

CHAPTER 7

Neuronal–Astrocytic Interactions (TCA Cycling)

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7.1 COMPARTMENTATION OF BRAIN METABOLISM

The brains of all vertebrates contain both neurons and glial cells, and the more evolved the organism the higher is the ratio of astrocytes to neurons (see Hertz and Schousboe¹). It is well known that there are different types of neurons, classified according to their neurotransmitter content. Furthermore, there are different types of glial cells: astrocytes, oligodendrocytes, and microglia. It is commonly believed that astrocytes are a homogeneous cell type, but increasing evidence suggests that heterogeneity also exists among these cells, particularly in different brain regions.² The classic concept of astrocytes acting as a scaffold, keeping neurons in their proper location, has during the last decades been replaced with the more dynamic view that astrocytes actively maintain and support neuronal activity.^{3,4} The present chapter will focus on metabolic interactions between astrocytes and glutamatergic or GABAergic neurons from cerebellum and cerebral cortex, respectively.

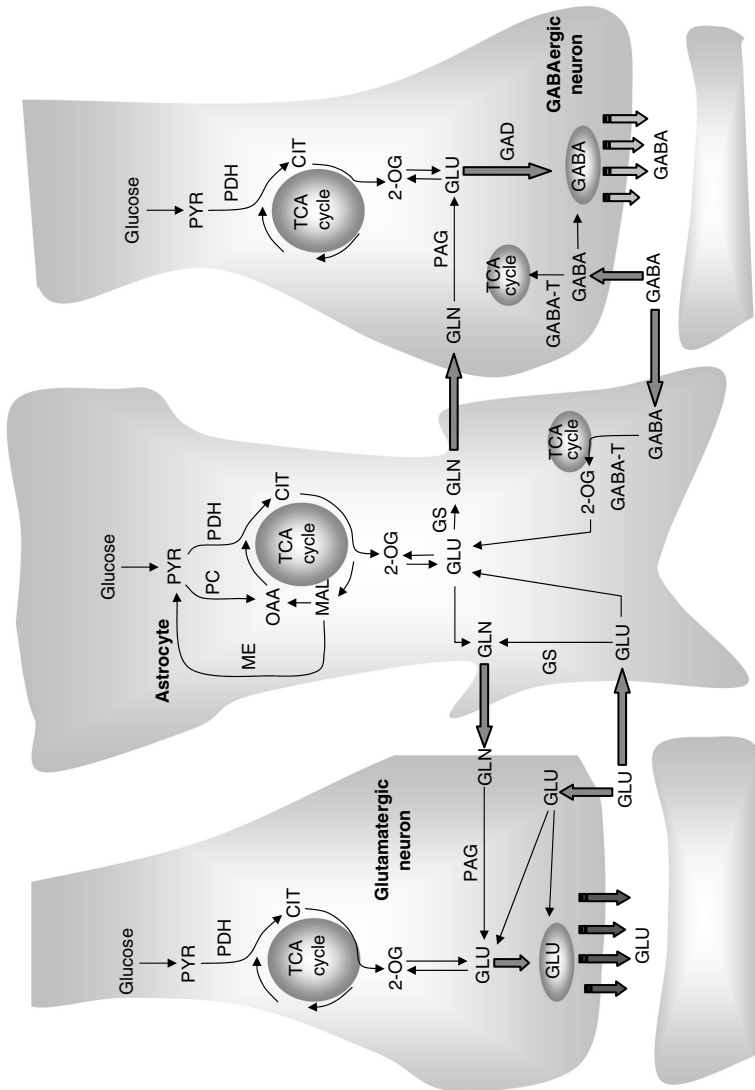


Figure 7.1 Schematic representation of key metabolic processes and release and uptake of neurotransmitters in glutamatergic and GABAergic synapses interacting with a surrounding astrocyte. The vesicular pools of glutamate and GABA are highlighted by ellipses. In the neuronal compartments, glucose and TCA cycle metabolism is indicated. Moreover, the glutamate–glutamine cycle including the glutamine synthetase (GS) reaction is indicated in the glutamatergic neuron–astrocyte interaction. Analogously, the GABA–glutamate–glutamine cycle including the GABA transaminase (GABA-T) and glutamate decarboxylase (GAD) reactions is indicated in the GABAergic neuron–astrocyte interaction. In the astrocytic compartment, pyruvate carboxylation to oxaloacetate via pyruvate carboxylase (PC) is indicated. Additionally, pyruvate recycling by conversion of malate to pyruvate catalyzed by malic enzyme (ME) is shown. One arrow does not imply one reaction only. Abbreviations: CIT, citrate; GAD, glutamate decarboxylase; GABA-T, GABA transaminase; GLN, glutamine; GLU, glutamate; GS, glutamine synthetase; MAL, malate; ME, malic enzyme; 2-OG, 2-oxoglutarate; PAG, phosphate-activated glutaminase; PC, pyruvate carboxylase; PDH, pyruvate dehydrogenase; PYR, pyruvate; TCA, tricarboxylic acid.

The original indication of metabolic compartmentation was based on the observation that a specific labeling of a metabolic product exceeds that of its precursor.⁵ Early studies of glutamate and glutamine metabolism in the brain, using ¹⁴C-labeled precursors such as glucose, acetate, and bicarbonate, clearly suggested that the metabolism of these amino acids had to take place in different compartments i.e., astrocytes and neurons.^{5,6} Subsequently, the concept of exchange of metabolites between these compartments was developed, advancing the proposal of a glutamate–glutamine cycle, metabolically linking glutamatergic neurons and astrocytes (for schematic representation, see Figure 7.1).^{7,8}

7.2 THE GLUTAMATE–GLUTAMINE CYCLE

An important function of astrocytes is to supply glutamatergic neurons with metabolic intermediates, since the neuronal metabolite pool is continuously drained by neurotransmitter release. It should be noted that net synthesis of TCA cycle intermediates, and thus compounds such as glutamate, GABA, and glutamine, depends on an anaplerotic pathway. In the brain, this is preferentially or exclusively achieved by the main anaplerotic enzyme, pyruvate carboxylase (PC), which has a glial localization.^{9–11} The carboxylation of pyruvate with carbon dioxide permits *de novo* synthesis of oxaloacetate, which may react with acetyl coenzyme A (acetyl CoA) to provide net synthesis of the TCA cycle intermediate 2-oxoglutarate, from which glutamate can be formed via transamination or NADH-requiring reductive amination by glutamate dehydrogenase.¹² However, the level of glutamate in astrocytes is low compared to neurons.¹³ Glutamate is converted to glutamine via glutamine synthetase (GS), which, like PC, is exclusively expressed in glial cells.^{14,15} Glutamine is released from astrocytes via astrocyte-specific glutamine transporters¹⁶ and subsequently taken up into neurons by high-affinity glutamine transporters characteristic for these cells.¹⁷ In the neuron, glutamine is converted to glutamate by phosphate-activated glutaminase (PAG).¹⁸ Using postembedding immunogold labeling, high intensities for PAG were found in mitochondria of perikarya and dendrites in most glutamatergic neurons of the cerebellum, whereas nonglutamatergic neurons (Purkinje and Golgi cells) exhibited less labeling for PAG. Glial cell mitochondria were devoid of specific PAG labeling and revealed a much lower glutamate–glutamine ratio than did the mitochondria of mossy fibers.¹⁹ Glutamine from astrocytes is used by the glutamatergic neurons for both neurotransmitter synthesis and energy production.²⁰ In spite of the above findings, it may be noted that PAG has been found in primary cultures of astrocytes, albeit at a level of activity lower than that in neurons.^{21,22}

The major part of glutamate is found in glutamatergic neurons from which it is released during depolarization in a Ca²⁺-dependent manner.^{13,23} Synaptically released glutamate is primarily taken up by astrocytes^{24–26} due to the predominant location on these cells of high-affinity glutamate transporters.^{27–29} Uptake of glutamate is an energy-demanding process requiring one molecule of adenosine triphosphate (ATP) for uptake of one molecule of glutamate.³⁰ In addition, the direct conversion of glutamate to glutamine by GS is also ATP dependent with a similar stoichiometry. In order to enter the TCA cycle, glutamate must be transported into mitochondria to be converted to 2-oxoglutarate and subsequently to oxaloacetate producing 9–12 molecules of ATP. The activities of glutamate dehydrogenase and amino transferases have been shown to be high in astrocytes. These enzymes are responsible for conversion of glutamate to 2-oxoglutarate, which may be further metabolized in the TCA cycle.^{31–34}

7.3 THE GABA–GLUTAMATE–GLUTAMINE CYCLE

In GABAergic neurons, glutamate is converted to GABA by glutamate decarboxylase (GAD), and glutamate is present in a relatively low concentration.^{13,35} Synaptically released GABA is taken up primarily by GABAergic neurons, and to a lesser extent by astrocytes.^{36–38} This is schematically

represented in Figure 7.1. GABA is metabolized by GABA-transaminase (GABA-T) in the so-called GABA shunt³⁹ (Figure 7.1), which allows four of the five C atoms from 2-oxoglutarate to reenter the TCA cycle as succinate. GABA-T is a ubiquitous enzyme present in both neurons and astrocytes, making astrocytes and neurons equally capable of metabolizing GABA.⁴⁰

It is well known that astrocytes are intimately involved in glutamate neurotransmission. However, the importance of astrocytic glutamine in GABA synthesis is controversial,^{41,42} but both *in vitro* and *in vivo* data imply that glutamine is an important precursor for GABA synthesis.^{43–46} Thus, the glutamate–glutamine cycle concept is extended to a GABA–glutamate–glutamine cycle, as seen in Figure 7.1. In keeping with the concept that the drain of GABA from neurons to astrocytes is relatively modest compared with the corresponding drain of glutamate from glutamatergic neurons,^{36,47,48} glutamine transport has been shown to be more intense in glutamatergic neurons than in neocortical, GABAergic neurons.⁴⁹

7.4 THE INTRACELLULAR COMPARTMENTATION CONCEPT

Mitochondria contain the respiratory chain enzyme complexes that carry out oxidative phosphorylation and produce the main part of cellular energy in the form of ATP. It is conceivable that different types of mitochondria might exist within the same cell due to the diversity of functions.⁵⁰ The existence of mitochondrial heterogeneity would expand the number of possible intracellular compartments. Mitochondrial heterogeneity is a well-known phenomenon in patients with defects of the mitochondrial genome (for review, see Taylor et al.⁵¹). Synaptic and nonsynaptic mitochondria isolated from a homogenate of adult rat brain exhibit differences in the activity of a number of TCA cycle enzymes.^{52,53} Intramitochondrial compartmentation could also exist. Free diffusion within the mitochondria has been shown to be unlikely due to the high apparent viscosity of the mitochondrial matrix.⁵⁴ Using electron microscopy it has also been suggested that the inner membrane proteins might be compartmentalized.⁵⁵ This phenomenon would further increase the number of possible intracellular compartments. Results from several studies indicate that both neuronal and astrocytic metabolism are compartmentalized,^{56–65} thus constituting further evidence that mitochondrial heterogeneity may exist. This notion is in line with recent studies demonstrating heterogeneity among mitochondrial populations with regard to expression of pyruvate and 2-oxoglutarate dehydrogenases.^{66–68} Needless to say, elucidation of the cellular compartmentation of these neuroactive amino acids is fraught with enormous experimental challenges. However, since the biosynthetic machinery for both glutamate and GABA involves neurons as well as astrocytes at the cytoplasmic and mitochondrial levels, it is imperative to fully understand this compartmentation if the regulatory mechanisms for biosynthesis of these neurotransmitters are to be worked out in detail.

7.5 METABOLIC INTERACTION IN BRAIN STUDIED BY ¹³C NMRS

Nuclear magnetic resonance spectroscopy (NMRS) has several appealing features for applications to metabolic studies. The nuclei that are most commonly used in NMRS for metabolic studies are ¹H, ³¹P, and ¹³C. ¹H and ³¹P are naturally abundant isotopes and therefore constitute the most common basis for studies involving examination of differences in the natural abundance spectra. In contrast, ¹³C has a natural abundance of 1.1%. This disadvantage normally makes its detection difficult, and ¹³C NMRS is thus of limited use for studies of endogenous metabolites unless they occur in large amounts. However, the low natural abundance can be an advantage in that ¹³C-enriched precursors can be used for metabolic pathway mapping with little or no background interference from metabolites endogenously labeled due to the natural abundance of ¹³C. Thus, ¹³C NMRS is a powerful tool for analysis of the metabolic trafficking between the heterogeneous cellular entities of the brain.

Information about biochemical pathways can be gained using ^{13}C -labeled substances.⁶⁹⁻⁷² These labeling experiments are analogous to those utilizing conventional ^{14}C labeling, but additional information about the location of the label within the molecule can be obtained. Moreover, the potential for *in vivo* applicability exists. Using ^{13}C NMR spectra in a qualitative manner, it is possible to detect unexpected compounds. An example of this is the finding of citrate in the culture medium of astrocytes, explicitly showing that astrocytes release citrate.⁷³ A wealth of additional information is, however, gained by quantitative analysis of ^{13}C spectra. With specifically labeled precursors, it is possible to distinguish between neuronal and glial pathways. Acetate is selectively taken up by astrocytes since they contain a specialized transport system, which is absent or less active in neurons.⁷⁴ In contrast, acetyl CoA derived from glucose has been calculated to be metabolized more actively in the neuronal tricarboxylic acid (TCA) cycle in rats.⁷⁵ Thus, by simultaneous injection of $[1-^{13}\text{C}]\text{glucose}$ and $[1,2-^{13}\text{C}]\text{acetate}$ and NMRS analysis of brain extracts, information about neuronal and astrocytic metabolism can be obtained in the same animal.⁷⁶

7.6 ^{13}C NMRS ANALYSES OF NEURONAL-GLIAL INTERACTIONS IN ANIMAL MODELS OF EPILEPSY

Epilepsy is one of the most common serious neurological disorders and is characterized by an imbalance of excitatory and inhibitory function mainly due to disturbed neurotransmitter metabolism.^{77,78} Animal models of epilepsy are often based on inhibition of the synthesis of GABA. Such inhibition has been shown to promote seizures, as does the administration of GABA_A receptor antagonists and glutamate receptor agonists.⁷⁹ In accordance with this, pentylenetetrazol (PTZ) is a frequently used chemical convulsant inducing tonic-clonic seizures.⁸⁰ It is generally believed that PTZ exerts its effects by binding to the picrotoxin-recognition site of GABA_A receptors.⁸¹ PTZ is known to decrease the effects of GABA and other inhibitory neurotransmitters, thus enhancing the probability of depolarization of neurons.⁷⁷ Kindling is another way to induce seizures.⁸⁰ The term *kindling* refers in this context to the continuous application of sub-threshold doses of PTZ leading to a behavior nearly identical to secondary generalized seizures,⁸² but other kindling paradigms exist.⁸⁰ A model for temporal lobe epilepsy is based on injection of kainic acid, which produces complex partial seizures.⁸³ Results of NMRS studies using these three models are presented in the following.

Injection of PTZ (70 mg/kg body weight) followed 30 min later by $[1-^{13}\text{C}]\text{glucose}$ plus $[1,2-^{13}\text{C}]\text{acetate}$ and decapitation 15 min thereafter showed impairment of amino acid metabolism in glutamatergic neurons, whereas astrocyte metabolism was unchanged at this early postictal stage.⁸⁴ Kindling in mice was achieved by 20 intraperitoneal injections of PTZ (35 mg/kg) over a period of 40 days. Metabolism in astrocytes seemed to be impaired as indicated by a decreased labeling of glutamine both from $[1-^{13}\text{C}]\text{glucose}$ and $[1,2-^{13}\text{C}]\text{acetate}$.⁸⁵ As mentioned above, astrocytes are closely involved in amino acid homeostasis and play a significant role in epileptogenic foci, where their proliferation (astrogliosis) is a well-known phenomenon.⁸⁶ This is especially true for limbic seizures or temporal lobe epilepsy and has been confirmed both in humans and in animal models.⁸⁷⁻⁸⁹ Thus, limbic seizures are an interesting subject for evaluation by ^{13}C NMRS and, consequently, the model of kainate-induced limbic seizures was used to study metabolic interactions between neurons and glia in this type of epilepsy. In agreement with the results from the PTZ-induced seizures, astrocytic metabolism was impaired 1 day after injection of the glutamate receptor agonist kainate. On day 14, however, no changes were observed in astrocytic metabolites, but labeling from $[1-^{13}\text{C}]\text{glucose}$, i.e., reflecting mainly neuronal metabolism, was increased in glutamate, GABA, glutamine, aspartate, and succinate. Thus, it was concluded that turnover of metabolites in the model of kainate-induced limbic seizures is time dependent. Early and only temporary enhanced astrocytic metabolic activity was followed by altered metabolism in neurons with an increased turnover of important metabolites such as the neurotransmitters GABA and glutamate.

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7.7 METABOLISM STUDIED BY ^{13}C NMRS IN BRAIN CELL CULTURE

The metabolic fate of glutamate has been studied in cultured neocortical astrocytes,^{90,91} cerebellar astrocytes,⁹² cerebellar granule neurons,⁹³ and neocortical neurons⁹⁴ using $[\text{U-}^{13}\text{C}]$ glutamate and NMRS. The main routes for glutamate metabolism is conversion into glutamine, GABA, or peptides, such as GSH, or oxidative metabolism via the TCA cycle for energy production and synthesis of aspartate and various other metabolites including lactate (for review see Sonnewald et al.⁹⁵). In neocortical and cerebellar astrocytes, it could be shown that glutamate not only was converted to glutamine but to a large extent entered the TCA cycle. ^{13}C labeling was found in aspartate, glutamate, and glutamine after metabolism of the uniformly labeled carbon skeleton in the TCA cycle. Surprisingly, labeled lactate obtained from glutamate metabolism in the TCA cycle was detected in the medium from these cells.⁹⁰⁻⁹² McKenna et al.⁹⁶ have shown that the route of glutamate metabolism is concentration dependent, in the sense that more glutamate is consumed for direct formation of glutamine at low concentrations (0.01–0.1 mM), whereas more is metabolized via the TCA cycle at higher concentrations (0.2–0.5 mM).

In cerebellar granule cells, $[\text{U-}^{13}\text{C}]$ glutamate was found to be metabolized in the TCA cycle since both aspartate and glutamate derived from precursors generated in the first and second turns of the TCA cycle were observed in cell extracts.⁹³ Minor amounts of uniformly labeled lactate were also found, but this could be explained by the small astrocytic contamination of the cultures⁹³ and thus may not reflect production in the granule neurons of pyruvate/lactate from malate, although these cells express malic enzyme.^{63,93}

The metabolism of $[\text{U-}^{13}\text{C}]$ glutamate in GABAergic cerebral cortical neurons has been found to be rather complex.^{45,94} Thus, much more label was seen in aspartate than in GABA,⁹⁴ and GABA synthesis has been shown to occur from both a cytoplasmic and a TCA cycle-derived pool of glutamate.⁴⁵ Moreover, the glutamate pool, which in these neurons is associated with energy metabolism, is at least partly separated from that acting as a precursor for GABA.⁴⁵ It may therefore be concluded that neurons exhibit signs of metabolic compartmentation with regard to glutamate.^{45,94}

7.8 PYRUVATE RECYCLING

Pyruvate recycling was first shown in the liver, where $[2\text{-}^{14}\text{C}]$ pyruvate could be converted to $[3\text{-}^{14}\text{C}]$ pyruvate and $[1\text{-}^{14}\text{C}]$ pyruvate, a process that can occur only if pyruvate is incorporated into the TCA cycle via acetyl CoA and subsequently regenerated from TCA cycle constituents⁹⁷ (see Figure 7.1). Recycling of pyruvate in the brain has been demonstrated by Cerdan et al.,⁹⁸ who found that $[1,2\text{-}^{13}\text{C}]$ acetate, a substrate that is specifically taken up and therefore metabolized in astrocytes (see above), can be converted in brain to monolabeled $[1\text{-}^{13}\text{C}]$ - and $[2\text{-}^{13}\text{C}]$ acetyl CoA and to glutamate labeled either in the C-4 or the C-5 position. This requires entry of acetate into the tricarboxylic acid (TCA) cycle (after formation of acetyl CoA) and exit of a TCA cycle intermediate to form pyruvate, which then is reintroduced into the TCA cycle. Based on the observation that this label from acetate was incorporated into glutamate but not into glutamine, it was concluded that pyruvate recycling took place in a compartment without glutamine synthetase activity, i.e., a neuronal but not an astrocytic compartment. Pyruvate recycling in the brain *in vivo* has been confirmed by Hassel et al.,⁹⁹ who found formation of labeled lactate from $[2\text{-}^{13}\text{C}]$ acetate and of $[2\text{-}^{13}\text{C}]$ lactate from $[1\text{-}^{13}\text{C}]$ glucose, and based on a more pronounced formation of TCA cycle-derived lactate from labeled acetate than from labeled glucose, it was concluded that pyruvate recycling was likely to occur in the astrocytic rather than the neuronal compartment. Cell culture work has confirmed this conclusion. Using $[\text{U-}^{13}\text{C}]$ glutamate or $[3\text{-}^{13}\text{C}]$ glutamate, it could be shown that neocortical and cerebellar astrocytes are able to generate pyruvate from a TCA cycle intermediate (malate or oxaloacetate) and subsequently reintroduce the carbon skeleton of this pyruvate into the

cycle as acetyl CoA.^{100,101} In contrast, neocortical and cerebellar neurons did not use this pathway. Incubation of neocortical cocultures (neurons and astrocytes) with [3-¹³C]glutamate did not show any upregulation of pyruvate recycling.¹⁰² Lack of pyruvate i.e., hypoglycemia could presumably lead to activation of pyruvate recycling. However, this was observed neither in cerebellar granule neurons nor in neocortical astrocytes.^{103,104}

7.9 ¹³C NMRS ANALYSIS OF THE EFFECTS OF THIOPENTAL ON BRAIN CELLS IN CULTURE

NMRS can also be used to examine the effects of pharmacological agents on the metabolic pathways in brain cells in culture. In the following discussion, thiopental is used as an example. Barbiturates such as thiopental are intravenously injected anesthetics interacting with the GABA_A receptor at the barbiturate-binding site, prolonging the opening of the Cl⁻ channel and thus extending the hyperpolarizing effect of GABA (for review, see Ito et al.¹⁰⁵).

7.9.1 Glutamate Uptake and Release

In neocortical astrocytes the amount of glutamate taken up was decreased during incubation with 1 mM thiopental.¹⁰⁶ In contrast, thiopental did not affect glutamate uptake in cultured cerebellar astrocytes and granule neurons.^{92,106} Glutamate release can occur either via a vesicular mechanism or reversal of the plasma membrane transporters involving the cytoplasmic pool of glutamate.¹⁰⁶⁻¹⁰⁹ In cerebellar granule neurons, thiopental did indeed decrease glutamate and aspartate release,¹⁰⁶ and in neocortical astrocytes, aspartate release was reduced.⁹¹ These findings may be compatible with the previous demonstration that thiopental inhibits evoked glutamate release from synaptosomes.¹¹⁰ However, since the paradigm used in the cerebellar granule neurons involved repetitive exposure to 200 μM glutamate in addition to a chronic exposure to 100 μM glutamate, it is likely to represent reversal of transport in an outward direction.¹⁰⁶ Thus, it appears that thiopental preferentially inhibits the glutamate transporters operating in the outward direction in cerebellar neurons and neocortical astrocytes, but not in cerebellar astrocytes.^{91,92,106}

7.9.2 Glutamate Metabolism

[U-¹³C]glutamate was found to be metabolized via the same pathways in cerebellar and neocortical astrocytes.^{91,92} However, the amounts of lactate formed from [U-¹³C]glutamate, especially in the presence of 1 mM thiopental, were higher in cerebellar than in neocortical astrocytes, and alanine formation was only observed in cerebellar astrocytes. Such regional differences were also reported in earlier studies.^{99,111,112} Furthermore, the distribution of glutamate into alternative metabolic pathways was different in the astrocyte cultures from these two regions. This underscores the importance of analyzing astrocytes from different brain regions. Regional differences in astrocyte function may reflect the fact that astrocytic properties with regard to expression of neurotransmitter transporters, for example, is influenced by the neuronal environment.⁴⁷ In cultured astrocytes, it may, however, be considered that differences in cell maturational stages could play a role.¹¹³ In keeping with the observation that in cerebellar granule neurons and astrocytes thiopental had no effect on glutamate uptake, it was found that the total amount consumed was unaffected by thiopental. However, the amounts of most metabolites synthesized from [U-¹³C]glutamate were increased in the presence of thiopental. This indicates that processes such as CO₂ production that are not detected by the present NMRS method may be decreased.^{92,106} Khazanov and Saratikov¹¹⁴ have shown that phenobarbital and benzonal depress energy production by rat brain mitochondria. Thus, it is not unlikely that the aforementioned results indicate a depression of the energy production by thiopental in mouse cerebellar cells in culture.

7.10 CONCLUDING REMARKS

Based on the original observation that the metabolism of glutamate in the brain must take place in separated pools (compartments) and that this most likely represents distinct metabolic patterns in neurons and glial cells (for references, see Waagepetersen et al.⁶⁸), it is clear that exchange of metabolites involved in this metabolism must occur between these two cell types. As exemplified in this chapter, toxic drugs may be utilized as tools to obtain information about such exchange reactions, as well as the metabolic processes in the individual cell types. Moreover, the use of such drugs combined with ¹³C labeling to follow the metabolic fate of individual metabolites and even individual C atoms has been instrumental in attempts to delineate a possible metabolic compartmentation at the cellular level occurring in neurons as well as glial cells. During the decade that has passed since the first indications of such cellular compartmentation,⁵⁷ it has become increasingly clear that such compartmentation does indeed exist. Future experimentation may lead to a better understanding of the functional implications of this phenomenon. One of the important challenges will be to elucidate the functional significance of regional diversity of astrocytes and the possibility that such diversity may be influenced by the neuronal environment. Any given individual synapse represents a unique microenvironment in which the functional status reflects the integration of such cross talk between the presynaptic, postsynaptic, and astrocytic entities.

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LIST OF ABBREVIATIONS

GABA-T — γ -Aminobutyric acid transaminase
 GAD — Glutamate decarboxylase
 GS — Glutamine synthetase
 NMR(S) — Nuclear magnetic resonance (spectroscopy)
 PAG — Phosphate-activated glutaminase
 PC — Pyruvate carboxylase
 PDH — Pyruvate dehydrogenase
 TCA — Tricarboxylic acid

REFERENCES

1. Hertz, L. and Schousboe, A., Ion and energy metabolism of the brain at the cellular level, *Int. Rev. Neurobiol.*, 18, 141, 1975.
2. Schousboe, A. and Divac, I., Difference in glutamate uptake in astrocytes cultured from different brain regions, *Brain Res.*, 177, 407, 1979.
3. Schousboe, A. and Waagepetersen, H., Role of astrocytes in homeostasis of glutamate and GABA during physiological and pathophysiological conditions, *Adv. Mol. Cell Biol.*, 31, 461, 2004.
4. Hansson, E. and Rönnbäck, L., Astrocytic receptors and second messenger systems, *Adv. Mol. Cell Biol.*, 31, 475, 2004.
5. Berl, S. and Clarke, D.D., The metabolic compartmentation concept, in *Glutamine, Glutamate and GABA in the Central Nervous System*, Hertz, L., Kvamme, E., McGeer, E.G., and Schousboe, A., Eds., Alan R. Liss, Inc., New York, 1983, p. 205.

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6. Berl, S. and Clarke, D.D., Compartmentation of amino acid metabolism, in *Handbook of Neurochemistry*, 1st ed., Vol. 2, Lajtha, A., Ed., Plenum Press, New York, 1969, p. 447.
7. Van den Berg, C.J. and Garfinkel, D., A simulation study of brain compartments: Metabolism of glutamate and related substances in mouse brain, *Biochem. J.*, 23, 211, 1971.
8. Balázs, R., Patel, A.J., and Richter, D., Metabolic compartments in the brain: Their properties and relation to morphological structures, in *Metabolic Compartmentation in the Brain*, Balázs, R. and Cremer, J.E., Eds., McMillan, London, 1973, p. 167.
9. Yu, A.C.H. et al., Pyruvate carboxylase activity in primary cultures of astrocytes and neurons, *J. Neurochem.*, 41, 1484, 1983.
10. Shank, R.P. et al., Pyruvate carboxylase: An astrocyte-specific enzyme implicated in the replenishment of amino acid neurotransmitter pools, *Brain Res.*, 329, 364, 1985.
11. Cesar, M. and Hamprecht, B., Immunocytochemical examination of neural rat and mouse primary cultures using monoclonal antibodies raised against pyruvate carboxylase, *J. Neurochem.*, 64, 2312, 1995.
12. Westergaard, N. et al., Evaluation of the importance of transamination versus deamination in astrocytic metabolism of [U-¹³C]glutamate, *Glia*, 17, 160, 1996.
13. Ottersen, O.P., Zhang, N., and Walberg, F., Metabolic compartmentation of glutamate and glutamine: morphological evidence obtained by quantitative immunocytochemistry in rat cerebellum, *Neuroscience*, 46, 519, 1992.
14. Norenberg, M.D. and Martinez-Hernandez, A., Fine structural localization of glutamine synthetase in astrocytes of rat brain, *Brain Res.*, 161, 303, 1979.
15. Tansey, F.A., Farooq, M., and Cammer, W., Glutamine synthetase in oligodendrocytes and astrocytes: new biochemical and immunocytochemical evidence, *J. Neurochem.*, 56, 266, 1991.
16. Chaudhry, F.A. et al., Molecular analysis of system N suggests novel physiological roles in nitrogen metabolism and synaptic transmission, *Cell*, 99, 769, 1999.
17. Varoqui, H. et al., Cloning and functional identification of a neuronal glutamine transporter, *J. Biol. Chem.*, 275, 4049, 2000.
18. Kvamme, E., Roberg, B., and Torgner, I.A., Phosphate-activated glutaminase and mitochondrial glutamine transport in the brain, *Neurochem. Res.*, 25, 1407, 2000.
19. Laake, J.H. et al., Postembedding immunogold labelling reveals subcellular localization and pathway-specific enrichment of phosphate activated glutaminase in rat cerebellum, *Neuroscience*, 4, 1137, 1999.
20. Hertz, L. et al., Astrocytes: glutamate producers for neurons, *J. Neurosci. Res.*, 57, 417, 1999.
21. Schousboe, A. et al., Phosphate activated glutaminase activity and glutamine uptake in primary cultures of astrocytes, *J. Neurochem.*, 32, 943, 1979.
22. Kvamme, E. et al., Properties of phosphate activated glutaminase in astrocytes cultured from mouse brain, *Neurochem. Res.*, 7, 761, 1982.
23. Cousin, M.A., Hurst, H., and Nicholls, D.G., Presynaptic calcium channels and field-evoked transmitter exocytosis from cultured cerebellar granule cells, *Neuroscience*, 81, 151, 1997.
24. Hertz, L., Functional interactions between neurons and astrocytes I. Turnover and metabolism of putative amino acid transmitters, *Prog. Neurobiol.*, 13, 277, 1979.
25. Schousboe, A., Transport and metabolism of glutamate and GABA in neurons and glial cells, *Int. Rev. Neurobiol.*, 22, 1, 1981.
26. Erecinska, M., The neurotransmitter amino acid transport systems. A fresh outlook on an old problem, *Biochem. Pharmacol.*, 36, 3547, 1987.
27. Gegelashvili, G. and Schousboe, A., High affinity glutamate transporters: regulation of expression and activity, *Mol. Pharmacol.*, 52, 6, 1997.
28. Gegelashvili, G. and Schousboe, A., Cellular distribution and kinetic properties of high-affinity glutamate transporters, *Brain. Res. Bull.*, 45, 233, 1998.
29. Tanaka, K., Functions of glutamate transporters in the brain, *Neurosci. Res.*, 37, 15, 2000.
30. Magistretti, P.J. et al., Energy on demand, *Science*, 283, 496, 1999.
31. Schousboe, A., Svenneby, G., and Hertz, L., Uptake and metabolism of glutamate in astrocytes cultured from dissociated mouse brain hemispheres, *J. Neurochem.*, 29, 999, 1977.
32. Hertz, L. et al., Astrocytes in primary cultures, in *Neuroscience Approached through Cell Culture*, Vol. 1, Pfeiffer, S.E., Ed., CRC Press, FL, 1982, p. 175.
33. Yu, A.C.H., Schousboe, A., and Hertz, L., Metabolic fate of ¹⁴C-labeled glutamate in astrocytes in primary cultures, *J. Neurochem.*, 39, 954, 1982.

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34. Hertz, L., Drejer, J., and Schousboe, A., Energy metabolism in glutamatergic neurons, GABAergic neurons and astrocytes in primary cultures, *Neurochem. Res.*, 13, 605, 1988.
35. Martin, D.L. and Rinvall, K., Regulation of gamma-aminobutyric acid synthesis in the brain, *J. Neurochem.*, 60, 395, 1993.
36. Hertz, L. and Schousboe, A., Primary cultures of GABAergic and glutamatergic neurons as model systems to study neurotransmitter functions. I. Differentiated cells, in *Model Systems of Development and Aging of the Nervous System*, Vernadakis, A., Privat, A., Lauder, J.M., Timiras, P.S., and Giacobini, E., Eds., Martinus Nijhoff Publishing, Boston, 1987, p. 19.
37. Borden, L.A., GABA transporter heterogeneity: pharmacology and cellular localization, *Neurochem. Int.*, 29, 335, 1996.
38. Schousboe, A., Pharmacological and functional characterization of astrocytic GABA transport: a short review, *Neurochem. Res.*, 25, 1241, 2000.
39. Balazs, R. et al., The operation of the γ -aminobutyrate bypath of the tricarboxylic acid cycle in brain tissue *in vitro*, *Biochem. J.*, 116, 445, 1970.
40. Baxter, C.F., Some recent advances in studies of GABA metabolism and compartmentation, in *GABA in Nervous System Function*, Roberts, E., Chase, T.N., and Tower, D.B., Eds., Raven Press, New York, 1976, p. 61.
41. Fonnum, F. and Paulsen, R.E., Comparison of transmitter amino acid levels in rat globus pallidus and neostriatum during hypoglycemia or after treatment with methionine sulfoximine or gamma-vinyl gamma-aminobutyric acid, *J. Neurochem.*, 54, 1253, 1990.
42. Preece, N.E. and Cerdan, S., Metabolic precursors and compartmentation of cerebral GABA in vigabatrin-treated rats, *J. Neurochem.*, 67, 1718, 1996.
43. Reubi, J.-C., Van der Berg, C., and Cuénod, M., Glutamine as precursor for the GABA and glutamate transmitter pools, *Neurosci. Lett.*, 10, 171, 1978.
44. Sonnewald, U. et al., Direct demonstration by [¹³C]NMR spectroscopy that glutamine from astrocytes is a precursor for GABA synthesis in neurons, *Neurochem. Int.*, 1, 19, 1993.
45. Waagepetersen, H.S. et al., Synthesis of vesicular GABA from glutamine involves TCA cycle metabolism in neocortical neurons, *J. Neurosci. Res.*, 57, 342, 1999.
46. Rothman, D.L. et al., *In vivo* nuclear magnetic resonance spectroscopy studies of the relationship between the glutamate-glutamine neurotransmitter cycle and functional neuroenergetics, *Phil. Trans. R. Soc. Lond.*, 354, 1165, 1999.
47. Schousboe, A. et al., Role of astrocytic transport processes in glutamatergic and GABAergic neurotransmission, *Neurochem. Int.*, in press, 2004.
48. Peng, L., et al., Utilization of glutamine and of TCA cycle constituents as precursors for transmitter glutamate and GABA, *Dev. Neurosci.*, 15, 367, 1993.
49. Su, T.Z., Campbell, G.W., and Oxender, D.L., Glutamine transport in cerebellar granule cells in culture, *Brain Res.*, 757, 69, 1997.
50. Sonnewald, U., Hertz, L., and Schousboe, A., Mitochondrial heterogeneity in the brain at the cellular level, *J. Cereb. Blood Flow Met.*, 18, 231, 1998.
51. Taylor, R.W. et al., Treatment of mitochondrial disease, *J. Bioenerg. Biomembr.*, 29, 195, 1997.
52. Leong, S.F. et al., The activities of some energy-metabolising enzymes in nonsynaptic (free) and synaptic mitochondria derived from selected brain regions, *J. Neurochem.*, 42, 1306, 1984.
53. Lai, J.C., Leung, T.K., and Lim, L., Heterogeneity of monoamine oxidase activities in synaptic and non-synaptic mitochondria derived from three brain regions: some functional implications, *Metab. Brain Dis.*, 9, 53, 1994.
54. Lopez-Beltran, E.A., Mate, M.J., and Cerdan, S., Dynamics and environment of mitochondrial water as detected by ¹H NMR, *J. Biol. Chem.*, 271, 10648, 1996.
55. Perkins, G.A. and Frey, T.G., Recent structural insight into mitochondria gained by microscopy, *Micron*, 31, 97, 2000.
56. Schousboe, A. et al., Glutamate and glutamine metabolism and compartmentation in astrocytes, *Dev. Neurosci.*, 15, 359, 1993.
57. Sonnewald, U. et al., NMR spectroscopic studies of ¹³C acetate and ¹³C glucose metabolism in neocortical astrocytes: evidence for mitochondrial heterogeneity, *Dev. Neurosci.*, 15, 351, 1993.
58. Waagepetersen, H.S. et al., Comparison of lactate and glucose metabolism in cultured neocortical neurons and astrocytes using ¹³C NMR spectroscopy, *Dev. Neurosci.*, 20, 310, 1998.

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59. Waagepetersen, H.S. et al., Multiple compartments with different metabolic characteristics are involved in biosynthesis of intracellular and released glutamine and citrate in astrocytes, *Glia*, 35, 246, 2001.
60. Bouzier, A.K. et al., Compartmentation of lactate and glucose metabolism in C6 glioma cells. A ^{13}C and ^1H NMR study, *J. Biol. Chem.*, 273, 27162, 1998.
61. McKenna, M.C. et al., The metabolism of malate by cultured rat brain astrocytes, *Neurochem. Res.*, 15, 1211, 1990.
62. McKenna, M.C. et al., New insight into the compartmentation of glutamate and glutamine in cultured rat brain astrocytes, *Dev. Neurosci.*, 18, 380, 1996.
63. McKenna, M.C. et al., Mitochondrial malic enzyme activity is much higher in mitochondria from cortical synaptic terminals compared with mitochondria from primary cultures of cortical neurons or cerebellar granule cells, *Neurochem. Int.*, 36, 451, 2000.
64. Cruz, F. et al., Intracellular compartmentation of pyruvate in primary cultures of cortical neurons as detected by (^{13}C) NMR spectroscopy with multiple (^{13}C) labels, *J. Neurosci. Res.*, 66, 771, 2001.
65. Zwingmann, C., Richter-Landsberg, C., and Leibfritz, D., ^{13}C isotopomer analysis of glucose and alanine metabolism reveals cytosolic pyruvate compartmentation as part of energy metabolism in astrocytes, *Glia*, 34, 200, 2001.
66. Margineantu, D.H. et al., Heterogeneous distribution of pyruvate dehydrogenase in the matrix of mitochondria, *Mitochondrion*, 1, 327, 2002.
67. Waagepetersen, H.S. et al., Mitochondrial heterogeneity in brain cells, *J. Neurochem.*, 85 (Suppl. 1), 56, 2003.
68. Waagepetersen, H.S., Sonnewald, U., and Schousboe, A., Compartmentation of glutamine, glutamate and GABA metabolism in neurons and astrocytes: Functional implications, *Neuroscientist*, 9, 398, 2003.
69. Badar-Goffer, R.S., Bachelard, H.S., and Morris, P.G., Cerebral metabolism of acetate and glucose studied by ^{13}C -n.m.r. spectroscopy. A technique for investigating metabolic compartmentation in the brain, *Biochem. J.*, 266, 133, 1990.
70. Hassel, B. et al., Trafficking of amino acids between neurons and glia *in vivo*. Effects of inhibition of glial metabolism by fluoroacetate, *J. Cereb. Blood Flow Metab.*, 17, 1230, 1997.
71. Håberg, A. et al., *In vivo* injection of $[1-^{13}\text{C}]$ glucose and $[1,2-^{13}\text{C}]$ acetate combined with *ex vivo* ^{13}C nuclear magnetic resonance spectroscopy: a novel approach to the study of middle cerebral artery occlusion in the rat, *J. Cereb. Blood Flow Metab.*, 18, 1223, 1998.
72. Chapa, F. et al., Metabolism of $(1-^{13}\text{C})$ glucose and $(2-^{13}\text{C}, 2-(2)\text{H}(3))$ acetate in the neuronal and glial compartments of the adult rat brain as detected by $[(^{13}\text{C}), (2)\text{H}]$ NMR spectroscopy, *Neurochem. Int.*, 37, 217, 2000.
73. Sonnewald U. et al., First direct demonstration of preferential release of citrate from astrocytes using $[^{13}\text{C}]$ NMR spectroscopy of cultured neurons and astrocytes, *Neurosci. Lett.*, 128, 235, 1991.
74. Waniewski, R.A. and Martin, D.L., Exogenous glutamate is metabolized to glutamine and exported by rat primary astrocyte cultures, *J. Neurochem.*, 47, 304, 1986.
75. Qu, H. et al., (^{13}C) MR spectroscopy study of lactate as substrate for rat brain, *Dev. Neurosci.*, 22, 429, 2000.
76. Taylor, A. et al., Approaches to studies on neuronal/glial relationships by ^{13}C -MRS analysis. *Dev. Neurosci.*, 18, 434, 1996.
77. Bradford, H.F., Glutamate, GABA and epilepsy, *Epilepsia*, 30, 17, 1989.
78. Sander, J.W. and Shorvon, S.D., Epidemiology of the epilepsies, *J. Neurol. Neurosurg. Psychiatry*, 61, 433, 1996.
79. Hosford, D.A., Models of primary generalized epilepsy, *Curr. Opin. Neurol.*, 8, 121, 1995.
80. Löscher, W., Animal models of epilepsy for the development of antiepileptogenic and disease-modifying drugs. A comparison of the pharmacology of kindling and post-status epilepticus models of temporal lobe epilepsy, *Epilepsy Res.*, 50, 105, 2002.
81. Macdonald, R.L. and Barker, J.L., Phenobarbital enhances GABA-mediated postsynaptic inhibition in cultured mammalian neurons, *Trans. Am. Neurol. Assoc.*, 102, 139, 1977.
82. Sonnewald, U. and Kondziell D., Neuronal glial interaction in different neurological diseases studied by *ex vivo* ^{13}C NMR spectroscopy, *NMR Biomed.*, 16, 424, 2003.

83. Ben-Ari, Y., Limbic seizure and brain damage produced by kainic acid: mechanisms and relevance to human temporal lobe epilepsy, *Neuroscience*, 14, 375, 1985.
84. Eloqayli, H. et al., Pentylentetrazole decreases metabolic glutamate turnover in rat brain, *J. Neurochem.*, 85, 1200, 2003.
85. Kondziella, D. et al., The pentylentetrazole-kindling model of epilepsy in SAMP8 mice: glial-neuronal metabolic interactions, *Neurochem. Int.*, 43, 629, 2003.
86. Khurgel, M. and Ivy, G.O., Astrocytes in kindling: relevance in epileptogenesis, *Epilepsy Res.*, 26, 163, 1996.
87. Hudson, L.P. et al., Amygdaloid sclerosis in temporal lobe epilepsy, *Ann. Neurol.*, 33, 622, 1993.
88. Amano, S. et al., Development of a novel rat mutant with spontaneous limbic-like seizures, *Am. J. Patol.*, 149, 329, 1996.
89. Garzillo, C.L. and Mello, L.E., Characterization of reactive astrocytes in the chronic phase of the pilocarpine model of epilepsy, *Epilepsia*, 43, 107, 2002.
90. Sonnewald, U. et al., Metabolism of [U-¹³C]glutamate in astrocytes studied by ¹³C NMR spectroscopy: incorporation of more label into lactate than into glutamine demonstrates the importance of the tricarboxylic acid cycle, *J. Neurochem.*, 61, 1179, 1993.
91. Qu, H. et al., Decreased glutamate metabolism in cultured astrocytes in the presence of thiopental, *Biochem. Pharmacol.*, 58, 1075, 1999.
92. Qu, H. et al., The effect of thiopental on glutamate metabolism in cerebellar astrocytes, *Neurosci. Lett.*, 304, 141, 2001.
93. Sonnewald, U. et al., MRS study of glutamate metabolism in cultured neurons/glia, *Neurochem. Res.*, 21, 987, 1996.
94. Westergaard, N. et al., Glutamate and glutamine metabolism in cultured GABAergic neurons studied by ¹³C NMR spectroscopy may indicate compartmentation and mitochondrial heterogeneity, *Neurosci. Lett.*, 185, 24, 1995.
95. Sonnewald, U., Westergaard, N., and Schousboe, A., Glutamate transport and metabolism in astrocytes, *Glia*, 21, 56, 1997.
96. McKenna, M.C. et al., Exogenous glutamate concentration regulates the metabolic fate of glutamate in astrocytes, *J. Neurochem.*, 66, 386, 1996.
97. Freidmann, B. et al., An estimation of pyruvate recycling during gluconeogenesis in the perfused rat liver, *Arch. Biochem. Biophys.*, 143, 566, 1971.
98. Cerdan, S., Künnecke, B., and Seelig, J., Cerebral metabolism of [1,2-¹³C₂]acetate as detected by *in vivo* and *in vitro* ¹³C NMR, *J. Biol. Chem.*, 365, 12916, 1990.
99. Hassel, B., Sonnewald, U., and Fonnum, F., Glial-neuronal interactions as studied by cerebral metabolism of [2-¹³C]acetate and [1-¹³C]glucose: an *ex vivo* ¹³C NMR spectroscopic study, *J. Neurochem.*, 64, 2773, 1995.
100. Sonnewald, U. et al., Metabolism of [U-¹³C₃] glutamine in cultured astrocytes studied by NMR spectroscopy: first evidence of astrocytic pyruvate recycling, *J. Neurochem.*, 67, 2566, 1996.
101. Håberg, A. et al., *In vitro* and *ex vivo* ¹³C-NMR spectroscopy studies of pyruvate recycling in brain, *Dev. Neurosci.*, 20, 389, 1998.
102. Waagepetersen, H.S. et al., Demonstration of pyruvate recycling in primary cultures of neocortical astrocytes but not in neurons, *Neurochem. Res.*, 11, 1431, 2002.
103. Bakken, I.J. et al., [U-¹³C]glutamate metabolism in astrocytes during hypoglycemia and hypoxia, *J. Neurosci. Res.*, 51, 636, 1998.
104. Bakken, I.J. et al., [U-¹³C] aspartate metabolism in cultured cortical astrocytes and cerebellar granule neurons studied by NMR spectroscopy, *Glia*, 23, 271, 1998.
105. Ito, T. et al., Pharmacology of barbiturate tolerance/dependence: GABAA receptors and molecular aspects, *Life Sci.*, 59, 169, 1996.
106. Qu, H. et al., Effects of thiopental on transport and metabolism of glutamate in cultured cerebellar granule neurons, *Neurochem. Int.*, 37, 207, 2000.
107. Nicholls, D. and Attwell, D., The release and uptake of excitatory amino acids, *Trends Pharmacol. Sci.*, 11, 477, 1990.
108. Belhage B. et al., ³H-D-aspartate release from cerebellar granule neurons is differentially regulated by glutamate- and K⁺-stimulation, *J. Neurosci. Res.*, 33, 436, 1992.

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109. Bak, L.K., Schousboe, A., and Waagepetersen, H.S., Characterization of depolarization-coupled release of glutamate from cultured mouse cerebellar granule cells using DL-threo- β -benzyloxyaspartate (DL-TBOA) to distinguish between the vesicular and the cytoplasmic pools, *Neurochem. Int.*, 43, 417, 2003.
110. Bak, L.K., Waagepetersen, H.S., and Schousboe, A., Role of astrocytes in depolarization-coupled release of glutamate in cerebellar cultures, *Neurochem. Res.*, 29, 257, 2004.
111. Pastuszko, A., Wilson, D.F., and Erecinska, M., Amino acid neurotransmitters in the CNS: effect of thiopental, *FEBS Lett.*, 177, 249, 1984.
112. Merle, M. et al., [1-¹³C]glucose metabolism in brain cells: isotopomer analysis of glutamine from cerebellar astrocytes and glutamate from granule cells, *Dev. Neurosci.*, 18, 460, 1996.
113. Martin, M., Canioni, P., and Merle, M., Analysis of carbon metabolism in cultured cerebellar and cortical astrocytes, *Cell Mol. Biol. (Noisy-le-grand)*, 43, 631, 1997.
114. Khazanov, V.A. and Saratikov, A.S., Effect of phenobarbital and benzonal on succinate and alpha-ketoglutarate oxidation by rat brain mitochondria, *Biull. Eksp. Biol. Med.*, 100, 692, 1985.

