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UPTAKE AND METABOLISM OF GABA IN ASTROCYTES CULTURED FROM DISSOCIATED MOUSE BRAIN HEMISPHERES

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Uptake kinetics and contents of GABA in cultured, normal (i.e. nontransformed) glia cells obtained from the brain hemispheres of newborn mice were measured together with the activity of the GABA transaminase. During three weeks of culturing the activity of the transaminase rose from a low neonatal value toward the level in the adult brain. The uptake kinetics indicated an unsaturable component together with an uptake following Michaelis–Menten kinetics. Both the K_m (40 μM) and the V_{max} (0.350 nmol $\times$ min^{-1} $\times$ mg^{-1} cell protein) were reasonably comparable to the corresponding values in brain slices, and the V_{max} was much higher than that reported for other glial preparations. The GABA content was low (<5 nmol/mg cell protein), which is in agreement with the high activity of the GABA transaminase.

INTRODUCTION

During recent years much evidence has accumulated that GABA is an inhibitory neurotransmitter in the mammalian central nervous system. GABA is thus liberated from brain tissue in vivo and in vitro by electrical or potassium-induced "stimulation" (1–6) and it exerts a depressant effect on spinal, cortical, and cerebellar neurons (7–13). Glutamate decarboxylase, which is the main GABA synthesizing enzyme, is highly concentrated in "GABA ergic" cerebellar synapses

TABLE I

KINETIC CONSTANTS FOR HIGH-AFFINITY UPTAKE OF GABA INTO BRAIN AND SPINAL CORD SLICES, SYNAPTOSOMES, AND NEURONAL AND GLIAL ENRICHED PREPARATIONS[a]

Tissue preparation	K_m (μM)	V_{max} (nmol × min^{-1} × g^{-1} wet wt.)	V_{max} (nmol × min^{-1} × mg^{-1} protein)
Adult brain slices[b]	11–31	34–167 (25°C)	—
Neonatal brain slices[b]	5–43	5 (25°C)	—
Spinal cord slices[b]	25	10–15 (25°C)	—
Synaptosomes[c]	13	—	2.2
Synaptosomes[b]	4	—	1.1 (27°C)
Synaptosomes[d]	0.42	—	—
Cerebellar glomeruli[e]	10	—	1.5
Bulk-prepared neurons[d]	0.72	—	—
Bulk-prepared glia cells[d]	0.27	—	—
Superior cervical ganglia[f]	7	0.2 (25°C)	—
Sensory ganglia[g]	10	2 (25°C)	—
Cultured cerebellar neurons[h]	0.33	20–80	—
Cultured cerebellar glia cells[h]	0.29	0.05–0.2	—
C-6 glioma cells[i]	32	—	0.023
C-6 glioma cells[j]	0.22	—	0.0014
Cultured mouse brain astrocytes[k]	40	28	0.350

[a] Most of the values are quoted verbatim from the literature. The V_{max} for synaptosomes from G. Levi and M. Raiteri (footnote *c*), however, was calculated from Fig. 4 in that paper and the V_{max} values for cultured cerebellar neurons and glia cells (23) were converted from nmol/mg DNA to nmol/g wet weight on the basis of the DNA contents in adult and neonatal rat brain reported by S. Zamenhof, L. Grauel, E. Van Marthens, and R. A. Stillinger, 1972. Quantitative determination of DNA in preserved brains and brain sections, *J. Neurochem.* 19:61–68. The constants obtained in the present work were derived from Fig. 2 and Eq. (1) by computer analysis using a Univac 1110 computer. In general V_{max} values for brain slices and for ganglia have been expressed on a wet weight basis, whereas those for isolated cellular or subcellular preparations (where wet weight determinations are uncertain) are based on protein (or DNA) contents. Since protein/wet weight ratios probably differ among all the preparations mentioned, no attempt has been made to recalculate. However, 1 g wet weight probably does correspond roughly to 100 mg protein. Measurements were performed at 37°C except when otherwise indicated. All results were obtained with preparations originating from cats, rats, rabbits, or mice. All GABA in superior cervical ganglia and sensory ganglia is known to be localized in glia cells (see, for example, reference 30).

[b] MARTIN, D. L. 1976. Carrier-mediated transport and removal of GABA from synaptic regions. Pages 347–386, *in* ROBERTS, E., CHASE, T. N., and TOWER, D. B. (eds.), GABA in the nervous system function, Raven Press, New York.

[c] LEVI, G., and RAITERI, M. 1973. Detectability of high and low affinity uptake systems for GABA and glutamate in rat brain slices and synaptosomes. *Life Sci.* 12:81–88.

[d] See reference 26.

[e] See reference 25.

[f] BOWERY, N. G., and BROWN, D. A. 1972. γ-aminobutyric acid uptake by sympathetic ganglia. *Nature New Biol.* 238:89–91.

[g] SCHON, F., and KELLY, J. S. 1974. The characterization of (^{3}H) GABA uptake into the satellite glial cells of rat sensory ganglia. *Brain Research* 66:289–300.

[h] See reference 23.

[i] See reference 33.

[j] See reference 28.

[k] Present work.

(14,15), and a binding of GABA to a postsynaptic receptor protein has been reported (16–19). In such a "GABA ergic" transmission process there must be a system that is able to remove GABA from the synaptic cleft. Since GABA metabolism per se is unlikely to be responsible for the removal (7,20), a re-uptake system for GABA seems to be necessary (11).

Evidence has been presented for a GABA transport into neurons (21–25), but it has also been suggested that glia cells take part in the removal of GABA (e.g., 26–34). The latter concept is mainly based on the demonstration of a GABA uptake into bulk-prepared glia cells and cultured glioma cells and also into peripheral ganglia, in which the uptake exclusively occurs into glia cells (30,31,34). The uptake rates are significantly higher into the bulk-prepared glia cells than into corresponding neurons (26), but no V_{max} values were given and those reported for GABA uptake into the glioma cells and the peripheral ganglia are at least one order of magnitude lower than the minimum values reported for brain slices (Table I). One may thus wonder whether this reflects a less active uptake of GABA into these types of glia cells or indicates that the GABA uptake into glia cells in general is of quantitatively minor importance.

The present study was undertaken to elucidate this question by investigating the kinetic characteristics of the GABA transport into cultured normal (i.e., nontransformed) glia cells from the brain hemispheres. It also sought to determine whether the activity of the GABA transaminase is high enough to enable the cells to metabolize the GABA taken up. The glia cells were obtained as primary cultures of dissociated brain cells (35) from the hemispheres of the neonatal mouse brain. Such cultures are practically void of neurons, and seem to be suitable for metabolic studies of normal glia cells (36–38).

A preliminary report has been given of some of the findings (39).

EXPERIMENTAL PROCEDURE

Tissue Culture. All experiments were carried out using the glia cell culture developed by Booher and Sensenbrenner (35) and shown to be highly enriched with the astrocyte-specific GFA protein (40). For preparation of ten cultures, cerebral hemispheres from three newborn Swiss mice were dissected and passed through a sterile nylon sieve (80 μm pore size) into 10 ml of a modified Eagle's minimum essential medium with double concentrations of the amino acids (except glutamine), quadruple concentrations of vitamins, 200,000 IU/liter penicillin, 20% (vol/vol) fetal bovine serum, 2.0 mM glutamine, no GABA, 7 mM glucose, and pH 7.3. One milliliter of this cell suspension plus 3 ml of the modified Eagle's medium were introduced into the tissue culture flask and cultured at 37°C in an atmospheric air/CO_2 mixture containing 5% (vol/vol) CO_2. The culture medium was changed three times a week. Unless otherwise stated the cultures were grown for 3 weeks;

during the last week serum was withdrawn and 0.1 mM dibutyryl cyclic AMP (BcAMP) was added to the culture medium, which led to the formation of abundant radial, branching, relatively short processes (cf. 41).

Uptake of GABA. Prior to the influx experiments the culture medium was exchanged with a solution (PBS) containing 150 mM NaCl, 3.0 mM KCl, 1.0 mM $CaCl_2$, 0.6 mM $MgCl_2$, 1.7 mM KH_2PO_4, 8.0 mM Na_2HPO_4, and 6 mM glucose; pH 7.4. The culture flask was opened and the layer of astrocytes loosened with a soft Teflon spatula having the same width as the flask. The culture was subsequently dissected into three or four samples, each of which contained approximately 30 μg of cell protein. These were incubated at 37°C (or, in one set of experiments, at 0°C) in 450 μl PBS containing varying concentrations of ^{3}H-labeled GABA. Sodium dependency was studied by replacing all NaCl with choline chloride, the effect of potassium by using media to which 5, 20, or 45 mM KCl was added, and the calcium requirement by omission of $CaCl_2$ from the incubation media. The incubation was terminated by centrifugation (10 sec) and rapid washing of the pellet (10 sec) with nonradioactive medium. The cells were dissolved in 100 μl 2 N KOH, and after appropriate dilution, radioactivity was determined as previously described (1), using a Packard Tri-Carb liquid scintillation spectrometer.

Determination of GABA. Ten cultures were extracted with 2.0 ml of 0.1 M perchloric acid (PCA) and the residue was washed twice with 2.0 ml of 0.1 M PCA. The combined supernatants were brought to pH 6.5 with KOH. After centrifugation, the supernatant was freeze-dried and redissolved in 2.5 ml of 0.2 M sodium citrate (pH 2.2). GABA was analyzed in a 0.5-ml aliquot on an amino acid analyzer (Bio-Cal 200) using a Stein and Moore technique.

Determination of GABA–Transaminase Activity. A 10% (wt/vol) homogenate of neonatal mouse brain was prepared in 0.05 M potassium phosphate, pH 7.2, containing 0.2 mM pyridoxal phosphate, 1.0 mM 2-aminoethylisothiouronium bromide hydrobromide (AET) and 0.1 mM EDTA. It was subsequently sonicated 2 × 15 sec at 0°C using a Branson sonifier (50 W). In other experiments, cultured astrocytes from one or two culture flasks were transferred to a test tube containing 200 ml of the buffer, and sonication was performed as described for the neonatal tissue. GABA–transaminase activity was assayed in 10-μl aliquots of the sonicated samples employing a modification of the method described by Hall and Kravitz (42). The assay mixture consisted of 0.1-*M* TRIS-HCl, pH 8.0, 1-mM AET, 0.02-mM pyridoxal phosphate, 4-mM α-ketoglutarate, 1-mM succinic acid, 3-mM NAD^+, and 25-mM ^{14}C-GABA[1] (spec. act. 100–200 cpm/μmol). The same buffer minus α-ketoglutarate was used for determination of blanks. After 30 min of incubation at 37°C the reaction was stopped by addition of 4-M perchloric acid, and the ^{14}C-labeled reaction products (succinic semialdehyde and succinate) were separated from [^{14}C] GABA on Dowex-50 × 2 (50–100 mesh) columns (0.7 × 7 cm). Radioactivity in an aliquot of the water eluate from the column was determined as described by Schousboe and Hertz (43) using a Packard Tri-Carb liquid scintillation spectrometer.

Protein was measured by the method of Lowry et al. (44) with the modification described by Miller (45) using bovine serum albumin as the standard.

Supplies. Plastic tissue culture T flasks (40 ml) were purchased from NUNC A/S, Denmark, and fetal bovine serum from Gibco/Bio-Cult Laboratories Ltd., Scotland. Dibutyryl-cyclic-3′,5′-AMP (BcAMP) and all amino acids and vitamins were obtained from Sigma Chemical Corp., St. Louis, Missouri, penicillin from LEO, Denmark, and (1-^{14}C) GABA (spec. act. 49.4 mCi/mmol) and (2,3-^{3}H) GABA (spec. act. 36.7 Ci/mmol) from New

[1] From the GABA content in the cultures (cf. Results) it can be calculated that endogenous GABA at most will increase the GABA concentration in the assay mixture by 0.025 mM, which was neglected.

England Nuclear Corp., Boston, Massachusetts. All other chemicals were of purest grade available from regular commercial sources.

Statistical significance (P) was calculated using Student's t test and computer analysis was performed with the aid of a Univac 1110 computer.

RESULTS

GABA Content

The content of GABA was determined in cultures grown for 2 weeks in a medium with serum and for a subsequent week in the absence of serum and presence of BcAMP. The amino acid analysis showed that such cultures contained less than 5 nmol GABA/mg cell protein.

Kinetics of GABA Uptake

The uptake of GABA was studied in similar cultures and found to be linear for at least 6–8 min. This is shown in Fig. 1, in which the zero time value represents entrapped GABA, i.e., the GABA content after 30 sec of incubation at 0°C. For determination of K_m and V_{max}, cultures were incubated for 5 min in media containing between 5 and 1000 μM GABA and the values for the GABA uptake were corrected for the entrapped GABA. In a typical experiment tissue corresponding to about 30 μg of cell protein and containing less than 5 nmol GABA/mg protein was

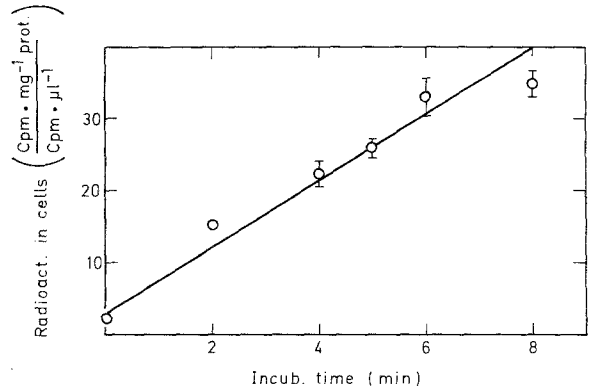

FIG. 1. Time course of the ^{3}H-GABA uptake into cultured astrocytes. The GABA concentration in the incubation medium was 50 μM and the extent of the uptake into the cells is given as (cpm × mg^{-1} cell protein/cpm × μl^{-1} incubation medium). Results are averages of 8–10 individual experiments with SEM indicated by vertical bars if they extend beyond the symbols.

incubated in 450-μl medium. The maximum augmentation of the external GABA concentration brought about by a possible release from the tissue thus corresponded to 0.3 μM, which could be neglected over the whole concentration range.

The GABA uptake (Fig. 2) conformed to the following equation:

$$V = V_{max}\,(1/(1 + K_m/S)) + k \times S \qquad (1)$$

where V indicates velocities (nmol $\times$ min^{-1} $\times$ mg^{-1} cell protein), K_m the Michaelis constant (μM), k the rate constant of an unsaturable mechanism (ml $\times$ min^{-1} $\times$ mg^{-1} cell protein), and S the substrate concentration (μM). The constant describing the unsaturable GABA uptake (k) was calculated by computer analysis of the experimental values to 7.3×10^{-4} ml $\times$ min^{-1} $\times$ mg^{-1} cell protein. K_m was in a similar way found to be 40 μM and V_{max} 0.350 nmol $\times$ min^{-1} $\times$ mg^{-1} cell protein or, recalculated on a wet weight basis and using a protein content of 8% of the wet weight (38), 28 nmol $\times$ min^{-1} $\times$ g^{-1} wet weight (Table I).

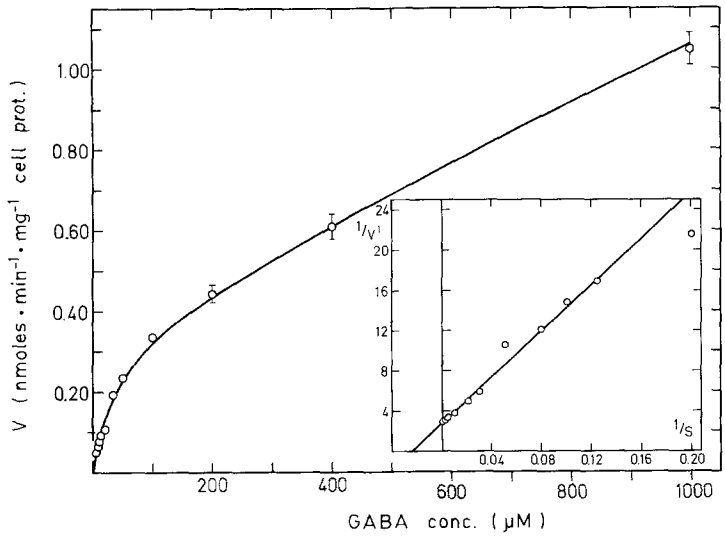

FIG. 2. GABA uptake (nmol $\times$ min^{-1} $\times$ mg^{-1} cell protein) into cultured astrocytes as a function of the external GABA concentration. Results are averages of 9–10 individual experiments with SEM indicated by vertical bars if they extend beyond the symbols. The curve was fitted to the experimental points by computer analysis, and is *the* curve described by Eq. (1) [a saturable component following Michaelis–Menten kinetics plus a possible (i.e., if $k \neq 0$) nonsaturable component] that most closely fits the experimental results. The insert shows $1/V'$ versus $1/S$ where V' is the observed V corrected for the unsaturable transport component ($k \times S$) calculated from S and the k value obtained by the computer analysis.

TABLE II
EFFECT OF CALCIUM AND POTASSIUM ON GABA UPTAKE INTO ASTROCYTES CULTURED FROM DISSOCIATED MOUSE BRAIN HEMISPHERES[a]

	Ca^{2+} (mM)	K^+ (mM)	uptake (%)	n	P
Control	1.0	5	100 ± 6.3	(9)	
	0	5	86 ± 7.1	(8)	n.s.
	1.0	10	117 ± 12.3	(6)	n.s.
	1.0	25	66 ± 3.7	(8)	<0.001
	1.0	50	53 ± 5.6	(7)	<0.001

[a] Values are expressed as percentages ± SEM of the value obtained after incubation for 5 min in a physiological medium containing 50 μM GABA. Corrections were made for the unsaturable component of the uptake. Numbers of experiments are given in parentheses.

Effect of Ions on GABA Uptake

The sodium dependency of the GABA uptake was investigated at a GABA concentration of 50 μM, where the unsaturable component of the uptake is almost negligible (cf. Fig. 2). The uptake proved to be highly sodium dependent. At 15 mM Na^+ the GABA uptake was thus only 13% of the value obtained after incubation in a physiological medium. A kinetic analysis of the sodium dependency (results not presented) suggested ordinary saturation kinetics with a K_m value for sodium of about 150 mM.

The 5-min values for the saturable GABA uptake (50 μM GABA) obtained in a Ca^{2+}-free medium and in media to which KCl was added are shown in Table II. It is seen that high concentrations of potassium inhibited the GABA uptake into the astrocytes by approximately 50%, whereas omission of calcium from the medium had little, if any, effect.

GABA–Transaminase Activity

The activity of the GABA–transaminase in neonatal brain and in dissociated brain cells after 1, 2, and 3 weeks in culture is shown in Table III. There is a decrease in the activity from the neonatal tissue to the cells cultured for 1 week, but after 2 weeks in culture the activity returned to the value observed in brains from newborn mice. If the cells were grown for an additional week in the presence of serum, the activity of the transaminase remain at this level, whereas serum withdrawal and addition of 0.1-mM BcAMP led to a significant increase (P <0.01) in the specific activity of the enzyme.

TABLE III
SPECIFIC ACTIVITY OF GABA–TRANSAMINASE IN NEONATAL MOUSE BRAIN (0 CULTURE PERIOD) AND IN ASTROCYTES CULTURED FROM NEONATAL MOUSE BRAIN HEMISPHERES FOR 1, 2, OR 3 WEEKS[a]

Culture period			GABA–transaminase activity (nmol × min^{-1} × mg^{-1} protein)
0 week			1.10 ± 0.05 (10)
1 week			0.21 ± 0.03 (10)
2 weeks			0.94 ± 0.08 (8)
3 weeks			0.93 ± 0.06 (9)
3 weeks	−serum	+BcAMP	1.64 ± 0.18 (7)

[a] During the third week of cultivation some cultures were grown in a serumfree medium to which 0.1-mM BcAMP was added. Enzyme activities were determined at 37°C as detailed in Methods. Values are means ± SEM with numbers of experiments given in parentheses.

DISCUSSION

The highest activity of the GABA–transaminase was observed in cultures grown for 2 weeks in the presence of serum and an additional week in a serumfree medium with BcAMP. This is analogous to what has been found for the (Na^+, K^+)-activated ATPase (37) and to other aspects of metabolic differentiation in the same type of cultures (38). Under these conditions the activity was significantly increased from the level in neonatal brain and approximately twice as high as the activity reported for bulk-prepared glia cells (46), dorsal root ganglia, and posterior pituitary gland [recalculated from Beart et al. (47) on the basis of an assumed protein content of 100 mg/g wet weight in their preparations]. It is, however, still only about one-third of the level (about 4 nmol × min^{-1} × mg^{-1} protein) found in homogenates from adult mice (48). Whether this indicates only partial metabolic differentiation cannot be resolved at present.

Since the activity of the transaminase was highest after a period of culturing in the absence of serum and presence of 0.1-mM BcAMP, the contents and uptakes of GABA were studied under these conditions. The GABA content of the cultured astrocytes (<5 nmol/mg protein) was low compared to a GABA content in the whole brain of about 3–4 μmol/g wet weight (30–40 nmol/mg protein) in adult mice and about 1.5 μmol/g wet weight in newborn animals (49). This is to be expected, however, since the cultures were grown in a medium without added GABA and

since the activity of the glutamate decarboxylase is low in neonatal mouse brain cells cultured for 1–3 weeks (50).

The uptake of GABA was, in contrast, quite high. The maximum velocity for GABA transport found in this paper is (Table I) somewhat lower than values previously obtained for adult brain cortex slices or rat brain synaptosomes but higher than values for neonatal brain cortex slices, spinal cord slices, and sensory ganglia, and much higher than the values reported for C-6 glioma cells and nonneuronal cells from cultured rat cerebellum.

Like transport systems for other amino acids in brain tissue (51–53), the GABA uptake into astrocytes was sodium dependent. Also, the observation that high concentrations of potassium (50 mM) inhibit the GABA uptake is comparable to an inhibitory effect of potassium on GABA uptake in bulk-prepared glia cells (54) and in brain slices (5,32).

The efficient, high-affinity uptake of GABA in cultured astrocytes from brain hemispheres supports the hypothesis that glia cells may be of physiological importance for the removal of GABA from the synaptic clefts (26–29,32,33). The intense uptake of GABA is not in discordance with the low GABA content in the cultures, since the activity of the GABA-transaminase was also found to be high. The precise physiological role of this GABA uptake remains unknown, since a major part of radioactive label is localized over nerve terminals after incubation of cortical tissue with ^{3}H-GABA (24) and also because neuronal elements possess GABA-transaminase activity (55,56) and a highly active mechanism for the uptake of GABA (cf. Table I). It is not known to what extent the measured uptakes represent a net uptake or a 1:1 homoexchange (57), but the low GABA contents do not favor a homoexchange. Further information about GABA contents, fluxes, and metabolism in glial and neuronal preparations under different conditions are thus required to elucidate the role of this amino acid as an inhibitory transmitter, a possible general "regulator" of neuronal activity (26, 29,58–60), and a metabolic intermediate in the brain.

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REFERENCES

1. ARNFRED, T., and HERTZ, L. 1971. Effects of potassium and glutamate on brain cortex slices: Uptake and release of glutamic and other amino acids. *J. Neurochem.* 18:259–265.
2. JASPER, H. H., and KOYAMA, I. 1969. Rate of release of amino acids from the cerebral cortex in the cat as affected by brain-stem and thalamic stimulation. *Can. J. Physiol. Pharmacol.* 47:889–905.
3. KATZ, R. J., CHASE, T. N., and KOPIN, I. J. 1969. Effects of ions on stimulus induced release of amino acids from mammalian brain slices. *J. Neurochem.* 16:961–967.
4. MACHIYAMA, Y., BALÁZS, R., and RICHTER, D. 1967. Effect of K^+-stimulation on GABA metabolism in brain slices *in vitro*. *J. Neurochem.* 14:591–594.
5. MACHIYAMA, Y., BALÁZS, R., HAMMOND, B. J., JULIAN, T., and RICHTER, D. 1970. The metabolism of γ-aminobutyrate and glucose in potassium ion-stimulated brain tissue *in vitro*. *Biochem. J.* 116:469–481.
6. SRINIVASAN, V., NEAL, M. J., and MITCHELL, J. F. 1969. The effect of electrical stimulation and high potassium concentrations on the efflux of (^{3}H) γ-aminobutyric acid from brain slices. *J. Neurochem.* 16:1235–1244.
7. CURTIS, D. R., and JOHNSTON, G. A. R. 1974. Amino acid transmitters in the mammalian central nervous system. *Ergeb. Physiol.* 69:97–188.
8. CURTIS, D. R., and WATKINS, J. C. 1960. The excitation and depression of spinal neurones by structurally related amino acids. *J. Neurochem.* 6:117–141.
9. GELLER, H. M., and WOODWARD, D. J. 1974. Responses of cultured cerebellar neurons to iontophoretically applied amino acids. *Brain Research* 74:67–80.
10. KRNJEVIĆ, K. 1970. Glutamate and γ-aminobutyric acid in brain. *Nature* 228:119–124.
11. KRNJEVIĆ, K., and SCHWARTZ, S. 1966. Is γ-aminobutyric acid an inhibitory transmitter? *Nature* 211:1372–1374.
12. KRNJEVIĆ, K., and SCHWARTZ, S. 1967. The action of γ-aminobutyric acid on cortical neurones. *Exptl. Brain Research* 3:320–336.
13. OBATA, K., ITO, M., OCHI, R., and SATO, N. 1967. Pharmacological properties of the postsynaptic inhibition by Purkinje cell axons and the action of γ-aminobutyric acid on Deiters neurones. *Exptl. Brain Research* 4:43–57.
14. MCLAUGHLIN, B. J., WOOD, J. G., SAITO, K., BARBER, R., VAUGHN, J. E., ROBERTS, E., and WU, J-Y. 1974. The fine structural localization of glutamate decarboxylase in synaptic terminals of rodent cerebellum. *Brain Research* 76:377–391.
15. SAITO, K., BARBER, R., WU, J-Y., MATSUDA, T., ROBERTS, E., and VAUGHN, J. E. 1974. Immunohistochemical localization of glutamate decarboxylase in rat cerebellum. *Proc. Natl. Acad. Sci. US* 71:269–273.
16. ENNA, S. J., KUHAR, M. J., and SNYDER, S. H. 1975. Regional distribution of postsynaptic receptor binding for gammaaminobutyric acid (GABA) in monkey brain. *Brain Research* 93:168–174.
17. PECK, E. J., JR., SCHAEFFER, J. M., and CLARK, J. H. 1973. γ-Aminobutyric acid, bicuculline, and post-synaptic binding sites. *Biochem. Biophys. Research Comm.* 52:394–400.
18. PECK, E. J., JR., SCHAEFFER, J. M., and CLARK, J. H. 1976. In pursuit of the GABA

receptor. Pages 319–336, *in* ROBERTS, E., CHASE, T. N., and TOWER, D. B. (eds.), GABA in the nervous system function, Raven Press, New York.

19. ZUKIN, S. R., YOUNG, A. B., and SNYDER, S. H. 1974. Gamma-aminobutyric acid binding to receptor sites in the rat central nervous system. *Proc. Natl. Acad. Sci. US* 71:4802–4807.
20. OTSUKA, M. 1972. γ-aminobutyric acid in the nervous system. Pages 249–289, *in* BOURNE, G. H. (ed.), The structure and function of nervous tissue, Vol. 4, Academic Press, New York.
21. HÖKFELT, T., and LJUNGDAHL, Å. 1972. Autoradiographic identification of cerebral and cerebellar cortical neurones accumulating labeled gamma-aminobutyric acid (^{3}H-GABA). *Exptl. Brain Research* 14:354–362.
22. IVERSEN, L. L., and BLOOM, F. E. 1972. Studies on the uptake of ^{3}H-GABA and ^{3}H-glycine in slices and homogenates of rat brain and spinal cord by electron microscopic autoradiography. *Brain Research* 41:131–143.
23. LASHER, R. S. 1975. Uptake of GABA by neuronal and nonneuronal cells in dispersed cell cultures of postnatal rat cerebellum. *J. Neurobiol.* 6:597–608.
24. STORM-MATHISEN, J. 1976. Distribution of the components of the GABA system in neuronal tissue: Cerebellum and hippocampus—Effects of axotomy. Pages 149–168, *in:* ROBERTS, E., CHASE, T. N., and TOWER, D. B. (eds.), GABA in the nervous system function. Raven Press, New York.
25. WILSON, J. E., WILKIN, G. P., and BALAZS, R. 1976. Metabolic properties of a purified preparation of large fragments of the cerebellar glomeruli: glucose metabolism and amino acid uptake. *J. Neurochem.* 26:957–965.
26. HENN, F. A., and HAMBERGER, A. 1971. Glial cell function: Uptake of transmitter substances. *Proc. Natl. Acad. Sci. US* 68:2686–2690.
27. HÖSLI, E., LJUNGDAHL, Å, HÖKFELT, T., and HÖSLI, L. 1972. Spinal cord tissue cultures—A model for autoradiographic studies on uptake of putative neurotransmitters such as glycine and GABA. *Experientia* 28:1342–1344.
28. HUTCHISON, H. T., WERRBACH, K., VANCE, C., and HABER, B. 1974. Uptake of neurotransmitters by clonal lines of astrocytes and neuroblastoma in culture. I. Transport of γ-aminobutyric acid. *Brain Research* 66:265–274.
29. IVERSEN, L. L., and KELLY, J. S. 1975. Uptake and metabolism of γ-aminobutyric acid by neurones and glial cells. *Biochem. Pharmacol.* 24:933–938.
30. ROBERTS, P. J. 1976. Amino acid transport in spinal and sympathetic ganglia. Pages 165–178, *in* LEVI, G., BATTISTIN, L., and LAJTHA, A. (eds.), Transport phenomena in the nervous system, physiological and pathological aspects, Vol. 69, Advances in experimental medicine and biology, Plenum Press, New York.
31. SCHON, F., and KELLY, J. S. 1974. Autoradiographic localization of (^{3}H)-GABA and (^{3}H)-glutamate over satellite glial cells. *Brain Research* 66:275–288.
32. SCHOUSBOE, A., LISY, V., and HERTZ, L. 1976. Postnatal alterations in effects of potassium on uptake and release of glutamate and GABA in rat brain cortex slices. *J. Neurochem.* 26:1023–1027.
33. SCHRIER, B. K., and THOMPSON, E. J. 1974. On the role of glial cells in the mammalian nervous system. *J. Biol. Chem.* 249:1769–1780.
34. YOUNG, J. A. C., BROWN, D. A., KELLY, J. S., and SCHON, F. 1973. Autoradiographic localization of sites of (^{3}H) γ-aminobutyric acid accumulation in peripheral autonomic ganglia. *Brain Research* 63:479–486.
35. BOOHER, J., and SENSENBRENNER, M. 1972. Growth and cultivation of dissociated neurons and glial cells from embryonic chick, rat and human brain in flask cultures. *Neurobiology* 2:97–105.

36. HERTZ, L., and SCHOUSBOE, A. 1975. Ion and energy metabolism of the brain at the cellular level. *Intern. Rev. Neurobiol.* 18:141–211.
37. SCHOUSBOE, A., FOSMARK, H., and FORMBY, B. 1976. Effect of serum withdrawal on Na^+–K^+ ATPase activity in astrocytes cultured from dissociated brain hemispheres. *J. Neurochem.* 26:1053–1055.
38. SCHOUSBOE, A., FOSMARK, H., and HERTZ, L. 1975. High content of glutamate and of ATP in astrocytes cultured from rat brain hemispheres: effect of serum withdrawal and of cyclic AMP. *J. Neurochem.* 25:909–911.
39. HERTZ, L., SVENNEBY, G., and SCHOUSBOE, A. 1976. Glutamate and GABA uptake into cultures of normal glia cells. *Trans. Am. Soc. Neurochem.* 7:192.
40. BOCK, E., JØRGENSEN, O. S., DITTMANN, L., and ENG, L. F. 1975. Determination of brain-specific antigens in short term cultivated rat astroglia cells and in rat synaptosomes. *J. Neurochem.* 25:867–870.
41. SCHOUSBOE, A., FOSMARK, H., and SVENNEBY, G. 1976. Taurine uptake in astrocytes cultured from dissociated mouse brain hemispheres. *Brain Research*, 116:158–164.
42. HALL, Z. W., and KRAVITZ, E. A. 1967. The metabolism of γ-aminobutyric acid (GABA) in the lobster nervous system. I. GABA-glutamate transaminase. *J. Neurochem.* 14:45–54.
43. SCHOUSBOE, A., and HERTZ, L. 1971. Effects of potassium on indicator spaces and fluxes in slices of brain cortex from adult and newborn rats. *J. Neurochem.* 18:67–77.
44. LOWRY, O. H., ROSEBROUGH, N. J., FARR, A. L., and RANDALL, R. J. 1951. Protein measurement with the folin phenol reagent. *J. Biol. Chem.* 193:265–275.
45. MILLER, G. L. 1959. Protein determination for large numbers of samples. *Anal. Chem.* 31:964.
46. SELLSTRÖM, Å., SJÖBERG, L-B., and HAMBERGER, A. 1975. Neuronal and glial systems for γ-aminobutyric acid metabolism. *J. Neurochem.* 25:393–398.
47. BEART, P. M., KELLY, J. S., and SCHON, F. 1974. γ-aminobutyric acid in the rat peripheral nervous system, pineal and posterior pituitary. *Biochem. Soc. Trans.* 2:266–268.
48. SCHOUSBOE, A., WU, J-Y., and ROBERTS, E. 1973. Purification and characterization of the 4-aminobutyrate-2-ketoglutarate transaminase from mouse brain. *Biochemistry* 12:2868–2873.
49. ROBERTS, E. 1962. γ-aminobutyric acid. Pages 636–656 *in* ELLIOTT, K. A. C., PAGE, I. H., and QUASTEL, J. H. (eds.), Neurochemistry, the chemistry of brain and nerve. 2nd ed. Charles C. Thomas, Springfield, Illinois.
50. WILSON, S. H., SCHRIER, B. K., FARBER, J. L., THOMPSON, E. J., ROSENBERG, R. N., BLUME, A. J., and NIRENBERG, M. W. 1972. Markers for gene expression in cultured cells from the nervous system. *J. Biol. Chem.* 247:3159–3169.
51. COHEN, S. R., and LAJTHA, A. 1972. Amino acid transport. Pages 543–572, *in* LAJTHA, A. (ed.), Handbook of neurochemistry, Vol. 7, Plenum Press, New York.
52. MARTIN, D. L., and SMITH, A. A., III. 1972. Ions and the transport of gamma-aminobutyric acid by synaptosomes. *J. Neurochem.* 19:841–855.
53. QUASTEL, J. H. 1974. Amino acids and the brain. *Biochem. Soc. Trans.* 2:765–780.
54. SELLSTRÖM, Å., and HAMBERGER, A. 1975. Neuronal and glial systems for γ-aminobutyric acid transport. *J. Neurochem.* 24:847–852.
55. HYDE, J. C., and ROBINSON, N. 1974. Appearance of gamma aminobutyrate transaminase activity in developing rat brain. *J. Neurochem.* 23:365–367.
56. VAN GELDER, N. M. 1965. The histochemical demonstration of γ-aminobutyric acid metabolism by reduction of a tetrazolium salt. *J. Neurochem.* 12:231–237.

57. LEVI, G., POCE, N., and RAITERI, M. 1976. Uptake and exchange of GABA and glutamate in isolated nerve endings. Pages 273–289, *in* LEVI, G., BATTISTIN, L., and LAJTHA, A. (eds.), Transport phenomena in the nervous system, physiological and pathological aspect, Vol. 69, Advances in experimental medicine and biology, Plenum Press, New York.
58. HEBB, C. 1970. CNS at the cellular level: Identity of transmitter agents. *Ann. Rev. Physiol.* 32:165–192.
59. ORREGO, F., and MIRANDA, R. 1976. Electrically induced release of (^{3}H) GABA from neocortical thin slices. Effects of stimulus waveform and of aminooxyacetic acid. *J. Neurochem.* 26:1033–1038.
60. WOOD, J. D. 1967. A possible role for gamma-aminobutyric acid in the homeostatic control of brain metabolism under conditions of hypoxia. *Exptl. Brain Research* 4:81–84.