly curtailed. This degree of inhibition could not have occurred by interruption of only the saturable component; although it is not known which component remained, it was clear that low sodium and low temperature affected both uptake mechanisms very substantially.

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**Table VI**

Effect of preloading of C-6 cells on influx of γ-aminobutyrate and taurine

C-6 glioma cells growing in Linbro wells were incubated for either 21 min or 30 s in Solution 1a (4 mM taurine for taurine uptake) or Solution 1b (3 mM γ-aminobutyrate for γ-aminobutyrate uptake) before addition of [14C]-labeled taurine (250 nCi, 207 μM) or γ-aminobutyrate (250 nCi, 159 μM) for 20-min uptake as described under "Materials and Methods." Wells contained an average of 71 and 98 μg of cell protein per cm² surface area for taurine and γ-aminobutyrate assays, respectively.

| Time (min) | Preload with   | Uptake (pmoles/min/mg protein) | Cell to medium ratio |
|-----------|----------------|--------------------------------|---------------------|
| 21        | γ-Aminobutyrate| 14,160                         | 0.32                |
| 0.5       | γ-Aminobutyrate| 24,015                         | 0.54                |
| 21        | Taurine        | 30,692                         | 0.52                |
| 0.5       | Taurine        | 39,580                         | 0.70                |

**Fig. 5.** Effect of preunloading of cultured glioma cells on uptake of putative neurotransmitters. C-6 glioma cells (Panel A, two wells per point, 33 μg of cell protein per cm² surface area) or C-21 cells (Panel B, two wells per point, 70.6 μg of cell protein per cm²) were grown in Linbro wells, washed, and incubated for the times shown in Solution 1, at which time the medium was replaced with fresh Solution 1 containing either 15.9 μM γ-aminobutyrate or 20.65 μM [1,2-14C]-taurine for a 20-min uptake assay as described under "Materials and Methods." Ranges of values are shown in brackets. GABA, γ-aminobutyric acid.
Effects of metabolic and nonspecific uptake inhibitors on γ-aminobutyrate and taurine uptake by glioma cells in culture

C-6 or C-2 glioma cells growing in Linbro wells were incubated (Experiments 1 to 3) with the indicated inhibitor for 30 min, or (Experiment 4) in low sodium medium (Solution 2, 0.94 meq of Na⁺ per liter) for 15 min, or (controls) in Solution 1 for 15 min before addition of either γ-aminobutyric acid (15.9 μM final concentration) or [1-14C]taurine (20.7 μM final concentration) for 20-min uptake evaluation as described under "Materials and Methods." Some wells of C-6 (Experiment 5) were incubated in Solution 1 with either 0.0 (low substrate) or 207 (high substrate) μM [14C]taurine or 6.0 or 159 μM [14C]-aminobutyrate for 20 min at either 37° or 0.7° (ice water bath) and then processed as usual. Protein content of wells ranged from 63 to 143 μg per cm² surface area for C-6 cells and from 60 to 98 μg for C-2 cells. Data are normalized from several different experiments and each datum represents the average of two or three wells normalized for protein content. The saturable uptake of γ-aminobutyrate in control cultures contributed 38, 36.5, and 8.1% to total uptake at 6.0, 15.9, and 159 μM, respectively, in C-6 cells; and 32, 36, and 11% at the same concentrations in C-2 cells. Similar values for taurine uptake were 92.3, 89.2, and 49.4% at 6.0, 20.7, and 207 μM, respectively, in C-6 cells; and 96.8, 94.7, and 71.6% at the same concentrations in C-2 cells.

Table VII

| Experiment | Inhibitor | Uptake by C-6 Cells | Uptake by C-2 Cells |
|------------|-----------|---------------------|---------------------|
|            |           | Amino-butyrate      | Taurine             | Amino-butyrate | Taurine |
| 1          | NaN₂ (10 mM) | 110               | 65                  | 131            | 92      |
| 2          | 2,4-Dinitrophenol (1 mM) | 108                | 71                  | 153            | 100     |
| 3          | Ouabain (10 μM) | 90                 | 76                  | 128            | 90      |
| 4          | Low sodium | 19                 | 30                  | 24             | 11      |
| 5          | Low temperature (0.7°) | 10                 | 6.5                 | 11             | 11      |
|            | Low substrate | 12                | 11                  | 11             | 11      |
|            | High substrate | 12                | 11                  | 11             | 11      |

Efflux of Putative Neurotransmitters from Glioma Cells—The data of Table II had shown that efflux into the medium accounted for a large proportion of the γ-aminobutyrate synthesized by intact C-6 glioma cells. We next evaluated whether putative transmitters which had been concentrated from the external milieu were similarly subject to efflux and whether such efflux had any relationship to β-adrenergic stimulation of cyclic adenosine 3′,5′-monophosphate levels in these cells.

Initially, the effect of time upon efflux of the neurotransmitters was studied. C-6 glioma cells (Fig. 6A) lost γ-aminobutyrate to the medium linearly for 30 min of the second incubation with a rate approximately one-third that of total uptake (at this substrate concentration about 35% of γ-aminobutyrate uptake was due to the saturable component). It was not determined whether an equilibrium situation could be reached with an excess γ-aminobutyrate concentration inside the cells, although the cell to medium ratio at 50% efflux was still in excess of 300. In contrast, these cells, atter loss of about 10% of concentrated taurine, did not allow any further net efflux of that compound. Cell to medium concentration ratios averaged 1686 throughout the 32-min test of taurine efflux. Similar results were obtained with C-2 cells (Fig. 6B) except that γ-aminobutyrate was extruded more rapidly than with C-6 and net excretion of taurine was also more rapid initially, but then decreased with time. γ-Aminobutyrate excretion by C-2, reached a plateau at an excretion of about 75% of the concentrated molecules, at which point the cell to medium concentration ratio was 84 and the external concentration was 0.703 μM. Previously determined uptake capabilities did not account entirely for the plateau, as the net excretion rate between 10 and 20 min in Fig. 6B was 22 pmol per min per mg of cell protein, while, at that external concentration, γ-aminobutyrate uptake would have been 2.08 pmol per min per mg of protein (65% saturable). That equilibrium was reached with a transmembrane gradient of more than 60-fold may indicate that a control mechanism exists for the maintenance of either intracellular or extracellular levels of γ-aminobutyrate. Similar calculations of transmembrane gradient and uptake rates for taurine (Fig. 6B, lower curve) show a maximum external concentration of 0.703 μM; precisely that found with γ-aminobutyrate. The internal concentration at 7 min in the second incubation was 332 μM taurine, or 472-fold greater than the extracellular concentration; after 32 min of the second incubation the internal concentration was 344 μM and the cell-medium ratio was 79. At an extracellular concentration of 0.703 μM the taurine uptake rates would have been 26 pmol per min per mg of protein (saturable) and 0.58 pmol per min per mg of protein (nonsaturable). The rate of decrease in net efflux from 22 to 32 min in the second incubation was estimated from the curve to be 0.344% per min of the total uptake from the first incubation or 26.8 pmol per min per mg of protein. Thus, the decrease in net efflux of taurine seen in Fig. 6B, is entirely consistent with simple reuptake from the medium. The cells not only maintained, but worked to increase, tissue-medium ratios of >300.

In order to study the process of efflux in more detail, the effects of various washes and incubation media were evaluated (Table VIII). The basal excretions of γ-aminobutyrate and taurine are shown in Experiment 1. Inclusion of 10 μM isoproterenol in the medium for the second incubation resulted in a small (26%) decrease in γ-aminobutyrate efflux and a 27% increase in the equilibrium level of taurine efflux. Incubation in substrate-free medium (Experiment 3) resulted in a 44% inhibition of γ-aminobutyrate efflux, indicating that exchange diffusion (as previously defined) may have contributed significantly to loss of that amino acid at 3 mM extracellular concentrations. In contrast, taurine efflux was unaffected by a change in substrate concentration in the external medium. Incubation in low Na⁺ media without substrate (Experiment 4) resulted in little change (from Experiment 3) in γ-aminobutyrate efflux. With taurine efflux, however, incubation in low Na⁺ medium profoundly inhibited the loss; the differences shown in Table VIII are understatements of the actual effect since normal reuptake of excreted taurine was essentially eliminated in low Na⁺ medium. Thus, taurine, but not γ-aminobutyrate, efflux by C-2, glioma cells appeared to be a Na⁺-dependent process. In other efflux experiments with C-6 cells, it was found that isoproterenol affected the rate of efflux neither of accumulated γ-aminobutyrate, nor of total counts from cells grown for four generations in [U-14C]-glucose.

Discussion

These data show that rat glial tumor cells are capable of: (a) functioning to remove glutamic and aspartic acids from extracellular milieu; (b) synthesis of the neuroactive molecules γ-aminobutyric acid, taurine, isethionic acid, glutamate, and glutamine; (c) concentrative uptake of γ-aminobutyrate and taurine by apparently different mechanisms and probably separate pathways; and (d) excretion of γ-aminobutyrate and taurine into the extracellular milieu. The data are consistent...
FIG. 6. Time course of efflux of putative neurotransmitters by glioma cells in culture. C-6 glioma cells (Panel A, three wells per point, 70 μg of cell protein per cm² surface area) or C-2 cells (Panel B, two wells per point, 92 μg of protein per cm²) were grown in Linbro wells, incubated for 20 min with either 15.9 μM γ-aminobutyric acid or 20.65 μM 1,2-3Htaurine, in Solution 1, and washed as described under "Materials and Methods" except that the wash solution was Solution 1 (without unlabeled γ-aminobutyrate or taurine). Cells were then given fresh Solution 1 and incubated for the times indicated; then the radioactivities of the medium and cells were measured and summed to obtain total uptake during the first incubation. These values were 3740 and 5290 pmoles/mg of protein for γ-aminobutyrate and taurine, respectively, with C-6, and 3550 and 7170 pmoles per mg of protein for γ-aminobutyrate and taurine, respectively, with C-2 cells. Ranges of values are shown in brackets. GABA, γ-aminobutyric acid.

TABLE VIII

Effects of wash solutions and incubation medium on efflux of γ-aminobutyrate and taurine by C-21 cells in culture

Rat C-21 glioma cells were grown in Linbro wells (2 wells per datum, 96 μg of cell protein per cm² surface area), washed, and incubated for 20-min uptake of either γ-aminobutyrate (15.9 μM, 25 nCi per well) or 1,2-3Htaurine (20.7 μM, 25 nCi per well) as described under "Materials and Methods." Wells were then washed with high substrate medium and incubated again for 24 min with the solutions indicated (see "Materials and Methods": Normal is Solution 1, High substrate is Solution la or lb, Low Na⁺ is Solution 2). After the second incubation, medium from each well was recovered and counted in its entirety with 10 ml of Triton-toluene scintillation fluid. Cells in the wells were then processed as usual and their content of radioactivity determined as described under "Materials and Methods." Total uptake (retained plus excreted) was 3540 ± 13% pmoles per mg of cell protein for γ-aminobutyrate and 10,040 ± 18% pmoles per mg for taurine.

| Experiment | Medium used for second incubation | Radioactive content excreted | γ-Aminobutyrate | Taurine |
|------------|----------------------------------|----------------------------|----------------|---------|
| 1          | High substrate                   | %                          | 57.2           | 19.9    |
| 2          | High substrate + L-isoproterenol (10 μM) | 42.2                        | 25.2           |
| 3          | Normal                           | 31.9                       | 19.7           |
| 4          | Low Na⁺                          | 38.3                       | 6.5            |

with the theory that, in the brain, the untransformed counterparts of these glioblastoma cells may have the capacity to control the extracellular concentrations of several neuroactive substances. Of the many binding and uptake studies performed with γ-aminobutyrate in various brain preparations (10, 38, 40, 42, 47-58), none is inconsistent with the data presented here. In fact, in the γ-aminobutyrate uptake studies of Iversen and Neal (40) with cortex slices, a total uptake of about 1.6 nmoles per min per mg of protein (115 nmoles per g of cortex per min) was found. At that same external concentration (2 μM) of γ-aminobutyrate, the uptake rate we found for glioma cells was 2.08 nmoles per min per mg of cell protein. Thus, it appears that a significant portion of brain uptake of γ-aminobutyrate may be due to glial cells which account for 50% of total neocortical volume (2).

Relevance of Glioma Cells to Glial Function—The significance of these data to brain function depends on the degree to which normal glial function is mirrored in these glioma cells. That the glioma cells actively transport γ-aminobutyrate, a property thought to be exclusive for brain (40), suggests that this may be a differentiated function retained by these cells. Data of Henn and Hamburger (10) apparently demonstrate such an uptake phenomenon in partially purified glial cells. That the γ-aminobutyrate uptake kinetics of the three glioma cell lines are virtually identical, although the lines originated from different tumors and have different growth characteristics and biochemical capabilities, lends support to the claim that this function is characteristic of normal glial cells. In line with these arguments are the apparent inhibition of γ-aminobutyrate uptake by bicuculline, an alkaloid known to inhibit γ-aminobutyrate action on its receptors (53, 59), and the absence of the saturable uptake component from RBF cells, a fibroblast-like line which originated from rat brain cell cultures. Although taurine uptake by glia...
may be important to brain function, that capability appears to be ubiquitous rather than specific for neural tissue; the presence of at least two components of taurine uptake in RBF cells, neuroblastoma cells (33), and some other cultured cells has been reported (37-39) in addition to its uptake by slices of brain from newborn and adult rats (34, 60). Additional differentiated functions which have been shown for one or more of these glial lines include synthesis of S-100 protein (19), presence of the enzymes adenosine 2':3'-cyclic monophosphate 3':phosphohydrolase (61), a putative marker for myelin-forming cells (62); catechol O-methyltransferase and monoamine oxidase (63), synthesis of glial fibrils (20), and of acid mucopolysaccharides (64) and the presence of the catecholamine-mediated stimulation of 3':5'-cyclic AMP accumulation (41). The cell lines C-6 and C-2 are presently referred to by many authors as astrocytoma cells, although the original tumor cells were thought to resemble more closely oligodendroglia (20). That origin could explain the presence of the phosphohydrolase activity as well as the ability to concentrate taurine (11). In addition, we have found that neither these glia nor RBF cells contain measurable amounts of the glial fibrillary acidic protein found in brain astrocytes (65) and in some of the cells in cultures of fetal rat brain cells. Thus, these glial tumour cells may be models of differentiated oligodendrocytes which have retained some of the functions of that cell type in the intact nervous system. Assuming this to be true, the importance of these data upon brain function may be assessed.

Functions of Glial Cells in Intact Nervous System—The data of this report lead to the conclusion that glia might affect the levels of neuroactive substances in the extracellular spaces of the nervous system. Glial control over the extracellular milieu could occur at several different levels of functional complexity. As has been noted previously (2, 3), the addition of glial cell volume to that of the extracellular space in brain (by the presence of purely passive glial cell membranes) would dilute substantially the concentration of neuroactive substances around neurons. To be effective, a disposal mechanism would also be required, although this could be as simple as diffusion into capillaries. A clearly more effective glial buffering of the extracellular fluid would be provided by an ability of the glial cell to concentrate neuroactive substances, and to maintain a transmembrane gradient, capabilities which have been suggested by other authors (5, 10, 11, 38, 66). Data of this report show these abilities for gliaoma cells with y-aminobutyrate and taurine, both of which are present in brain tissue in millimolar concentrations (32, 34, 52, 60, 67, 68) and have been considered by many authors to be more or less likely candidates as neurotransmitters (an excellent review for taurine is Ref. 69). As a test of the ability of cells with these characteristics to influence brain function, the following should be noted. The concentration of taurine in cerebrospinal fluid, which may be representative of extracellular fluid, has been measured at 5 to 55 μM (69).

In this range the saturable component of taurine uptake for NT-1 cells comprised 95 to 99% of total taurine uptake. If the same concentration range of y-aminobutyrate were encountered by NT-1 cells, saturable uptake would comprise 50 to 22% of the total uptake. The KT values for the saturable processes fall within this range for both amino acids with all the glioma lines. Clearly, these uptake phenomena may function physiologically to remove these neuroactive substances from the immediate milieu of neurons.

Further increments in complexity of glial function seem to require that the cells respond to a stimulus. Such a response would demand the function of a sensor, a transducer, and an effector, in the simplest case all three functions might reside within the same macromolecule. Examples of such responses, with increasing complexity, would be: (a) maintenance by glial cells of a particular extracellular concentration(s) of one or more neuroactive substances. (b) In response to neuronal activity or changed extracellular levels of a neuroactive substance(s), or both, glia might modify their influx or efflux rate for that or some other neuroactive substance. This response would function to complement or counteract the effects of the local milieu on neuronal membranes. (c) Several spatially related glial cells might respond to local or general hyper- or hypoactivity of neurons (through sensing of K+ ions, neuroactive substances, electrical, or other changes in their domains) by the uptake or excretion (or both) of neuroactive substances within those domains in order to modify in concert the state of over-all excitability. (d) Gliial cells might communicate with each other concerning over all excitability of the nervous system, and then take appropriate damping or activating responses. The data of this report do not define the precise level at which gliial cells function. However, it is perhaps not unrealistic to assume that cells which comprise approximately 90% of the nervous system might be able to exert considerable control over the status of that system.

Probably the ultimate possibility for glial function would be that glial cells control brain function absolutely, including memory, etc., with neurons only as messengers, and all information storage and processing occurring in glial cells. While this possibility seems very remote at present, there are, as far as we know, no data which preclude its demonstration at some future time. It is reasonable to conclude that glial function may contribute substantially to the working of the nervous system.

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