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Biochemical Aspects of the Neurotransmitter Function of Glutamate

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The metabolism of glutamate in CNS tissues has been studied extensively over the past 40 years. Prior to the late 1960s, most studies focused on the central role of glutamate in nitrogen metabolism (35,37), the involvement of this amino acid in energy metabolism (6), its metabolic relationship to GABA (4), and its metabolic compartmentation (6). Since the appearance of neurophysiological (10,22) and neurochemical (13,20,21) evidence that glutamate may function as an excitatory neurotransmitter, interest has developed in the metabolism of glutamate as it relates specifically to this putative synaptic function (1,5,7,12,29,31,32,36). Unfortunately, investigations into the metabolic processes that underlie the neurotransmitter function of glutamate are hindered because of the complexity of glutamate metabolism and the complex morphology of CNS tissues.

At the present time our knowledge is not sufficient to draw many definite conclusions regarding the biochemical aspects of the neurotransmitter function of glutamate. Despite this limited amount of definitive information, a model has emerged that outlines a series of the biochemical events that appear to be associated with the transmitter function (Fig. 1). This model was developed from experimental observations reported by a number of investigators. Notable contributors include Berl and his colleagues (6), Van den Berg and Garfinkel and their colleagues (36), Balázs et al. (1), Quastel and Benjamin (5,29), Bradford and his colleagues (7), Shank and Baxter (32), Shank and Aprison (31), and, more recently, Hamberger et al. (15).

BIOCHEMICAL MODEL OF THE NEUROTRANSMITTER ROLE OF GLUTAMATE

According to this model, the neurotransmitter pool of glutamate resides within the synaptic vesicles of the nerve terminals of those neurons utilizing glutamate as a neurotransmitter (hereafter such neurons will be referred to as glutamate neurons). The glutamate molecules within these vesicles are presumed to be derived from

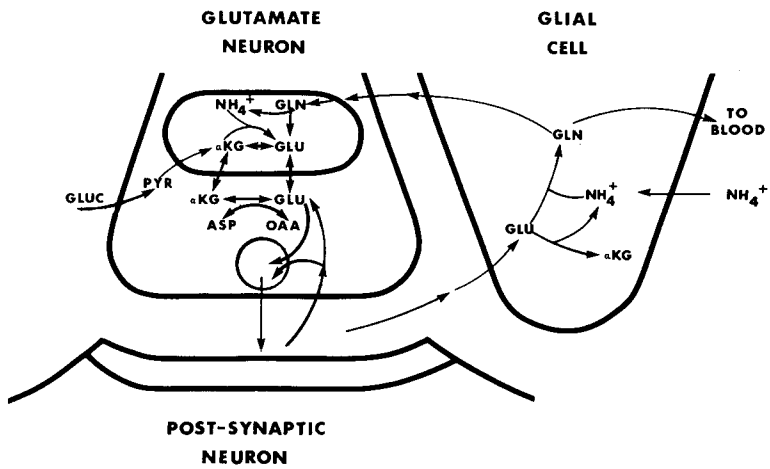

FIG. 1. A model illustrating some of the presumed biochemical events that occur at a glutamate synapse.

cytoplasmic and mitochondrial pools. Since glutamate in these latter pools serves several other cellular functions, it is unlikely that the transmitter pool exists as a distinct metabolic entity, but instead is part of a common metabolic pool.

The transmitter pool of glutamate is thought to have its metabolic origin in both glucose and glutamine. Each molecule of glutamine may serve as a source of amino nitrogen for two molecules of glutamate. This model implies that the carbon portion of glutamate is supplied by glutamine and α-ketoglutarate in equal amounts. However, the metabolism of glutamate is probably far too complex for such a simple stoichiometric relationship to exist.

The mechanism by which glutamate is released from nerve terminals is not yet established, although the favored view is that excitation-secretion coupling in glutamate neurons is mediated by a Ca^{2+}-dependent exocytotic process. Present evidence indicates that the release of glutamate is at least partially dependent on extracellular Ca^{2+} (27). However, glutamate can also be released to some extent in the absence of extracellular Ca^{2+}, indicating either that Ca^{2+} released from intracellular storage sites can mediate exocytosis or that glutamate can be released by a carrier mechanism.

Subsequent to being released from the presynaptic neuron, the molecules of glutamate must be rapidly cleared out of the synaptic cleft. Present evidence leaves little doubt that this inactivation process occurs by a combination of two mechanisms: (a) transport back into the nerve terminal and (b) diffusion out of the cleft followed by uptake into glial cells. Much of the glutamate taken up by glial cells is apparently converted to glutamine, which is subsequently released into the extracellular fluid. This glutamine is then available to be taken up into the nerve terminal in order to replenish the neurotransmitter pool. This model therefore suggests that a metabolic cycle exists between glutamate neurons and glial cells in which there is a *net* flow of glutamate from neurons to glia that is compensated by a

net flow of glutamine in the reverse direction. The model does not necessarily imply that there is a stoichiometric relationship between the fluxes of these compounds.

In this review we will focus on three key elements of this model: (a) glucose and glutamine as metabolic precursors of the neurotransmitter pool of glutamate, (b) uptake as the mechanism of transmitter inactivation, and (c) the role that glial cells play in supporting the neurotransmitter function.

GLUCOSE: PRECURSOR OF THE NEUROTRANSMITTER POOL OF GLUTAMATE

It is experimentally well established that glucose is the principal substrate of energy metabolism in CNS tissues and that much of the glucose carbon passes through glutamate before being oxidized to CO_2. Since energy metabolism is especially vigorous in the CNS, the synthesis of glutamate from precursors derived from glucose is quite rapid. There are at least two metabolic pathways that can account for this role of glutamate as an intermediate in energy metabolism. Both of these pathways can be thought of as attachments to the normal citric acid cycle (Fig. 2). One involves the synthesis of GABA and is frequently referred to as the "GABA

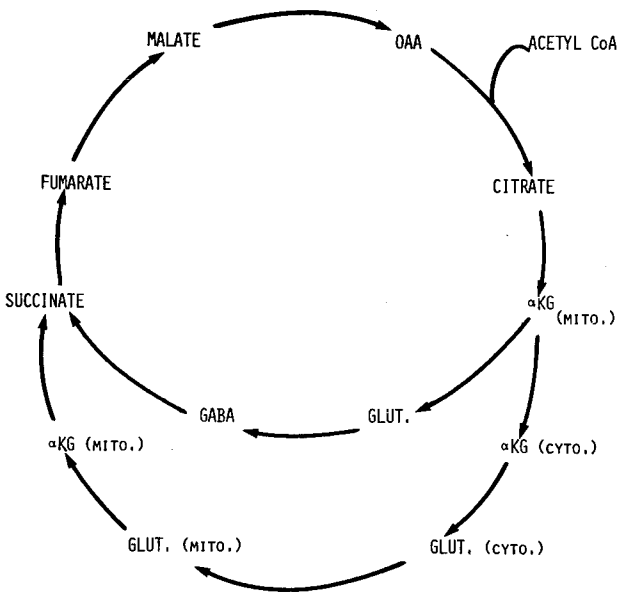

FIG. 2. Depiction of the role of glutamate as an intermediate in energy metabolism. In CNS tissues much of the glucose carbon is incorporated into glutamate prior to being oxidized to CO_2. Therefore, glutamate may be regarded as an intermediate in an expanded citric acid cycle. There are at least two functions served by this metabolic role of glutamate. One is the synthesis of GABA (*inner loop*) in those neurons utilizing GABA as a neurotransmitter. The second function is to aid the transfer of reducing equivalents from the cytoplasm into mitochondria by serving as a component of the malate-aspartate shuttle (see ref. 8). The involvement of glutamate in the malate-aspartate shuttle is depicted by the outer loop.

shunt." This pathway is probably restricted to neurons utilizing GABA as a neurotransmitter. Present evidence indicates that about 10% of the carbon oxidized in the CNS passes through the GABA shunt (1). The second pathway reflects the involvement of glutamate in the malate-aspartate shuttle, which serves as a mechanism by which reducing equivalents are transferred from the cytosol into mitochondria (8).

Although these pathways result in a rapid turnover of glutamate, they are not necessarily useful for restoring the transmitter pool. For every molecule of glutamate formed via these pathways, another one must be converted to the next intermediate (GABA or α-ketoglutarate). Otherwise, the content of each of the intermediates in the pathways would eventually be depleted unless a mechanism were available to replenish the carbon drained off through the loss of glutamate. One possible mechanism by which the content of the intermediates in these pathways can be replenished is the carboxylation of pyruvate to form oxaloacetate.

Because glucose carbon is incorporated into glutamate so extensively, it is likely that a very high percentage of the glutamate molecules within the transmitter pool have glucose as their metabolic origin. However, the actual value that glutamate derived from the citric acid cycle has in restoring the transmitter pool is more likely to be reflected in the rate at which pyruvate is carboxylated to form oxaloacetate. Although it is well established that CNS tissues have the capacity to convert pyruvate to oxaloacetate (28), the extent to which this occurs in the terminals of glutamate neurons is yet to be determined.

GLUTAMINE: PRECURSOR OF THE NEUROTRANSMITTER POOL OF GLUTAMATE

In absolute terms, the rate at which glutamate is formed from glutamine is probably much less than the rate at which it is formed from glucose. However, in terms of the *net* synthesis of glutamate needed to replace the molecules lost through transmitter release, the conversion of glutamine to glutamate can conceivably equal or exceed that from glucose.

Studies designed to provide evidence regarding the possible role of glutamine as a precursor for the neurotransmitter pool of glutamate have been reported recently by several investigators (7,15,29,31). In our own experiments we have examined the uptake and metabolism of ^{14}C-glutamine by the isolated toad-brain preparation and by a synaptosomal preparation obtained from the rat brain. When the isolated toad brain was incubated in a medium containing 0.2 mM glutamine, there was no net uptake of this amino acid; indeed, there was a marked net efflux of glutamine from the brain (Fig. 3). Such an efflux is to be expected since glutamine is probably the major end product of nitrogen metabolism in the CNS (26,36). Despite the net efflux of glutamine there was an accumulation of ^{14}C-glutamine in the tissue that could reflect a net uptake of glutamine into some cellular compartment, such as the terminals of glutamate neurons. Much of the ^{14}C-glutamine taken up was metabolized, as evidenced by an accumulation of label in glutamate (Fig. 3),

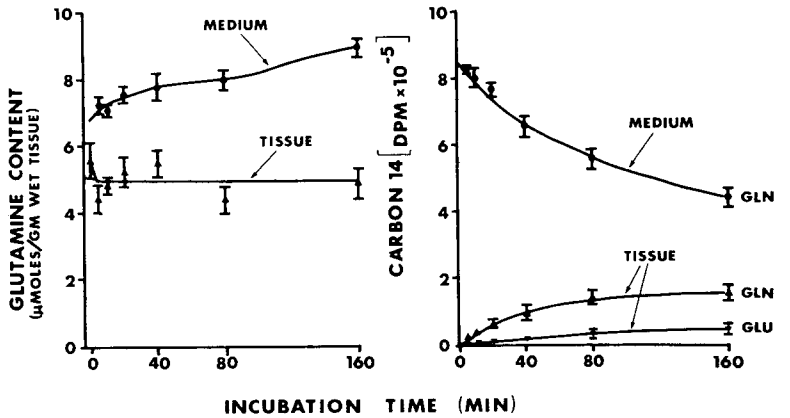

FIG. 3. The time course of change in glutamine content (**left**) and radioactivity (**right**) in isolated toad-brain tissue and the medium when the tissue was incubated in a medium initially containing 0.2 mM L-glutamine and 0.4 μCi (U-^{14}C)L-glutamine. Hemisected toad brains (average mass of approximately 50 mg) were incubated in 2 ml oxygenated bicarbonate-buffered medium at 23° C. The values for the content of glutamine in the medium were obtained by dividing the amount (μmoles) of glutamine in the medium by the tissue mass. Each data point is the mean ± SEM of 6 brain hemisections. In addition to the accumulation of ^{14}C in glutamine and glutamate in the tissue, a considerable amount also accumulated in GABA and aspartate, and presumably much of the ^{14}C was eventually expelled as CO_2. (From Shank and Aprison, ref. 31.)

GABA, and aspartate (31). When the specific radioactivity of glutamate was plotted relative to that of glutamine in the tissue, an unusual metabolic relationship was revealed. The value of this ratio (S.A.[1] glutamate/S.A. glutamine) rose very quickly for a brief period (5 min or less), but increased slowly thereafter (Fig. 4). When the toad-brain tissue was incubated in a standard bicarbonate buffered medium, the average specific activity of glutamate was 7% of that for glutamine after a 5-min incubation period, but after a 160-min incubation period, the specific activity of glutamate had risen to only 15% of that for glutamine. In one set of experiments, the toad-brain tissue was incubated in a medium in which sucrose was substituted for NaCl, and K^+ was made 29 mM. This incubation condition should promote the release of glutamate from nerve terminals and greatly reduce the reuptake of glutamate into either neurons or glial cells. This condition markedly increased the amount of glutamine rapidly converted to glutamate, but did not markedly effect the slope during which the relative specific activity of glutamate rose slowly (Fig. 4).

Since the metabolism of glutamine and glutamate in CNS tissues is quite complex, it is likely that any single explanation for the biphasic rise in the relative specific radioactivity of glutamate shown in Fig. 4 will be an oversimplification. One reasonable explanation for the rapid initial rise would be that a small portion (~10%) of the glutamine taken up by the tissue is converted to glutamate before

[1] S.A., specific radioactivity.

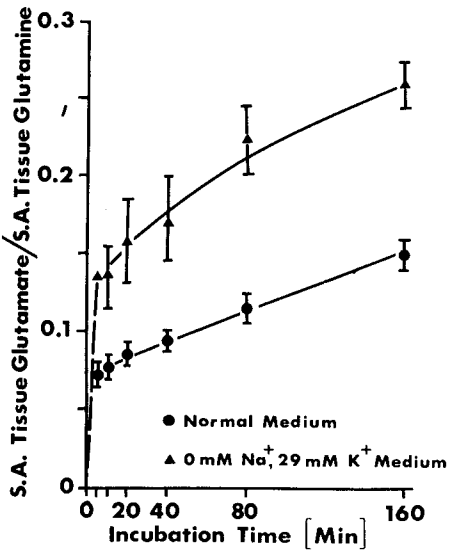

FIG. 4. The change in the specific radioactivity of glutamate in the toad-brain tissue is shown relative to the specific radioactivity of glutamine in the tissue. The data for the normal medium relate to the experiments described in Fig. 3. The data for the 0 mM Na^+, 29 mM K^+ medium were obtained when the toad-brain tissue was incubated in a medium in which sucrose was substituted for NaCl and $KHCO_3$ was substituted for $NaHCO_3$. This incubation medium was used in order to enhance the release of glutamate and block the uptake. (From Shank and Aprison, ref. 31.)

mixing with the bulk of the endogenous glutamine. This could happen in either of two ways. Some of the glutamine could be converted to glutamate during transit across the cell membrane. In this situation the glutamine rapidly metabolized to glutamate would mix with very little of the endogenous pool of glutamine before being converted to glutamate. A biochemical system that could both transport glutamine across the cell membrane and deamidate it to glutamate is the γ-glutamyl cycle described by Meister and colleagues (26). A second way in which glutamine could be metabolized to glutamate without mixing with the bulk of the endogenous glutamine is that the exogenous glutamine could be transported into a compartment that contains little glutamine but is rich in glutaminase activity.

Because the specific radioactivity of glutamate remained much lower than that of glutamine, it is likely that most (~90%) of the exogenous glutamine was transported into a tissue compartment where it was fully or partially mixed with a large pool of endogenous glutamine. Furthermore, the glutamine in this pool must be metabolized to glutamate quite slowly.

The marked increase in the relative amount of glutamine rapidly converted to glutamate when the toad-brain tissue was incubated in the low Na^+-high K^+ medium suggests, but certainly does not establish, that the terminals of glutamate neurons constitute one site where glutamine is rapidly converted to glutamate. Isolated synaptosomes are known to be rich in glutaminase activity (7), and have the capacity to metabolize considerable amounts of glutamine to glutamate (Table 1) (3,7). In addition, the content of glutamine in synaptosomes is relatively low (Table 1) (7). These observations further suggest, but unfortunately do not establish, that the terminals of glutamate neurons represent one site where glutamine is rapidly converted to glutamate.

TABLE 1. *Metabolism of (U-^{14}C) glutamine by synaptosomes from rat brain*[a]

Amino acid	Content (nmoles/mg protein)	Total radioactivity (dpm/mg protein)	R.S.A.[b]
Glutamine	3.55	14,600	1.00
Glutamate	14.55	29,100	0.49
Aspartate	7.78	8,010	0.31
GABA	4.44	7,360	0.20

[a] Synaptosomes were obtained from the whole brain of rats by a procedure similar to that of Gray and Whittaker (14). The synaptosomes were incubated for 15 min at 37° C in an oxygenated bicarbonate-buffered medium. After incubation, a tissue pellet was obtained by centrifugation. The tissue was homogenized in 80% ethanol, and the content and radioactivity in each amino acid was determined by the method of Shank and Aprison (30).

[b] R.S.A., specific radioactivity relative to glutamine which is arbitrarily assigned a value of 1.0. From Shank and Aprison, *unpublished*.

NEURONAL AND GLIAL UPTAKE OF GLUTAMATE

Until the early 1970s, studies pertaining to the uptake of glutamate were primarily concerned with the transport of glutamate across the blood-brain barrier (BBB) and the role of transport mechanisms in regulating the intracellular concentration of glutamate. These studies demonstrated that the flux of glutamate between the blood and CNS parenchyma is severely restricted by the BBB and that CNS tissues can accumulate large amounts of glutamate even when there is a large intra- to extracellular concentration ratio (23).

In recent years, most uptake studies have been concerned with the role that transport mechanisms serve in supporting the neurotransmitter function of glutamate. These studies leave little doubt that transport systems present in both neuronal and glial cell membranes are responsible for inactivating glutamate subsequent to release from nerve terminals. These transport systems presumably also serve to ensure that the extracellular steady-state concentration of glutamate is maintained below levels that can cause neuronal excitation. A matter that has yet to be resolved is the relative amount of glutamate taken back into the nerve terminal and returned to the transmitter pool.

Initial uptake studies first demonstrated that CNS slices (2,16), synaptosomes (6), and glial preparations (17) take up glutamate by two transport systems with widely different affinity constants. One of the transport systems exhibits a relatively high affinity for glutamate ($K_m \sim 20$ μM) and was Na^+ dependent. The other system has a low affinity for glutamate, but the V_{max} is greater. More recently, a third transport system has been identified. This system has a K_m value of ~ 2 μM and has been observed in granule cells and glial cells obtained from the cerebellum of mice (9), and in CNS tissue slices (11). Data obtained with cerebellar granule cells and glial-enriched preparations suggest that although all three systems may be instrumental in inactivating glutamate (Table 2), the highest affinity system is particularly

TABLE 2. *Relative uptake capacity of glutamate transport systems during synaptic resting and active states*[a]

Transport system	K_m (μM)	Relative V_{max}	Relative uptake activity: Resting state[b]	Relative uptake activity: Synaptic activation[c]
Very high affinity	2	1	100	200
High affinity	20	2	36	390
Low affinity	500	5	4	670

[a] Data based on values obtained for granule cell and glial-enriched preparations obtained from the cerebellum of 10-day-old mice.
[b] Assumed concentration of glutamate in synaptic cleft is 0.002 mM.
[c] Assumed concentration of glutamate in synaptic cleft is 1.0 mM.

instrumental in keeping the extracellular concentration at very low levels during synaptic resting periods.

That CNS tissues do indeed have the ability to rapidly remove glutamate from extracellular fluids has been shown by net uptake studies using the isolated toad brain. In these studies, whole or hemisected toad brains were incubated in a bicarbonate-buffered physiological solution containing 0.04 or 0.1 mM L-glutamate (33). During incubation there was a rapid net uptake of glutamate, and eventually a steady-state extracellular concentration of less than 0.002 mM was attained (Fig. 5). When no glutamate was initially present in the medium, there was a net efflux into

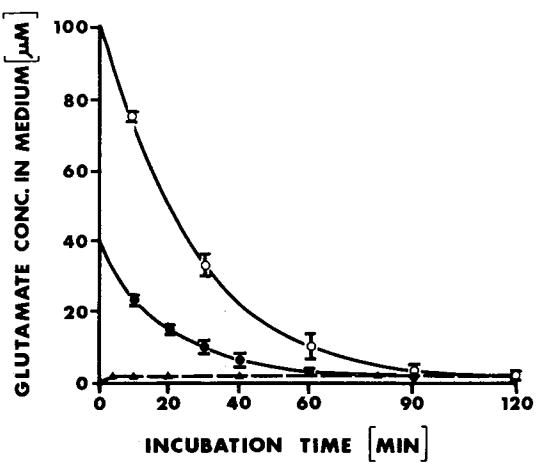

FIG. 5. Time course of changes in the concentration of glutamate in the medium in which isolated toad brains were incubated. Each toad brain (~60 mg wet tissue) was incubated in 2 ml oxygenated bicarbonate-buffered medium initially containing glutamate concentrations of 100 (○), 40 (●), or 0 μmoles/liter (△). Each point is the mean of 2 to 7 experiments. The vertical lines represent the range (where $N = 2$) or SEM of the data. In some instances, the SEM values were smaller than the size of the symbol. (From Shank et al., ref. 33; and Shank and Aprison, ref. 31.)

the medium until a similar steady-state level was achieved. Therefore, on the basis of these results, it would appear that the CNS regulates extracellular glutamate at a level between 0.001 and 0.002 mM. Physiological studies indicated that 0.002 mM is the concentration at which glutamate just begins to cause membrane depolarization (19). This extracellular concentration (0.002 mM) is about 0.05% of the intracellular concentration of glutamate; thus, CNS tissues have a remarkable capacity to remove this amino acid from the extracellular fluid.

METABOLISM OF GLUTAMATE TAKEN UP BY CNS TISSUES

Exogenous glutamate taken up by the isolated toad brain does not equilibrate with the endogenous glutamate before being metabolized. Most of the exogenous glutamate is rapidly metabolized to glutamine, and much of the glutamine so formed is subsequently released without being further metabolized (Fig. 6). Mammalian brain preparations have also been shown to selectively metabolize exogenous glutamate to glutamine (6). Glutamine synthetase is now known to be located predominantly, if not exclusively, within neuroglia (25,34); consequently, it is reasonable to conclude that most of the exogenous glutamate was taken into glial cells. This does not necessarily mean that glutamate released from nerve terminals is selectively taken

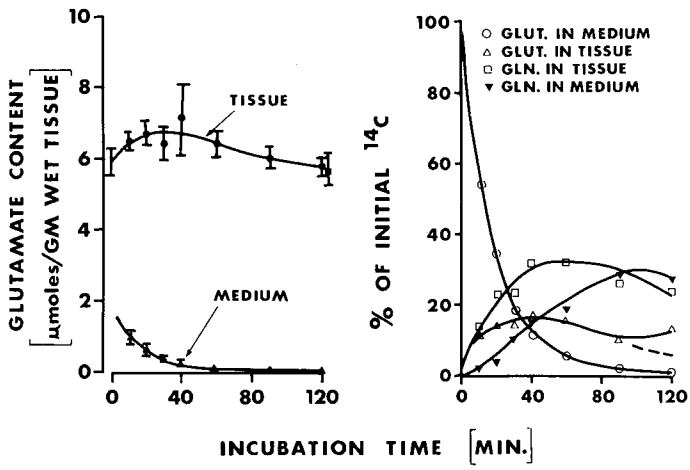

FIG. 6. Uptake and metabolism of glutamate by the isolated toad brain. **Left:** The change in the content of glutamate in the tissue and medium. **Right:** The change in the distribution of radioactivity in glutamate and glutamine in the tissue and medium are plotted. Each toad brain (~60 mg wet mass) was incubated in a bicarbonate-buffered medium (2 ml) initially containing 0.04 mM L-glutamate and 0.02 μCi of (U-^{14}C)L-glutamate. The content of glutamate (μmoles/g wet tissue) in the medium was obtained by multiplying the actual concentration of glutamate in the medium by the ratio of the medium volume (in ml) to the tissue mass (in g). The data are the mean ± SEM of 3 or 4 experiments. The last data point for the content of glutamate in the tissue represents the mean content of glutamate when the tissue was incubated 120 min without glutamate initially present in the medium. (From Shank and Baxter, ref. 32, and *unpublished;* and Shank et al., ref. 33.)

up by glia. The exogenous glutamate is likely to have a greater probability of being exposed to glial uptake sites than neuronal uptake sites, whereas the reverse is the case for endogenous glutamate released from nerve terminals.

CONCLUSION

At the present time, our model of the biochemical processes associated with the neurotransmitter function of glutamate serves more as a working hypothesis than an established and well-understood series of biochemical events. It does appear reasonably certain that uptake into the presynaptic terminal and surrounding glial cells functions as the mechanism by which glutamate is inactivated after being released into the synaptic cleft. However, we still have much to learn about the transport systems that mediate this uptake. It is likely that glutamine and α-ketoglutarate serve as the major precursors of the transmitter pool of glutamate; however, virtually nothing is known about the mechanisms that regulate the synthesis of glutamate from these percursors. With regard to the role that glial cells serve, we as yet have no appreciation of the quantitative significance of their involvement in the inactivation of glutamate or in supplying glutamine for the restoration of the transmitter pool.

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