A.C.H. Yu, L. Hertz, M.D. Norenberg, E. Syková and S.G. Waxman (Eds.)
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CHAPTER 17

Regulatory role of astrocytes for neuronal biosynthesis and homeostasis of glutamate and GABA

A. Schousboe[1], N. Westergaard[1], U. Sonnewald[2], S.B. Petersen[2], A.C.H. Yu[3] and L. Hertz[4]

[1] PharmaBiotec Research Center, The Neurobiology Unit, Department of Biological Sciences, Royal Danish School of Pharmacy, DK-2100 Copenhagen, Denmark; [2] SINTEF, MR-Center, N-7030 Trondheim, Norway; [3] Department of Pathology, Stanford University School of Medicine, Stanford, CA 94305, U.S.A.; and [4] Departments of Pharmacology and Anesthesia, University of Saskatchewan, Saskatoon, S7N OWO Sask., Canada

Introduction

Studies of incorporation of radioactivity from different [^{14}C] labeled precursors (glucose, acetate, bicarbonate) into glutamate and glutamine in brain tissue preparations performed two decades ago indicated that glutamate metabolism was extremely complicated (Berl and Clarke, 1969). It was observed that glutamine could attain a much higher specific radioactivity than its precursor glutamate and it was concluded that two or more metabolically distinct pools of glutamate must exist in the brain, each of which is metabolized to glutamine with different turnover rates. It was further envisaged that these pools might well represent different cell types and it was predicted that glutamine synthesis from glutamate would be particularly prominent in one of these pools which in all likelihood would be localized in glial cells rather than in neurons. That this is indeed the case was firmly established by the elegant demonstration by Martinez-Hernandez et al. (1977) and Norenberg and Martinez-Hernandez (1979) that the glutamine synthesizing enzyme glutamine synthetase (GS) is localized in astrocytes and not in neurons. As a consequence of this there must be a cycling of glutamate, glutamine and presumably GABA between neurons and astrocytes and this cycling has been defined as the glutamate/glutamine cycle (Balazs et al., 1972; Benjamin and Quastel, 1974).

The use of relatively intact brain tissue preparations or bulk-prepared cellular preparations did not allow studies to be performed of exact fluxes of the amino acids between these compartments. The subsequent development of primary cultures of different types of neurons and of astrocytes (cf. Hertz et al., 1985) has made it possible to study these parameters as well as the biochemical conversions of these amino acids in the individual cell types. These studies have allowed a quantitative assessment of these parameters to be made (cf. Schousboe et al., 1983a; Hertz and Schousboe, 1986). The fact that it has recently become feasible to also combine individual cell types in well defined co-cultures (Westergaard et al., 1991, 1992) has offered an additional tool by which these parameters can be studied. The present review will provide an overview of the current status of the knowledge about transmembrane fluxes of glutamate, glutamine and GABA and their apparent metabolic interconversions.

Transmembrane fluxes of amino acids

Glutamate/glutamine

Based upon a large number of studies of

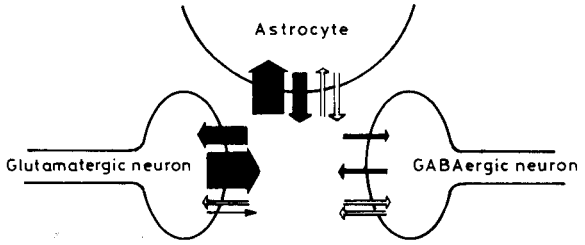

Fig. 1. Schematic drawing of release and uptake of glutamate (➡) and glutamine (⇒) from glutamatergic or GABAergic neurons and astrocytes. The release of glutamate from the glutamatergic neurons corresponds to that observed during excitation. The sizes of the arrows give an estimate of the relative magnitudes of the respective fluxes. It can be seen that the majority of the neuronally released glutamate is accumulated into astrocytes. Moreover, it should be noted that very little glutamine is being released from glutamatergic neurons. (From Schousboe et al., 1988.)

glutamate uptake into primary cultures of astrocytes from different brain areas and different types of neurons it can be concluded that the rate of astrocytic glutamate uptake is higher than the uptake rate into neurons. This is illustrated in Fig. 1 which shows glutamate uptake into the two cell types at an extracellular glutamate concentration of 50 μM. Fig. 1 also shows the corresponding release of glutamate from neurons and astrocytes and it can be seen that in glutamatergic neurons there must be a net loss of glutamate during excitation and that this loss of glutamate is matched by a corresponding net uptake into astrocytes. As expected there does not appear to be a major net release of glutamate from GABAergic neurons as uptake and release rates are of the same relatively small magnitude. Analogous studies in the cultured cells of fluxes of glutamine has allowed similar calculations of uptake and release of this amino acid to be performed, assuming that the extracellular glutamine concentration is 500 μM. Fig. 1 shows that transmembrane fluxes of glutamine in neurons and astrocytes are smaller than those of glutamate. It also shows that there is a net loss of glutamine from astrocytes and a net uptake of glutamine in both glutamatergic and GABAergic neurons. However, in the glutamatergic neurons the uptake of glutamine does not quantitatively correspond to the loss of glutamate. It has therefore been postulated (Schousboe and Hertz, 1983; Hertz and Schousboe, 1986, 1987; Schousboe et al., 1988; Shank and Aprison, 1988) that other precursors for biosynthesis of transmitter glutamate may exist such as, e.g., TCA cycle constituents. In order for a neuron to utilize TCA constituents for biosynthesis of glutamate it must possess a biosynthetic pathway allowing a net synthesis of TCA constituents from glucose. This requires the presence of pyruvate carboxylase. However, pyruvate carboxylase is not present in neurons but only in astrocytes (Yu et al., 1983; Shank et al., 1985). Therefore, neurons are dependent on supply of such precursors from astrocytes. This means that regardless of whether the precursor for transmitter glutamate is glutamine or a tricarboxylic acid constituent, a communication between neurons and astrocytes must take place as the astrocytes control the availability of these metabolites. This aspect will be dealt with in more detail below.

GABA

From numerous studies of GABA transport in neurons and astrocytes it seems reasonable to conclude that most synaptically released GABA is reaccumulated into the pre-synaptic nerve endings although a smaller fraction is taken up into surrounding astrocytes (Schousboe and Hertz, 1983; Hertz and Schousboe, 1987). It is therefore likely that GABAergic neurons during activity lose some GABA. This is indirectly indicated by the demonstration that selective inhibitors of astroglial GABA uptake are able not only to increase the synaptic GABA pool but also to protect against seizures caused by a reduction in the efficacy of GABA mediated neurotransmission (cf. Schousboe, 1990). Since there appears to be a net uptake of glutamine in GABAergic neurons (cf. Fig. 1) it is possible that the loss of GABA is compensated for by influx of glutamine which is a good precursor for neurotransmitter GABA (Besson et al., 1981; Hertz et al., 1992a).

Glutamatergic neurons and astrocytes

Cerebellar granule neurons, which have glutamatergic properties (Hertz and Schousboe, 1987) develop morphologically and functionally when seeded on top of a preformed confluent layer of cerebellar astrocytes but not on astrocytes from cerebral cortex (Westergaard et al., 1991). The exact reason for this has not been worked out but in all likelihood it involves differences in cell surface characteristics in the two different types of astrocytes. A full discussion of this is, however, beyond the scope of this review.

Table I shows that the biochemical and functional properties of the neurons with regard to their glutamatergic nature have been preserved in the co-culture system. It is seen that the activity of aspartate aminotransferase (AAT), which is a key enzyme in the biosynthesis of transmitter glutamate (Palaiologos et al., 1988), was similar in cerebellar granule cells cultured alone or on top of cerebellar astrocytes. The ability to release neurotransmitter, monitored by depolarization coupled, Ca^{2+}-dependent release of preloaded [^{3}H]D-aspartate was found to be higher in granule neurons cultured alone compared to the neurons cultured on top of astrocytes. This may be explained by an uptake of the transmitter into the astrocytes but further experimentation is needed to fully understand this phenomenon. Similarly, the astrocytes had retained their high activity of dexamethasone-inducible glutamine synthetase (Table II), which is a glia-specific enzyme not expressed in neurons (Norenberg and Martinez-Hernandez, 1979). From these findings it is concluded that this co-culture model of glutamatergic neurons and astrocytes may serve as a tool to investigate the metabolic interaction between these two cell types with regard to biosynthesis of transmitter glutamate (cf. below).

TABLE I

Specific activity of aspartate aminotransferase and transmitter release in co-cultures of cerebellar granule cells and astrocytes from cerebellum

Culture type	Aspartate amino-transferase activity (nmol/min per mg)	Transmitter release (% of basal)
Granule cells (G)	382 ± 25	3150 ± 482
Astrocytes, cerebellum (A_{ce})	172 ± 6	–
G/A_{ce})	276 ± 12	257 ± 21

Specific activity (nmol/min per mg protein) of AAT and K^+-stimulated, Ca^{2+}-dependent [^{3}H]-D-aspartate release (% of basal) in cerebellar granule cells or astrocytes cultured alone or as sandwich co-cultures where neurons were seeded on top of a preformed layer of astrocytes. Results are averages of 7 – 9 cell preparations ± S.E.M. (From Westergaard et al., 1991.)

TABLE II

Specific activity of GS in co-cultures of cerebellar granule cells and astrocytes from cerebellum

Culture type	Glutamine synthetase activity (nmol/min per mg)	
	Plain medium	Dexamethasone (10 μM)
Granule cells (G)	2.6 ± 0.1	5.4 ± 0.4
Astrocytes, cerebellum (A_{ce})	9.3 ± 0.7	49.8 ± 2.8
G/A_{ce}	7.3 ± 0.5	33.7 ± 3.7

Specific activity (nmol/min per mg protein) of GS in cerebellar granule cells or astrocytes cultured alone or as sandwich co-cultures. In some cases the culture medium contained 10 μM dexamethasone during the last 48 h of the culture period. Results are averages of 5 – 14 cell preparations ± S.E.M. (From Westergaard et al., 1991.)

GABAergic neurons and astrocytes

In an analogous study it has been shown that neurons from cerebral cortex survive and differentiate equally well when cultured on top of a preformed layer of astrocytes from either cerebral cortex or cerebellum (Westergaard et al., 1992). Apparently, different types of neurons exhibit different requirements with regard to the astrocytic matrix on which they grow. Again, a detailed discussion of this interesting question is beyond the scope of this review. As shown in Table III the neurons expressed

TABLE III

Specific activity of glutamate decarboxylase in sandwich co-cultures or cerebral cortical neurons and astrocytes from cerebellum or cerebral cortex as well as in the respective pure cell cultures

Culture type	Glutamate decarboxylase activity	
	mU/mg protein	mU/dish
Cortical neurons (N)	0.52 ± 0.06	0.48 ± 0.08
Astrocytes, cerebellum (A_{ce})	< 0.05	
N/A_{ce}	0.46 ± 0.06	0.52 ± 0.04
Astrocytes, cortex (A_{co})	< 0.05	
N/A_{co}	0.34 ± 0.04	0.32 ± 0.01

Specific activity (mU/mg protein or mU/dish) of GAD in cortical neurons or astrocytes from cerebellum or cerebral cortex cultured alone or as sandwich co-cultures. Values are averages of 7–15 cell preparations ± S.E.M. (From Westergaard et al., 1992.)

glutamate decarboxylase (GAD) activity under all culture conditions indicating that they behave in these culture systems like GABAergic neurons, as has previously been reported for cultured cerebral cortical neurons (Yu et al., 1984a; Drejer et al., 1987; Hertz and Schousboe, 1987; Kuriyama and Ohkuma, 1987). The finding that the co-cultures expressed GAD to the same extent as the pure neuronal cultures despite the fact that they contained fewer neurons (~ 50% less) indicates that the presence of astrocytes in these cultures may lead to an enhanced expression of GAD in the neurons. The astrocytes in the co-cultures were shown to express glutamine synthetase to the same extent as their neuron-free counterparts (Table IV). With regard to a further characterization of the GABAergic property of the neurons it was shown that in the co-cultures potassium- or glutamate-stimulated, Ca^{2+}-dependent GABA release was comparable to that observed in pure cultures of cerebral cortical neurons (Westergaard et al., 1992).

These co-cultures have been used to investigate interactions between neurons and astrocytes with regard to GABA homeostasis during a depolarization coupled release of GABA. Fig. 2 shows the effect of the astroglial selective GABA uptake inhibitor 4,5,6,7-tetrahydroisoxazolo[4,5-*c*]pyridin-3-ol (THPO) on potassium stimulated [^{3}H]GABA release in co-cultures of neurons and astrocytes. It is seen that in the presence of 1 mM THPO, K^+-stimulated GABA release was enhanced, suggesting that under normal conditions a fraction of the released GABA is likely to be taken up into the astrocytes. Since these co-cultures apparently constitute a model system in which there appears to be a dynamic interaction between GABAergic neurons and astrocytes they have recently been employed to illustrate metabolic interactions between these cell types, employing the NMR technique to follow the fate of [^{13}C]labeled metabolites (Sonnewald et al., 1991, 1992). An overview of these studies will be given below.

TABLE IV

Specific activity of glutamine synthetase in co-cultures of cortical neurons and astrocytes from cerebellum or cerebral cortex

Culture type	Glutamine synthetase activity (nmol/min per mg)	
	Plain medium	Dexamethasone (10 μM)
Cortical neurons (N)	1.6 ± 0.1	5.5 ± 0.7
Astrocytes, cerebellum (A_{ce})	22.4 ± 4.5	114.7 ± 8.0
N/A_{ce}	15.3 ± 1.4	49.2 ± 2.0
Astrocytes, cortex (A_{co})	12.4 ± 1.9	79.7 ± 9.6
N/A_{co}	10.9 ± 0.6	28.8 ± 1.9

Specific activity (nmol/min per mg protein) of GS in cortical neurons or astrocytes from cerebellum or cerebral cortex cultured alone or as sandwich co-cultures. In some cases the culture medium contained 10 μM dexamethasone during the last 48 h of the culture period. Values are averages of 6–12 cell preparations ± S.E.M. (From Westergaard et al., 1992.)

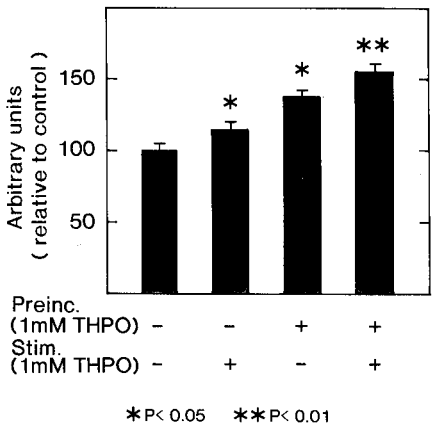

Fig. 2. Evoked release of [^{3}H]GABA as percent of basal release in sandwich co-cultures of cerebral cortical neurons and cerebellar astrocytes in the presence of 1 mM THPO in the preincubation media (Preinc.) and/or in the stimulation media (Stim.) together with 55 mM K^+. Results are expressed in arbitrary units relative to the control value (100) which was 215% of the basal release. Results are averages of 7 – 16 experiments with S.E.M. indicated by vertical bars. The asterisks indicate statistically significant differences compared to the control value (* $P < 0.05$; ** $P < 0.01$; ANOVA F-test). (From Westergaard et al., 1992.)

Precursors for glutamate and GABA formation

Glutamate

Based on a large number of studies of labeling of releaseable glutamate from many different brain tissue and brain cell preparations it can be concluded that glutamine plays a major role as a precursor for transmitter glutamate (cf. Hertz and Schousboe, 1986, 1988). However, as can be seen from Fig. 3 also other compounds such as TCA cycle constituents (cf. Shank and Aprison, 1988) may serve this function. As pointed out above, due to the fact that neurons lack the capacity to synthesize these compounds, they are totally dependent on a supply from astrocytes which contain the enzymes glutamine synthetase and pyruvate carboxylase required for this synthesis (Norenberg and Martinez-Hernandez, 1979; Yu et al., 1983; Shank et al., 1985).

Based on a study of the ability of the aspartate aminotransferase inhibitor aminooxyacetic acid and the mitochondrial dicarboxylate carrier inhibitor phenylsuccinate to inhibit release of transmitter glutamate from cultured cerebellar granule cells, Palaiologos et al. (1988) demonstrated that synthesis of transmitter glutamate from glutamine requires the activity of these two proteins. On the basis of this finding, it was proposed that glutamate formed by glutaminase catalyzed hydrolysis of glutamine enters the mitochondria in exchange for aspartate. Subsequently glutamate is transaminated in the matrix to form α-ketoglutarate and aspartate, and the α-ketoglutarate can be transported into the cytosol in exchange with malate utilizing the dicarboxylate carrier. Finally glutamate is formed from α-ketoglutarate in the cytosol by transamination with aspartate catalyzed by the cytoplasmic AAT (cf. Fig. 3). If this model is correct, it would be predicted that if α-ketoglutarate is supplied to the cells together with an amino acid donor, transmitter glutamate should be formed in an phenylsuccinate-insensitive manner. This has recently been shown to be the case (Peng et al., 1991), indicating not only that this mechanism for synthesis of transmitter glutamate may be correct but also that α-ketoglutarate can be used as a precursor as previously suggested (Hertz and Schousboe, 1988; Shank and Aprison, 1988). Operation of this mechanism is further supported by the demonstration that phenylsuccinate completely blocks potassium-stimulated glutamate release in rat hippocampus in vivo (Christensen et al., 1991). Moreover, it has been shown by Kihara and Kubo (1989) that release of transmitter glutamate from brain slices is increased in the presence of α-ketoglutarate.

Also other TCA cycle constituents may serve as precursors for transmitter glutamate. In cerebellar granule cells [^{14}C]malate is taken up and rapidly converted into [^{14}C]glutamate in an aminooxyacetic acid-sensitive manner, indicating that α-ketoglutarate formed from malate in the mitochondria undergo transamination rather than reductive amination in order to be converted to glutamate (Hertz and Schousboe, 1988; Hertz et al., 1992b). Since a net synthesis of TCA constituents from glucose only can take place in astrocytes, it is assumed

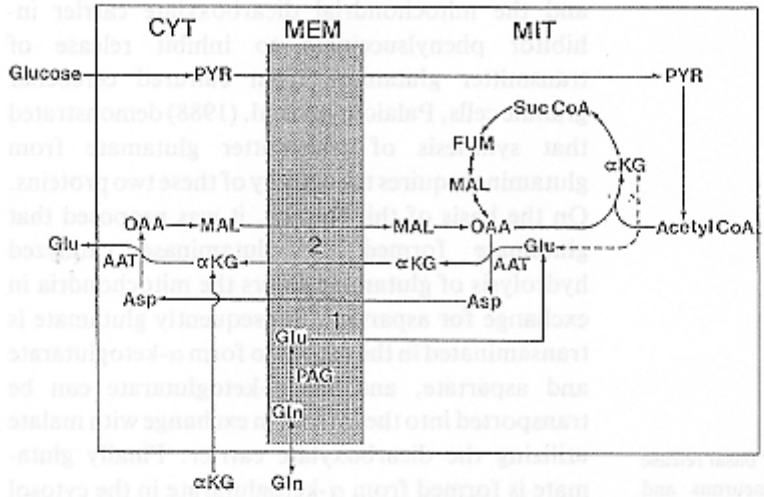

Fig. 3. Schematic representation of reactions involved in biosynthesis of transmitter glutamate from glutamine or from αKG. For details, see text. CYT, Cytoplasm; MEM, mitochondrial membrane; MIT, intra-mitochondrial; OAA, oxaloacetate; MAL, malate; αKG, α-ketoglutarate; PYR, pyruvate; FUM, fumarate; Asp, aspartate; Glu, glutamate; Gln, glutamine; 1, aspartate/glutamate carrier; 2, ketodicarboxylate carrier; PAG, phosphate activated glutaminase; AAT, aspartate aminotransferase. (From Hertz et al., 1992a.)

that any neuronal synthesis of glutamate based on TCA cycle constituents as precursors would require release of these compounds from astrocytes. That such a release can indeed take place has recently been demonstrated by Sonnewald et al. (1991), using cultured neurons and astrocytes separated or in co-culture, and incubation with 1-[^{13}C]-glucose followed by [^{13}C]NMR spectroscopy of cell extracts and lyophilized and redissolved media. As demonstrated in Fig. 4 media from astrocytes and co-cultures of astrocytes and neurons, but not from neurons, were found to contain relatively large amounts of [^{13}C]labeled citrates derived from glucose. Whether also other TCA cycle constituents may be released from the astrocytes remains to be shown but regardless of this, the ability to release citrate clearly demonstrates that astrocytes can perform a net synthesis of TCA cycle constituents at the expense of glucose whereas neurons cannot. In relation to the demonstration (Peng et al., 1991) that alanine can serve as an amino group donor for synthesis of transmitter glutamate, it is interesting that the media from astrocytes also contained [^{13}C]alanine whereas media from the neurons did not. This indicates that there could be a transfer of alanine from astrocytes to neurons. In this context it should be noted that neurons have a large capacity for alanine transport via a carrier with relatively high affinity for alanine (N. Westergaard and A. Schousboe, unpublished observations). Not surprisingly the media from astrocytes but not neurons contained [^{13}C]glutamine confirming the astrocytic localization of glutamine synthetase (Norenberg and Martinez-Hernandez, 1979).

GABA

Transmitter GABA is synthesized from glutamate by the action of glutamate decarboxylase, which is found only in GABAergic neurons (Roberts, 1979). However, it appears that in the brain in vivo (Reubi et al., 1978; Besson et al., 1981), in synaptosomes (Hamberger et al., 1978; Ward et al., 1983) as well as in cultured GABAergic neurons (Yu et al., 1984b) glutamine is a better precursor for GABA than glutamate. The exact mechanism by which glutamine is transformed into GABA is not

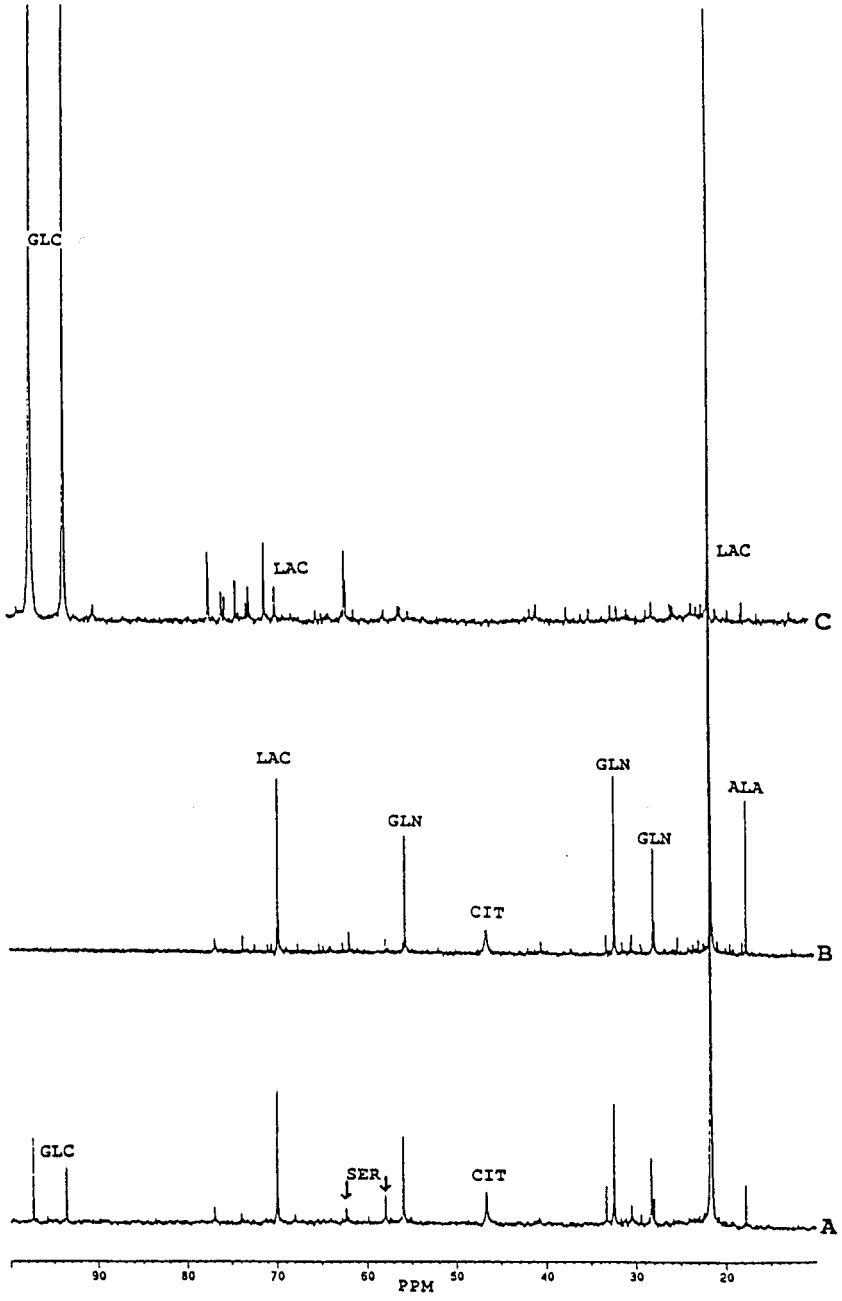

Fig. 4. [^{13}C]NMR spectra of lyophilized media collected from co-cultures of cerebral cortical neurons and astrocytes (*A*) and cultures of cortical astrocytes (*B*) or neurons (*C*) after incubation of the cells for 48 h (*A, B*) or 20 h (*C*) in culture media containing 6 mM [1-^{13}C]glucose. GLC, Glucose; LAC, lactate; GLN, glutamine; ALA, alanine; SER, serine; CIT, citrate. (From Sonnewald et al., 1991.)

quite clear. It is possible that it could require the participation of the complex exchange processes shown in Fig. 3 but direct evidence for this is so far lacking. There are, however, some indications that this may not be the prevailing synthetic route for GABA derived from glutamine. Kihara and Kubo (1989) failed to demonstrate an enhanced GABA synthesis in brain slices after inclusion of α-ketoglutarate in the media, and labeled malate is not incorporated into GABA in cultured GABAergic neurons in spite of the fact that malate is oxidatively metabolized in these cells (Hertz et al., 1992b). However, it has been shown that [^{15}N] can be incorporated into glutamate from alanine and leucine indicating that transamination of α-ketoglutarate does occur (Yudkoff et al., 1990). It also appears that the glutamate dehydrogenase reaction is operating in both directions since [^{14}C]glutamine can be oxidized to $^{14}CO_2$ in an aminooxyacetic acid-insensitive manner (Yu et al., 1984b) and $^{15}NH_3$ can be incorporated into glutamate (Yudkoff et al., 1990). These findings indicate that some parts of oxidative metabolism may occur in a compartment different from that responsible for conversion of glutamine to GABA. Since both of these processes require mitochondrial enzymes it can be hypothesized that GABAergic neurons may contain different types of mitochondria, some of which are primarily used for oxidative metabolism whereas others are mainly involved in synthesis of GABA. Further metabolic studies using different labeled precursors in combination with specific inhibitors are required in order to obtain information pertinent to this hypothesis.

Recently GABA synthesis from 1-[^{13}C]glucose and 2-[^{13}C]acetate has been studied in co-cultures of GABAergic neurons and astrocytes (cf. above) using NMR spectroscopy (Sonnewald et al., 1991, 1992). In the co-cultures as well as in cultures of neurons alone labeling of GABA in positions C-2, C-3 and C-4 and glutamate in the corresponding positions could be detected in cell extracts after incubation with 1-[^{13}C]glucose for 20 – 48 h (Fig. 5). Since the cultures were incubated for a long period of time no conclusions can be drawn regarding the character of the communication between neurons and astrocytes, but the results unequivocally show that in the neurons there is metabolic communication between a mitochondrial compartment accessible to endogenous acetyl-CoA produced from glucose and a glutamate pool from which GABA is synthesized extramitochondrially. In another set of experiments (Sonnewald et al., 1992) it was demonstrated that in co-cultures, labeling of GABA from 2-[^{13}C]acetate could be completely blocked by methionine sulphoximine which inhibits glutamine synthesis (Ronzio et al., 1969). This shows that glutamine derived from acetate in astrocytes serves as a precursor for GABA indicating that indeed a metabolic interaction takes place between the neurons and the astrocytes in these cultures and confirming that the astrocytes comprise the metabolic compartment in which acetate is metabolized (Berl and Clarke, 1969). Further studies of metabolic processes in these cultures using NMR spectroscopy may throw new light on the mechanism by which neurotransmitter GABA may be formed from metabolites originating from astrocytes and it may be possible to elucidate further the regulatory role which is played by astrocytes with regard to synthesis of transmitter amino acids in neurons.

Role of alanine in neuronal-glia communication

As mentioned above alanine has been shown to function as an amino group donor for synthesis of transmitter glutamate in cerebellar granule neurons (Peng et al., 1991) and it is selectively synthesized from glucose and released into the media in astrocytes and not in neurons (Sonnewald et al., 1991). This prompted a study of the ability of neurons and astrocytes to transport alanine. It was found that both GABAergic and glutamatergic neurons have a high capacity for alanine uptake with very high V_{max} values and K_m values around 200 μM (N. Westergaard and A. Schousboe, unpublished observations). Astrocytes also possess such an uptake system for alanine but the V_{max} is lower. This is compatible with a preferential uptake of alanine into neurons. In this context it may be of

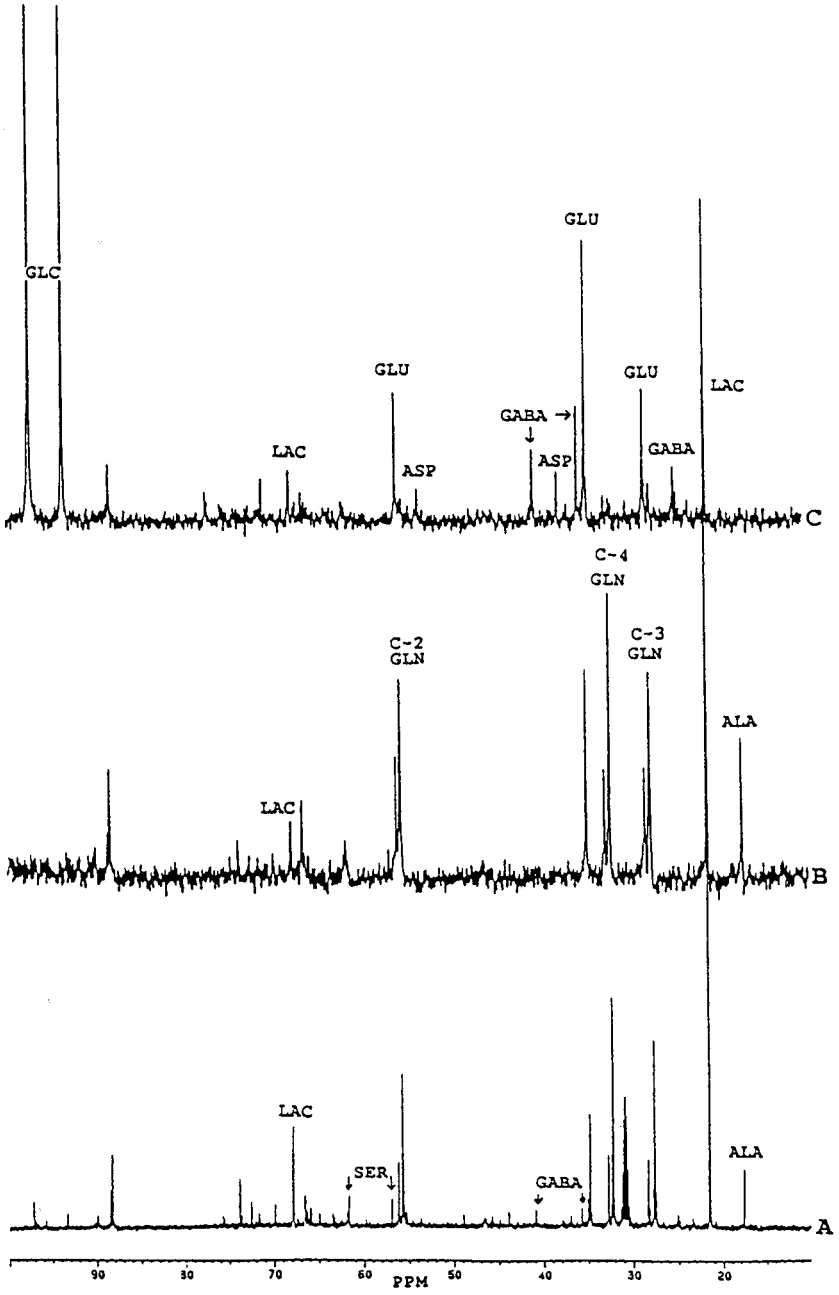

Fig. 5. [^{13}C]NMR spectra of PCA extracts of co-cultures of cerebral cortical neurons and astrocytes (*A*) and cultures of cortical astrocytes (*B*) or neurons (*C*) after incubation of the cells for 48 h (*A, B*) or 20 h (*C*) in culture media containing 6 mM [1-^{13}C]glucose. GLC, Glucose; LAC, lactate; GLU, glutamate; ASP, aspartate; GABA, γ-aminobutyrate; GLN, glutamine; ALA, alanine; SER, serine. (From Sonnewald et al., 1991.)

interest that following ischemia there is a large overflow of alanine in hippocampus (Benveniste et al., 1984). This could indicate that post-ischemic conversion of lactate to pyruvate and subsequent transamination to form alanine takes place preferentially in astrocytes. Since this process requires glutamate it may play a role in the maintenance of low astrocytic glutamate levels following ischemia which in turn may decrease glutamatergic activity. At the moment no direct proof for this is available but further metabolic studies in co-cultures of neurons and astrocytes may provide clues to this hypothesis.

Pathological consequences of astrocyte malfunction

Glutamate and neurodegeneration

It is well established that glutamate and other endogenous excitatory amino acids can cause extensive neuronal damage if the extracellular concentration in the brain becomes elevated (Rothman and Olney, 1986; Choi, 1988; Schousboe et al., 1991a). Such increases in extracellular brain glutamate and aspartate levels are known to occur during pathological states such as ischemia, hypoxia and hypoglycemia (Benveniste et al., 1984; Sandberg et al., 1986). One important factor contributing to this increase in extracellular glutamate is likely to be a failure in high affinity glutamate uptake present primarily in astrocytes but also significantly in glutamatergic neurons (Schousboe et al., 1988). This glutamate carrier is dependent on the transmembrane sodium gradient and accordingly sensitive to changes in the energy state of the cells (Nicholls and Attwell, 1990). Direct evidence that a considerable part of the glutamate overflow accompanied by ischemia originates from a non-transmitter pool has recently been provided by Christensen et al. (1991). Using the microdialysis technique combined with HPLC analysis of glutamate it was shown that the drug phenylsuccinate (cf. above) completely blocked K^+-stimulated glutamate overflow in rat hippocampus whereas it had no effect on glutamate overflow associated with 20 min ischemia. Since phenylsuccinate selectively inhibits de novo synthesis of glutamate in the transmitter-related pool leaving the non-transmitter-related glutamate pool intact it is likely that this latter pool contributes a significant amount of the glutamate released into the extracellular space during ischemia which is associated with energy failure (Siesjö, 1978). In line with this conclusion, it has been shown that in cultured neurons the cytotoxicity of glutamate and aspartate is greatly increased by a blocker of high affinity glutamate/aspartate uptake whereas the toxicity of non-transportable glutamate receptor agonists was unaffected (Frandsen and Schousboe, 1990). It must therefore be concluded that an intact uptake system for glutamate is of key importance with regard to protection against the neurotoxic action of glutamate and other endogenous excitatory amino acids. Since glutamate uptake is primarily an astrocytic function, normal function of the astrocytes is of critical importance. Any increase in release of glutamate from these cells is therefore likely to have pathological consequences and efforts should be made to devise methods by which such release can be prevented.

GABA and epilepsy

Seizure activity and epilepsy are generally associated with an imbalance in excitatory and inhibitory activity (Schousboe, 1990). With regard to the latter, diminished GABAergic activity will almost inevitably lead to seizure activity (Meldrum, 1975; Wood, 1975; Schousboe, 1990). Since removal of GABA from the synaptic cleft by the astrocytic GABA carrier will cause a drain of GABA from the neurotransmitter GABA pool, it was suggested (Schousboe, 1979) that one way to enhance GABAergic activity might be to prevent GABA from being taken up into astrocytes. Subsequently, using GABA analogues of restricted conformation the neuronal and glial GABA uptake systems were characterized (Schousboe et al., 1978, 1979, 1981, 1983b) and two bicyclic isoxazoles, THPO (cf. above) and THAO (4,5,6,7-tetrahydroisoxazolo-[4,5-*c*]azepin-3-ol) were found to be selective glial

GABA uptake inhibitors. It was later demonstrated by different groups of investigators (Horton et al., 1979; Krogsgaard-Larsen et al., 1981; Wood et al., 1983; Schousboe et al., 1983b, 1986; Seiler et al., 1985; Gonsalves et al., 1989a,b) that these compounds indeed protect mice and chicks against different chemically and light-induced seizures associated with failures in GABAergic neurotransmission. Accordingly, it is possible that drugs whose action is related to astrocytic high affinity GABA uptake may be of potential interest as antiepileptic agents (cf. Krogsgaard-Larsen et al., 1987; Schousboe, 1990; Schousboe et al., 1991b).

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References

Balazs, R., Patel, A.J. and Richter, D. (1972) Metabolic compartments in the brain: their properties and relation to morphological structures. In: R. Balazs and J.E. Cremer (Eds.), *Metabolic Compartmentation in the Brain,* MacMillan, New York, pp. 167 – 184.

Benjamin, A.M. and Quastel, J.H. (1974) Fate of L-glutamate in the brain. *J. Neurochem.,* 23: 457 – 464.

Benveniste, H., Drejer, J., Schousboe, A. and Diemer, N.H. (1984) Elevation of the extracellular concentrations of glutamate and aspartate in rat hippocampus during transient cerebral ischemia monitored by intracerebral microdialysis. *J. Neurochem.,* 43: 1369 – 1374.

Berl, S. and Clarke, D.D. (1969) Compartmentation of amino acid metabolism. In: A. Lajtha (Ed.), *Handbook of Neurochemistry,* Plenum Press, New York, pp. 447 – 472.

Besson, M.J., Gauchy, C., Kemel, M.L. and Glowinski, J. (1981) In vivo release of ^{3}H-GABA synthesized from ^{3}H-glutamine in the substantia nigra and the pallido entopeduncular nuclei in the cat. *Adv. Biochem. Psychopharmacol.,* 30: 95 – 103.

Choi, D. (1988) Glutamate neurotoxicity and diseases of the nervous system. *Neuron,* 1: 623 – 634.

Christensen, T., Bruhn, T., Diemer, N.H. and Schousboe, A. (1991) Effect of phenylsuccinate on potassium- and ischemia-induced release of glutamate in rat hippocampus monitored by microdialysis. *Neurosci. Lett.,* 134: 71 – 74.

Drejer, J., Honoré, T. and Schousboe, A. (1987) Excitatory amino acid-induced release of ^{3}H-GABA from cultured mouse cerebral cortex interneurons. *J. Neurosci.,* 7: 2910 – 2916.

Frandsen, A. and Schousboe, A. (1990) Development of excitatory amino acid induced cytotoxicity in cultured neurones. *Int. J. Dev. Neurosci.,* 8: 209 – 216.

Gonsalves, S.F., Twitchell, B., Harbaugh, R.E., Krogsgaard, P. and Schousboe, A. (1989a) Anticonvulsant activity of intracerebroventricularly administered glial GABA uptake inhibitors and other gabamimetics in chemical seizure models. *Epilepsy Res.,* 4: 34 – 41.

Gonsalves, S.F., Twitchell, B., Harbaugh, R.E. and Krogsgaard-Larsen, P. (1989b) Anticonvulsant activity of the glial GABA uptake inhibitor, THAO, in chemical seizures. *Eur. J. Pharmacol.,* 168: 265 – 268.

Hamberger, A., Chiang, G., Nylen, E.S., Scheff, S.W. and Cotman, C.W. (1978) Stimulus-evoked increase in the biosynthesis of the putative neurotransmitter glutamate in the hippocampus. *Brain Res.,* 143: 549 – 555.

Hertz, L. and Schousboe, A. (1986) Role of astrocytes in compartmentation of amino acid and energy metabolism. In: S. Fedoroff and A. Vernadakis (Eds.), *Astrocytes, Vol. II,* Academic Press, New York, pp. 179 – 208.

Hertz, L. and Schousboe, A. (1987) Primary cultures of GABAergic and glutamatergic neurons as model systems to study neurotransmitter function. I. Differentiated cells. In: A. Vernadakis, A. Privat, J.M. Lauder, P.S. Timiras and E. Giacobini (Eds.), *Model Systems of Development and Aging of the Nervous System,* Nijhoff, Boston, MA, pp. 19 – 31.

Hertz, L. and Schousboe, A. (1988) Metabolism of glutamate and glutamine in neurons and astrocytes in primary cultures. In: E. Kvamme (Ed.), *Glutamine and Glutamate in Mammals, Vol. II,* CRC Press, Boca Raton, FL, pp. 39 – 55.

Hertz, L., Juurlink, B.H.J. and Szuchet, S. (1985) Cell cultures. In: A. Lajtha (Ed.), *Handbook of Biochemistry, Vol. 8,* Plenum, New York, pp. 603 – 661.

Hertz, L., Peng, L., Westergaard, N., Yudkoff, M. and Schousboe, A. (1992a) Neuronal-astrocytic interactions in metabolism of transmitter amino acids of the glutamate family. In: A. Schousboe, N.H. Diemer and H. Kofod (Eds.), *Alfred Benzon Symposium, 32: Drug Research Related to Neuroactive Amino Acids,* Munksgaard, Copenhagen, pp. 30 – 48.

Hertz, L., Yu, A.C.H. and Schousboe, A. (1992b) Uptake and metabolism of malate in neurons and astrocytes in primary cultures. *J. Neurosci. Res.,* in press.

Horton, R.W., Collins, J.F., Anlezark, G.M. and Meldrum, B.S. (1979) Convulsant and anticonvulsant actions in DBA/2 mice of compounds blocking the reuptake of GABA. *Eur. J. Pharmacol.,* 59: 75 – 83.

Kihara, M. and Kubo, T. (1989) Aspartate aminotransferase for synthesis of transmitter glutamate in the medulla oblongata: effect of aminoxyacetic acid and 2-oxoglutarate. *J. Neurochem.*, 52: 1127 – 1134.

Krogsgaard-Larsen, P., Labouta, I.M., Meldrum, B., Croucher, M. and Schousboe, A. (1981) Uptake inhibitor as experimental tools and potential drugs in epilepsy research. In: P.L. Morselli, E.M. Reynolds, K.G. Lloyd, W. Lösher and B.S. Meldrum (Eds.), *Neurotransmitters, Seizures, and Epilepsy,* Raven Press, New York, pp. 23 – 35.

Krogsgaard-Larsen, P., Falch, E., Larsson, O.M. and Schousboe, A. (1987) GABA uptake inhibitors: relevance to antiepileptic drug research. *Epilepsy Res.*, 1: 77 – 93.

Kuriyama, K. and Ohkuma, S. (1987) Development of cerebral cortical GABAergic neurons in vitro. In: A. Vernadakis, A. Privat, J.M. Lauder, P.S. Timiras and E. Giacobini (Eds.), *Model Systems of Development and Aging of the Nervous System,* Nijhoff, Boston, MA, pp. 45 – 56.

Martinez-Hernandez, A., Bell, K.P. and Norenberg, M.D. (1977) Glutamine synthetase: glial localization in brain. *Science,* 195: 1356 – 1358.

Meldrum, B.S. (1975) Epilepsy and γ-aminobutyric acid-mediated inhibition. *Int. Rev. Neurobiol.*, 17: 1 – 36.

Nicholls, D. and Attwell, D. (1990) The release and uptake of excitatory amino acids. *Trends Pharmacol. Sci.*, 3: 462 – 468.

Norenberg, M.D. and Martinez-Hernandez, A. (1979) Fine structural localization of glutamine synthetase in astrocytes of rat brain. *Brain Res.*, 161: 303 – 310.

Palaiologos, G., Hertz, L. and Schousboe, A. (1988) Evidence that aspartate amino transferase activity and ketodicarboxylate carrier function are essential for biosynthesis of transmitter glutamate. *J. Neurochem.*, 51: 317 – 320.

Peng, L., Schousboe, A. and Hertz, L. (1991) Utilization of alpha-ketoglutarate as a precursor for transmitter glutamate in cultured cerebellar granule cells. *Neurochem. Res.*, 16: 29 – 34.

Reubi, J.-C., Van den Berg, C. and Cuenod, M. (1978) Glutamine as precursor for the GABA and glutamate transmitter pools. *Neurosci. Lett.*, 10: 171 – 174.

Roberts, E. (1979) New directions in GABA research, I: immunocytochemical studies of GABA neurons. In: P. Krogsgaard-Larsen, J. Scheel-Krüger and H. Kofod (Eds.), *GABA-Neurotransmitters. Pharmacochemical, Biochemical and Pharmacological Aspects,* Munksgaard, Copenhagen, pp. 28 – 45.

Ronzio, R.A., Rowe, B.W. and Meister, A. (1969) Studies on the mechanism of inhibition of glutamine synthetase by methione sulfoximine. *Biochemistry,* 8: 1066 – 1075.

Rothman, S.M. and Olney, J.W. (1986) Glutamate and the pathophysiology of hypoxic-ischemic brain damage. *Ann. Neurol.*, 19: 105 – 111.

Sandberg, S., Butcher, S.P. and Hagberg, H. (1986) Extracellular overflow of neuroactive amino acids during severe insulin-induced hypoglycemia: in vivo dialysis of the rat hippocampus. *J. Neurochem.*, 47: 178 – 184.

Schousboe, A. (1979) Effects of GABA analogs on the high-affinity uptake of GABA in astrocytes in primary culture. In: P. Mandel and F.V. De Feudis (Eds.), *GABA-Biochemistry and CNS Function,* Plenum, New York, pp. 219 – 237.

Schousboe, A. (1990) Neurochemical alterations associated with epilepsy or seizure activity. In: M. Dam and L. Gram (Eds.), *Comprehensive Epileptology,* Raven Press, New York, pp. 1 – 16.

Schousboe, A. and Hertz, L. (1983) Regulation of glutamatergic and GABAergic neuronal activity by astroglial cells. In: N.N. Osborne (Ed.), *Dale's Principle and Communication between Neurones,* Pergamon Press, Oxford, pp. 113 – 141.

Schousboe, A., Krogsgaard-Larsen, P., Svenneby, G. and Hertz, L. (1978) Inhibition of the high-affinity, net uptake of GABA into cultured astrocytes by β-proline, nipecotic acid and other compounds. *Brain Res.*, 153: 623 – 626.

Schousboe, A., Thorbek, P., Hertz, L. and Krogsgaard-Larsen, P. (1979) Effects of GABA analogues of restricted conformation on GABA transport in astrocytes and brain cortex slices and on GABA receptor binding. *J. Neurochem.*, 33: 181 – 189.

Schousboe, A., Larsson, O.M., Hertz, L. and Krogsgaard-Larsen, P. (1981) Heterocyclic GABA analogues as new selective of astroglial GABA transport. *Drug Dev. Res.*, 1: 115 – 127.

Schousboe, A., Larsson, O.M., Drejer, J., Krogsgaard-Larsen, P. and Hertz, L. (1983a) Uptake and release processes for glutamine, glutamate and GABA in cultured neurons and astrocytes. In: L. Hertz, E. Kvamme, E.G. McGeer and A. Schousboe (Eds.), *Glutamine, Glutamate, and GABA in the Central Nervous System,* Alan R. Liss, New York, pp. 297 – 315.

Schousboe, A., Larsson, O.M., Hertz, L. and Krogsgaard-Larsen, P. (1983b) Transport and metabolism of gamma-aminobutyric acid in neurons and glia: implications for epilepsy. *Epilepsia,* 24: 531 – 538.

Schousboe, A., Hjeds, H., Engler, J., Krogsgaard-Larsen, P. and Wood, J.D. (1986) Tissue distribution, metabolism, anticonvulsant efficacy, and effect on brain amino acid levels of the glia-selective γ-aminobutyric acid transport inhibitor 4,5,6,7-tetrahydroisoxazolo[4,5-*c*]pyridin-3-ol in mice and chicks. *J. Neurochem.*, 47: 758 – 763.

Schousboe, A., Drejer, J. and Hertz, L. (1988) Uptake and release of glutamate and glutamine in neurons and astrocytes in primary cultures. In: E. Kvamme (Ed.), *Glutamine and Glutamate in Mammals, Vol. II,* CRC Press, Boca Raton, FL, pp. 21 – 39.

Schousboe, A., Frandsen, A., Wahl, P. and Krogsgaard-Larsen, P. (1991a) Excitatory amino acid induced cytotoxicity in cultured neurons: role of intracellular Ca^{2+} homeostasis. In: P. Ascher, D. Choi and Y. Christen (Eds.), *Glutamate, Cell Death and Memory,* Springer, Berlin, pp. 137 – 152.

Schousboe, A., Larsson, O.M. and Krogsgaard-Larsen, P.

(1991b) GABA uptake inhibitors as anticonvulsants. In: I. Tunnicliff and B.U. Raess (Eds.), *GABA Mechanisms in Epilepsy,* Alan R. Liss, New York, pp. 165 – 187.

Seiler, N., Sarhan, S., Krogsgaard-Larsen, P., Hjeds, H. and Schousboe, A. (1985) Amplification by glycine of the anticonvulsant effect of THPO, a GABA uptake inhibitor. *Gen. Pharmacol.,* 15: 509 – 511.

Shank, R.P. and Aprison, M.H. (1988) Glutamate as a neurotransmitter. In: E. Kvamme (Ed.), *Glutamine and Glutamate in Mammals, Vol. II,* CRC Press, Boca Raton, FL, pp. 3 – 19.

Shank, R.P., Bennet, G.S., Freytag, S.D. and Campbell, G.L. (1985) Pyruvate carboxylase: an astrocyte-specific enzyme implicated in the replenishment of amino acid neurotransmitter pools. *Brain Res.,* 329: 364 – 367.

Siesjö, B.K. (1978) *Brain Energy Metabolism,* Wiley, New York.

Sonnewald, U., Westergaard, N., Krane, J., Unsgard, G., Petersen, S.B. and Schousboe, A. (1991) First direct demonstration of preferential release of citrate from astrocytes using [^{13}C]NMR spectroscopy of cultured neurons and astrocytes. *Neurosci. Lett.,* 128: 235 – 239.

Sonnewald, U., Westergaard, N., Schousboe, A., Svendsen, J.S., Unsgård, G. and Petersen, S.B. (1992) Direct demonstration by [^{13}C]NMR spectroscopy that glutamine from astrocytes is a precursor for GABA synthesis in neurons. *Neurochem. Int.,* in press.

Ward, H.K., Thanki, T.M. and Bradford, H.F. (1983) Glutamine and glucose as precursors of transmitter amino acids: ex vivo studies. *J. Neurochem.,* 40: 855 – 860.

Westergaard, N., Fosmark, H. and Schousboe, A. (1991) Metabolism and release of glutamate in cerebellar granule cells co-cultured with astrocytes from cerebellum or cerebral cortex neurons. *J. Neurochem.,* 56: 59 – 66.

Westergaard, N., Larsson, O.M., Jensen, B. and Schousboe, A. (1992) Synthesis and release of GABA in cerebral cortical neurons co-cultured with astrocytes from cerebral cortex or cerebellum. *Neurochem. Int.,* 20: 567 – 575.

Wood, J.D. (1975) The role of gamma-aminobutyric acid in the mechanism of seizures. *Prog. Neurobiol.,* 5: 79 – 95.

Wood, J.D., Johnson, D.D., Krogsgaard-Larsen, P. and Schousboe, A. (1983) Anticonvulsant activity of the glial-selective GABA uptake inhibitor, THPO. *Neuropharmacology,* 22: 139 – 142.

Yu, A.C.H., Drejer, J., Hertz, L. and Schousboe, A. (1983) Pyruvate carboxylase activity in primary cultures of astrocytes and neurons. *J. Neurochem.,* 41: 1484 – 1487.

Yu, A.C.H., Hertz, E. and Hertz, L. (1984a) Alterations in uptake and release rates for GABA, glutamate and glutamine during biochemical maturation of highly purified cultures of cerebral cortical neurons, a GABAergic preparation. *J. Neurochem.,* 42: 951 – 960.

Yu, A.C.H., Fisher, T.E., Hertz, E., Tildon, J.T., Schousboe, A. and Hertz, L. (1984b) Metabolic fate of [^{14}C]-glutamine in mouse cerebral neurons in primary cultures. *J. Neurosci. Res.,* 11: 351 – 357.

Yudkoff, M., Nissim, I. and Hertz, L. (1990) Glutathione turnover in cultured astrocytes; studies with [^{15}N]glutamate. *J. Neurochem.,* 55: 137 – 145.