Journal of Neurochemistry
Raven Press, New York

Short Review

Glutamate: A Neurotransmitter in Mammalian Brain

Frode Fonnum

Norwegian Defence Research Establishment, Division for Environmental Toxicology, Kjeller, Norway

Glutamate is ubiquitously distributed in brain tissue, where it is present in a higher concentration than any other amino acid. During the last 50 years glutamate in brain has been the subject of numerous studies, and several different functions have been ascribed to it.

Early studies by Krebs (1935) suggested that glutamate played a central metabolic role in brain. The complex compartmentation of glutamate metabolism in brain was first noted by Waelsch and coworkers (Berl et al., 1961). These studies were precipitated by the claim that glutamate improved mental behaviour and was beneficial in several neurological disorders including epilepsy and mental retardation. Other scientists pointed out its function in the detoxification of ammonia in brain (Weil-Malherbe, 1950). Glutamate is also an important building block in the synthesis of proteins and peptides, including glutathione (Meister, 1979). The toxic effect of administered glutamate and its analogues kainic acid, ibotenic acid, and *N*-methyl aspartic acid on CNS neurones has become a large and independent line of research (Lucas and Newhouse, 1957; Olney et al., 1974; Lund-Karlsen and Fonnum, 1976; Coyle, 1983). Attention has also been focused on the role of glutamate as a precursor for the inhibitory neurotransmitter γ-aminobutyric acid (GABA) (Roberts and Frankel, 1950).

Electrophysiological studies (Curtis and Watkins, 1961) focused early on the powerful and excitatory action of glutamate on spinal cord neurones. Since the action was widespread and effected by both the D- and L- forms, it was at first difficult to believe that glutamate could be a neurotransmitter. During the last 15 years, however, several studies have provided support for the concept that glutamate is a transmitter in brain (for review see Curtis and Johnston, 1974; Fonnum, 1978; 1981; Roberts et al., 1981; DiChiara and Gessa, 1981). Glutamate satisfies today to a large extent the four main criteria for classification as a neurotransmitter: (1) it is presynaptically localized in specific neurones; (2) it is specifically released by physiological stimuli in concentrations high enough to elicit postsynaptic response; (3) it demonstrates identity of action with the naturally occurring transmitter, including response to antagonists; and (4) mechanisms exist that will terminate transmitter action rapidly.

The evidence for glutamate as a transmitter at the locust neuromuscular junction has recently been carefully evaluated by Usherwood (1981). In that case the identity of action of glutamate with the naturally occurring transmitter on the neuromuscular receptor, the release from nerve terminals, and its similarity to acetylcholine at the mammalian neuromuscular junction with regard to presynaptic pharmacology and denervation supersensitivity are compelling evidence for glutamate as a neurotransmitter.

The main methods used to identify glutamergic pathways in brain will be critically reviewed and discussed. The effect of lesions on high-affinity uptake and release are particularly important, but immunohistochemical methods to study enzymes and glutamate itself are becoming more interesting. The release of glutamate has been demonstrated by several different procedures using both *in vivo* and *in vitro* preparations. The synthesis of large groups of specific agonists and antagonists has been important both for identification and characterization of the glutamate receptor by electrophysiological techniques and for the isolation of glutamate receptors. High and perhaps low-affinity uptake into nerve terminals and glial cells is important for the termination of transmitter action. Particular attention is given in this review to the complex compartmentation of glutamate synthesis and the possibility of identifying the transmitter pool of glutamate.

HIGH-AFFINITY UPTAKE

A common property of transmitter amino acids such as GABA and glycine and transmitter amines

Address correspondence and reprint requests to Frode Fonnum, Norwegian Defence Research Establishment, Division for Environmental Toxicology, N-2007 Kjeller, Norway.

Abbreviations used: GABA, δ-Aminobutyric acid; HRP, Horseradish peroxidase; NMDA, *N*-Methyl-D-aspartate.

such as dopamine, serotonin, and noradrenaline is a sodium-dependent high-affinity uptake system (for review see Fonnum et al., 1980). A similar uptake system also exists for glutamate. The uptake of glutamate shows an absolute requirement for sodium (Bennet et al., 1973), and studies with $^{22}Na^+$ show that two sodium ions are required for the uptake of one glutamate molecule (Stallcup et al., 1979). The order of association of the ions with the transport carrier has by kinetic studies been determined to be sodium-sodium-glutamate (Wheeler and Hollingsworth, 1978).

The uptake carrier of glutamate exhibits a high degree of specificity. L-Glutamate and D- and L-aspartate are taken up by the same uptake mechanism, whereas no other amino acid is taken up to any significant extent by this carrier (Logan and Snyder, 1971; Balcar and Johnson, 1972, 1973; Davies and Johnston, 1976). The active uptake of D-aspartate has allowed us to use this relatively metabolically stable compound as a false transmitter in uptake and release studies (Lund-Karlsen and Fonnum, 1978; Malthe-Sørenssen et al., 1979).

The high-affinity uptake can be measured biochemically in a homogenate or in a synaptosome preparation, and autoradiographically in thin slices (<200 μm). Alternatively, the number of sodium-dependent binding sites can be used in studying postmortem material (Vincent and McGeer, 1980). With these techniques the uptake seems to be highly specific for glutamergic nerve terminals. In more than 20 regions of the brain there is a reduction in glutamate uptake after lesions of presumed glutamergic pathways (see Table 1). Lesions of specific pathways are often accompanied by a reduction of 70–90% in uptake of glutamate or aspartate in a homogenate, without any changes in uptake of other transmitters such as GABA (Lund-Karlsen and Fonnum, 1978; Fonnum et al., 1981*a*; Walaas, 1981; Young et al., 1981). Quantitative autoradiographic studies from hippocampal slices show a three- to sixfold higher grain density over unmyelinated axons and boutons than average for the slice. In fact, 80% of the grains were over neuronal structures, whereas only 20% were over glial structures (Storm-Mathisen and Iversen, 1979). Very specific autoradiographic localization of [^{3}H]D-aspartate has been found in brain (Søreide and Fonnum, 1980; Fonnum et al., 1981*b*).

An exception to this rule is described in several cerebellar studies where glial cells show a higher labelling than neuronal structures. In these studies care was taken to preserve cell structures (de Barry et al., 1982; Levi et al., 1982; Wilkins et al., 1982). Recently, however, Shank and Campbell (1983) have shown that in cerebellar fractions parallel fibre synaptosomes showed a more than threefold higher uptake than the astrocytes. After destruction of the parallel fibre terminals with X-ray radiation (Rohde et al., 1979), viral infection (Young et al., 1974), or in mutant mice with reduced number of parallel fibres (Hudson et al., 1976), there is a significant reduction in uptake in cerebellar homogenate.

Glial cell preparations from sensory ganglia (Schon and Kelly, 1974), isolated glial cells (Henn and Hamberger, 1971), or primary cultures of astrocytes (Hertz et al., 1979) all show high-affinity uptake of glutamate. Injection of [^{3}H]glutamate intracerebrally also leads to the preferential labelling of glial cells (Hökfelt and Ljungdahl, 1972).

There is apparently no difference in the substrate and inhibitor specificity in the glutamate uptake of nerve terminals or glial cell preparations (Balcar et al., 1977). Recently 4-acetamino-4-isothiocyano-2,2-disulphonic acid stilbene has been suggested as a specific glial inhibitor (Waniewski and Martin, 1983). The compound has previously been used as an inhibitor of high-affinity uptake of glycine by human erythrocytes (Ellory et al., 1981) and the specificity in brain has not been thoroughly explored. The different findings of high-affinity uptake of glutamate both into nerve terminals and glial cell preparations can be reconciled by assuming that both structures have an uptake activity. The glial cell uptake is, however, very labile to mechanical disruption such as homogenization or even rough tissue slice preparation. *In vivo* the glial cell uptake is probably important in removing the released neurotransmitter.

High-affinity uptake studies cannot be used to differentiate between the transmitters aspartate and glutamate. The uptake ratio between D-aspartate and L-glutamate is similar in different brain regions (McGeer et al., 1977) and only small changes are observed after lesions (Lund-Karlsen and Fonnum, 1978; Storm-Mathisen and Woxen-Opsahl, 1978; Thangnipon et al., 1983). The small differences noted may be due to changes in the amino acids of the homogenate. Goldfish rod photoreceptors, however, showed a preferential uptake of L-glutamate compared with L-aspartate (Marc and Lam, 1981). Also, injection of labelled aspartate and glutamate in the cochlea gave labelling of different structures in cochlear nucleus (Kane, 1979). Further, Streit (1980) reported labelling of neurones in different cortical layers after injection with D,L-[^{3}H]glutamate and D-[^{3}H]aspartate. It was, however, not excluded in any of these studies that different rate of metabolism rather than differences in uptake specificity of the two amino acids could explain these unexpected results.

The importance of the uptake process in terminating the excitatory effect of exogenous aspartate and glutamate has been established. The uptake inhibitors L-glutamate dimethylester (Haldeman and McLennan, 1973) and L- and D-threo-3-hydroxy aspartate (Johnston et al., 1980), all prolonged the excitatory action of L-glutamate.

TABLE 1. *Summary of evidence for identification of glutamate neurones in brain*

	Uptake	Release	Immunocyto-chemistry + injection	Content
Auditory nerve		1	3	2
Cerebellar fibres				
Climbing fibres	4	4		
Parallel fibres	6,7	8		7
Corticofugal fibers				
Amygdala	9			9
Cuneate nucleus	10		11	
Dentate gyrus	12	13		
Lateral geniculate body	14	15	15	14
Nucleus accumbens	16,7			16
Olfactory tubercle	9			9
Pontine nucleus	18			
Red nucleus	10			
Spinal cord	10			
Neostriatum	19	20,22,23	21	19
Substantia nigra	24			
Thalamus	19		25	
Hippocampal fibres				
Commissural		13,26		
Schaffer collateral	12	28		
Fornix fibers				
Bed nl of stria				
Terminals	16			16
Hypothalamus	16			16
Lateral septum	16	29,32		16
Mammillary body	16			16
Nucleus accumbens	16			16
Nucleus of diagonal band	16			16
Lateral olfactory tract		30		
Spinal cord interneurons		31	11	

(1) Canzek and Reubi, 1980; (2) Wenthold, 1978; (3) Altschuler et al., 1981; (4) Wiklund et al, 1982; (5) Rea et al., 1980; (6) Rohde et al., 1979; (7) Young et al., 1974; (8) Sandoval and Cotman, 1978; (9) Walker and Fonnum, 1983; (10) Young et al., 1981; (11) Rustioni and Cuenod, 1982; (12) Storm-Mathisen, 1977; (13) Nadler et al., 1978; (14) Lund-Karlsen and Fonnum, 1978; (15) Baughman and Gilbert, 1981; (16) Walaas and Fonnum, 1979; (17) Walaas, 1981; (18) Thangnipon et al., 1983; (19) Fonnum et al., 1981*a*; (20) Godukhin et al., 1980; (21) Streit, 1980; (22) Reubi and Cuenod, 1979; (23) Rowlands and Roberts, 1980; (24) Fonnum et al., 1981*b*; (25) Rustioni et al., 1982; (26) Skrede and Malthe-Sørenssen, 1981; (27) Nitsch et al., 1979; (28) Malthe-Sørenssen et al., 1979; (29) Malthe-Sørenssen et al., 1980; (30) Collins and Probett, 1981; (31) Davidoff et al., 1967; (32) Fonnum and Walaas, 1978.

SYNAPTIC RELEASE

Endogenous glutamate or exogenously labelled glutamate/aspartate has been released in a Ca^{2+}-dependent manner from slices or synaptosome preparations by several different depolarization methods such as electric field stimulation (de Belleroche and Bradford, 1972; Potashner, 1978), high potassium concentration (Nadler et al., 1977, 1978), veratridine (Toggenburger et al., 1982), or in the absence of depolarization agents by the ionophore A23187 and Ca^{2+} (Levi et al., 1976). Several toxins such as the fungal toxins verrucologen and penitrenin (Norris et al., 1980), the scorpion venom *Tityus* toxin, and the snake venom β-bungarotoxin (Dolly et al., 1978; Smith et al., 1980) all release transmitter amino acids including glutamate from cortical synaptosomes.

The transmitter amino acids including glutamate are also released by depolarization of glial cell preparations, but in a Ca^{2+}-independent manner (Blaustein, 1975; Sellström and Hamberger, 1977; Bowey et al., 1979; Duce and Keen, 1983).

There are several examples of synaptic release of aspartate/glutamate after specific stimulation of nerve pathways in *in vitro* preparations. Exogenously added D-aspartate or L-glutamate is released by stimulation of the Schaffer collaterals

(Malthe-Sørenssen et al., 1979; Wieraszko and Lynch, 1979) and of the commissural pathway (Skrede and Malthe-Sørenssen, 1981) in the hippocampal slice preparations, or from lateral septum in the septum-fimbria slice preparation (Malthe-Sørenssen et al., 1980). Endogenous aspartate/glutamate are released by stimulation of the lateral olfactory tract both directly and by activation of the deep and superficial pyramidal cells in the olfactory cortical slice (Collins, 1980).

There are several examples of release of glutamate from *in vivo* experiments. Glutamate was released together with smaller amounts of glycine, taurine, and aspartate but not together with other amino acids from the cortical surface after stimulation of the medial reticular formation in an encephali isolé preparation (Jasper and Koyama, 1969). Glutamate was also released from the hippocampal surface after stimulation of the entorhinal cortex (Crawford and Connor, 1973). Likewise, glutamate and GABA were released from sensorimotor cortex by contralateral, but not by ipsilateral, bronchial plexus stimulation (Abdul-Ghani et al., 1979). There are examples of release of glutamate together with GABA and taurine after direct electrical stimulation or KCl application to the visual cortex (Clarke and Collins, 1975, 1976).

SUBCELLULAR DISTRIBUTION

Subcellular fractionation has not shown a specific localization of glutamate to synaptosomes or to synaptic vesicle fractions, which is in contrast to the findings for acetylcholine (Mangan and Whittaker, 1966; Rassin, 1972; de Belleroche and Bradford, 1972; Kontro et al., 1980). It is particularly striking that newly synthesized glutamate after injection of labelled glucose immediately prior (10 min) to preparation of the tissue did not give a preferential labelling of glutamate in the synaptosome fraction (van den Berg, 1973; Fonnum and Minchin, unpublished observation). The results may in part be explained by the fact that glutamate is ubiquitously localized in brain, but it may well be that the transmitter amino acids are bound to synaptic vesicles differently than is acetylcholine. The experiment on the different effects of cold and warm buffer on the release of transmitter amines and amino acids supports such an idea (Raiteri and Levi, 1973). Glutamate-like immunoreactivity has been indicated in some synaptic vesicles (Ottersen and Storm-Mathisen, 1983). The fast retrograde transport of D-aspartate described in many presumed glutamergic pathways (Streit, 1980) may also be taken as an indication for localization of glutamate in synaptic vesicles. It may be that the isolation of proper synaptosomal and vesicular pools of glutamate requires a different technique than used for acetylcholine. Recently, however, Naito and Ueda (1983) have demonstrated ATP-dependent uptake of glutamate into protein 1-associated synaptic vesicles.

LOCALIZATION OF GLUTAMATE NEURONES

The most commonly used technique to identify glutamate pathways is the combination of lesions and high-affinity uptake studies as discussed above. The results obtained using this technique agree with the general conclusions drawn from other techniques. Lesions combined with Ca^{2+}-dependent release have also been of considerable value and have even been claimed to differentiate between aspartate and glutamate as the transmitter (see, however, Reubi et al., 1980). Lesions will also result in a decreased level of the neurotransmitter itself. There are several examples where a lesion is accompanied by a loss in the endogenous level of glutamate, and this method may also be used to differentiate between aspartate and glutamate (Lund-Karlsen and Fonnum, 1978; Walaas and Fonnum, 1980; Fonnum et al., 1981*a*; Hassler et al., 1982). Interestingly, earlier studies in hippocampus did not reveal changes in the endogenous level of acidic amino acids (Nadler et al., 1977, 1978, but see Nitsch et al., 1979), but recent results have shown that lesion of the hippocampal commissural pathway is accompanied by a loss of synaptosomal glutamate (Nadler and Smith, 1981).

Retrograde transport of D-aspartate after microinjection in terminal region is a method that may be of great value in tracing neurotransmitter pathways (Streit, 1980). The success of this method must depend on specific uptake rather than specific transport; otherwise horseradish peroxidase (HRP), wheat germ agglutinin conjugated-HRP, and other proteins used in anatomical transport studies would have to be regarded as neurotransmitters. The technique should preferably be limited to metabolically stable transmitters or false transmitters such as D-aspartate.

Phosphate-activated glutaminase and cytoplasmic aspartate aminotransferase have been successfully localized in some presumed glutamate nerve terminals by immunohistochemical methods (Altschuler et al., 1981; Wendtholdt, personal communication). This is interesting, since the enzymes have an ubiquitous localization in brain (Johnson, 1972) and are at most only slightly concentrated in glutamergic nerve terminals (see Glutamate Synthesis). It should be kept in mind that small differences in endogenous concentrations of a substance may be responsible for negative or positive immunocytochemical staining.

Recently Storm-Mathisen et al. (1983) have succeeded in histochemical localization of glutamate-like immunoactivity in hippocampus with results similar to those obtained by high-affinity uptake of

[^{3}H]aspartate. The method is based on the assumption that glutamate, as are other neurotransmitters, is localized in a high concentration in glutamate nerve terminals (for GABA see Fonnum and Walberg, 1973). Studies have shown results in agreement with the general view on glutamergic terminal localization in regions such as cerebellum or neostriatum. Discrepancies are found in nucleus interpeduncularis, which exhibits a high glutamate-like immunoactivity (Ottersen and Storm-Mathisen, 1983), but low glutamate uptake activity (Fonnum, unpublished).

The results on localization of glutamergic neurones are summarized in Table 1. It should be borne in mind that it is at present difficult to differentiate between aspartate and glutamate as the transmitter. Cortical afferent fibres are often glutamergic. The fibres apparently come from areas throughout the entire cortex although medial frontal cortex appears to be especially rich in afferent glutamergic neurones.

Fornix fibres derived from subiculum and hippocampus are another important glutamergic pathway (Walaas and Fonnum, 1980). Within the hippocampus itself there is strong evidence for glutamate, or aspartate, as transmitters in the commissural and mossy fibres, as well as in the perforant path. The lateral olfactory tract, the auditory nerve, and the cerebellar climbing fibres are listed in Table 1, although the aspartergic component may dominate in these fibers (Wenthold, 1979; Collins and Probett, 1981; Wiklund et al., 1982). It is appropriate in connection with Table 1 to recall the strong evidence for glutamate as a transmitter of the optic nerve in pigeon (for review see Cuenod et al., 1981), and also that an acidic amino acid may be released from afferent nerve fibers from arterial baroreceptors terminating in nucleus tractus solitaire (Talman et al., 1980).

SYNTHESIS OF GLUTAMATE IN BRAIN

The transport of circulating glutamate to the brain normally plays only a minor role in regulating the brain glutamate level. In fact, the influx from plasma across the blood–brain barrier is much lower than the efflux of glutamate from brain (Oldendorf, 1971; Oldendorf and Szabo, 1976; Pratt, 1976).

In view of the many different roles assigned to glutamate, it is not surprising that its synthesis and metabolism are compartmentalized in a very complex pattern. Administration of the two general precursors, glucose and acetate, in a labelled form gives very different incorporation of radioactivity into the two related amino acids, glutamate and glutamine. Glucose leads to a low specific radioactivity of glutamine relative to glutamate whereas the opposite is true for acetate. This has been interpreted as if glucose and acetate are synthesizing glutamate via two different citrate cycles in brain. The list of precursors behaving as acetate include propionate, butyrate, citrate, leucine, GABA, aspartate, protein, and ammonia, whereas glycerol, lactate, pyruvate, α-ketoglutarate, and β-hydroxybutyrate behave as glucose (Clarke et al., 1975). Glucose is believed to be metabolized in the large glutamate compartment with little glutamine synthesis. This compartment consists probably of neuronal structures including nerve terminals (Balazs et al., 1970; Machiyama et al., 1970). Acetate, on the other hand, is metabolized in a small glutamate compartment high in glutamine synthesis (Berl et al., 1961; van den Berg et al., 1975). The immunohistochemical localization of glutamine synthetase, the enzyme synthesizing glutamine, to astroglial cells is compatible with the view that the small compartment is the astroglial cells (Norenberg and Martinez-Hernandez, 1979). The autoradiographic picture after incubation of peripheral ganglia with labelled glucose and acetate (Minchin and Beart, 1974), and the rate of conversion of labelled acetate and glucose into glutamate and glutamine in normal and deafferentated substantia nigra (Minchin and Fonnum, 1979) are consistent with these morphological interpretations.

The large compartment contains 85–98% of the total glutamate pool in brain (summarized by Cremer et al., 1975). A further subdivision of the two compartments is, however, necessary to accommodate the several different functions and turnover rates of glutamate (van den Berg et al., 1975; Fonnum, 1981). Two of these subcompartments contain transmitter glutamate and precursor glutamate for GABA, respectively. Lesions of glutamergic pathways to different regions of the brain are often accompanied by a loss of 20–45% of the endogenous glutamate content, indicating the size of the transmitter glutamate pool (Lund-Karlsen and Fonnum, 1978; Walaas and Fonnum, 1980; Fonnum et al., 1981*a;* Hassler et al., 1982). In contrast, the loss of glutamate in substantia nigra is negligible after lesion of the GABAergic striatonigral tract (Korf and Venema, 1983).

The compartmentation studies indicate the following immediate precursors for glutamate synthesis: (1) from glutamine by phosphate-activated glutaminase, (2) from 2-oxoglutarate and aspartate by aspartate aminotransferase, and (3) from 2-oxoglutarate by ornithine δ-aminotransferase.

Several studies have shown that glutamine is an excellent precursor both for the releasable pool (transmitter pool) of glutamate as well as for GABA (Hamberger et al., 1979*a,b;* Reubi, 1980). Phosphate-activated glutaminase belongs to the large group of mitochondrial enzymes that are preferentially but not exclusively localized to nerve terminals (Reijnierse et al., 1975; Bradford and Ward,

1976). Degeneration of the glutamergic terminals in neostriatum or cochlear nucleus was accompanied by a small but significant fall (approximately 20%) of glutaminase (Walker and Fonnum, unpublished; Ward et al., 1982; Wenthold, 1980). Immunohistochemical localization has shown that the enzyme is at least preferentially localized to glutamergic terminals in cochlear nucleus (Wenthold, personal communication). Glutaminase activity is strongly regulated by its products, glutamate and ammonia. It may be that end-product regulation is the dominating factor for regulation of transmitter glutamate synthesis (Kvamme and Olsen, 1980).

Several experiments have shown that [U-^{14}C]-glucose can function as a precursor for the releasable pool of glutamate (Potashner, 1978; Bradford et al., 1978; Hamberger et al., 1979*a*). Glucose is metabolized to precursors for glutamate such as oxaloacetate or 2-oxoglutarate. The keto acid could be produced from glucose either via citrate cycles or, as argued by Shank and Campbell (1982), via pyruvate carboxylase which is localized in astroglial cells.

2-Oxoglutarate, either synthesized in the nerve terminal or transported from the astroglial cell, could be transaminated by aspartate aminotransferase to transmitter glutamate. Aspartate aminotransferase is localized in brain as two isoenzymes with widely different kinetic properties (Fonnum, 1968). One isoenzyme shows product inhibition and is predominantly present in synaptic mitochondria whereas the other isoenzyme is localized like the cytoplasmic marker lactate dehydrogenase (Fonnum, 1968). An immunocytochemical technique has shown that the cytoplasmic isoenzyme is preferentially localized to presumed glutamergic terminals in retina and cochlear nucleus (Altschuler et al., 1982). Kinetic studies with an inhibitor, aminooxyacetic acid, also suggest that the enzyme is associated more with neuronal than glial structures (Berl and Clarke, 1978).

Kinetic studies by infusion of [^{15}N]ammonium acetate (Berl et al., 1962) and subcellular fractionation (Reijnierse et al., 1975) suggest that glutamate dehydrogenase is localized to nonsynaptic mitochondria and therefore does not participate in the formation of transmitter glutamate.

The basic amino acid ornithine may be converted to glutamate in brain via glutamate semialdehyde (Yoneda et al., 1982). The slow conversion, at least in climbing fibre endings, makes this substance less likely to be a precursor for transmitter glutamate (Shank and Campbell, 1983). One should note that quantitative data on influx of ornithine from circulation and its metabolism in brain are sparse.

Double labelling experiments with glucose and glutamine in hippocampal dentate gyrus slices have shown that 66% of the released glutamate pool was derived from glutamine and only 16% from glucose (Hamberger et al., 1979*a*,*b*). Similar experiments with cortex synaptosomes have shown that 80% of the released glutamate was derived from glutamine and less than 20% from glucose (Bradford et al., 1978). Characteristic of these studies is that the presence of glutamine in addition to glucose enhanced the released glutamate several-fold. Due to the rapid diffusion in slices and synaptosomes, it may be that the strong endogenous regulation of glutaminase is not working and that the contribution from glutamine is overemphasized in *in vitro* studies.

In *ex vivo* studies the two precursors were infused into the lateral ventricles *in vivo*, but the release experiments were carried out with the prelabelled slices *in vitro*. In such experiments the released pool of glutamate was labelled to the same extent by glutamine and glucose (Ward et al., 1983). In an *in vivo* study where the sensorimotor cortex was first perfused with 0.5 m*M* glutamine and then stimulated by *Tityus* toxin (1 μ*M*), the specific activities of the released glutamate were only about 1% of that of the original glutamine solution. The low specific activities could be due either to a tremendous dilution of the infused glutamine by endogenous glutamine or to a low conversion rate of glutamine to transmitter glutamate (Thanki et al., 1983).

In conclusion, the relative contribution of glutamine or glucose to transmitter glutamate *in vivo* therefore remains an open question. It may well be that transmitter pool of glutamate will accept any glutamate available independent of source and that the synthesis of the transmitter itself is not a rate-limiting factor under normal conditions.

RECEPTOR AND BINDING STUDIES

The development of new agonists and antagonists have suggested the existence of at least three distinct classes of receptors for the dicarboxylic amino acids glutamate and aspartate. The three receptors are activated preferentially by *N*-methyl-D-aspartate (NMDA), quisqualate, and kainate (Watkins, 1981; McLennan, 1981).

The NMDA receptor has a slight preference for aspartate compared with glutamate, whereas the opposite is true for the quisqualate and kainate receptors. The NMDA receptor may be characterized by the potent and specific antagonists D-α-aminoadipic acid and 2-amino-5-phosphonovaleric acid. NMDA antagonists have blocked excitation of corticofugal fibres to cuneate nucleus, caudate nucleus, and cerebral cortex (Stone, 1979). The quisqualate receptor is antagonized by L-glutamate diethylester, which has low potency, but has been shown to have antagonistic action against the synaptic excitation in neostriatum (Spencer, 1976), and other CNS cells (Haldeman and McLennan, 1972).

Cotman et al. (1981) have shown that different glutamergic/aspartergic pathways in hippocampus are blocked by different groups of antagonists. Glutamate and quisqualate gave depolarization with fast onset and recovery with a small increase in membrane conductance whereas NMDA showed depolarization slow in onset and recovery with a large and stable increase in membrane conductance (Lambert et al., 1981). The kainate receptor may have different physiological functions and is not discussed further.

Several different groups have reported sodium-independent binding of glutamate and aspartate to brain membranes (Sharif and Roberts, 1981; Michaelis et al., 1983; Baudry et al., 1983). The specific binding is modified by Ca^{2+} and Cl^- ions (Fagg et al., 1982). Activation of proteinases by Ca^{2+} ions also increases the number of binding sites (Baudry et al., 1983).

There is a poor correlation between the pharmacological activity of the agonists and antagonists and the binding to glutamate sites in several studies (Sharif and Roberts, 1981; Michaelis et al., 1983). But recently a close correspondence between the binding of glutamate and ω-phosphonoamino acids to synaptic membranes in the presence of Cl^- and Ca^{2+} ions and pharmacological activity have been demonstrated (Fagg et al., 1982). It will be of great interest to see whether the present complexity in binding studies will result in the series of subpopulations of receptors for the acidic amino acids. In particular, it will be interesting to see whether such studies will lead to a better differentiation between aspartate and glutamate as transmitters.

FUTURE STUDIES

During the last 5 years we have come a long way in showing that glutamate satisfies the criteria for classification as a neurotransmitter in mammalian brain and in identification of glutamate neurones and pathways. One of the most intriguing problems, which has not yet found a satisfactory solution, is the differentiation between aspartate and glutamate as transmitters. The two amino acids are metabolically closely related through transamination reaction and they are taken up by the same high-affinity uptake mechanism. At least *in vitro* they are released by the same mechanism and can act as false transmitter for each other. The quisqualate and the NMDA receptors do not differentiate well between them. The localization of aspartate transaminase is independent of the relative levels of aspartate and glutamate (Graham and Aprison, 1969). Lesions combined with endogenous release or together with determination of amino acid levels have been suggested to discriminate between them (see Reubi et al., 1980).

Several different precursors have been discussed for the synthesis of transmitter glutamate, glutamine being the strongest candidate. It is, however, not yet known to what extent the transmitter glutamate pool is well differentiated from the metabolic pool (Engelsen and Fonnum, 1982). A solution to these problems may enable us to develop a method for the determination of turnover of transmitter glutamate.

The use of subcellular fractionation technique to identify the different pools of glutamate has so far been of little value. Progress in this direction will also help in understanding the storage and release of the transmitter glutamate pool.

Further work on the glutamate receptor binding may well result in the isolation of subgroups of receptors that will better differentiate between the actions of glutamate and aspartate, and in developing pharmacas that may be important in mental health and memory. In this respect it should be borne in mind that glutamate is particularly important as a transmitter in cortical and hippocampal regions.

REFERENCES

Abdul-Ghani A. S., Bradford H. F., Cox D. W. G., and Dodd P. R. (1979) Peripheral sensory stimulation and the release of transmitter amino acids *in vivo* from specific regions of cerebral cortex. *Brain Res.* **171**, 55–56.

Altschuler F. R., Neises G. R., Harmison G. C., Wenthold R. J., and Fex J. (1981) Immunocytochemical localization of aspartate aminotransferase immunoreactivity in cochlear nucleus of the guinea pig. *Proc. Natl. Acad. Sci. USA* **78**, 6553–6557.

Altschuler R. A., Mosinger J. L., Harmison G. G., Parakkal M. H., and Wenthold R. J. (1982) Aspartate aminotransferase-like immunoreactivity as a marker for aspartate/glutamate in guinea pig photoreceptors. *Nature* **298**, 657–659.

Balazs R., Machiyama Y., Hammond B. J., Julian T., and Richter D. (1970) The operation of the γ-aminobutyrate bypath of the tricarboxylic acid cycle in brain tissue *in vitro*. *Biochem. J.* **116**, 445–467.

Balcar V. J. and Johnston G. A. R. (1972) The structural specificity of the high affinity uptake of L-glutamate and L-aspartate by rat brain slices. *J. Neurochem.* **19**, 2657–2666.

Balcar V. J., and Johnston G. A. R. (1973) High affinity uptake of transmitters: studies on the uptake of L-aspartate, GABA, L-glutamate and glycine in cat spinal cord. *J. Neurochem.* **20**, 529–539.

Balcar V. J., Borg J., and Mandel P. (1977) High affinity uptake of L-glutamate and L-aspartate by glial cells. *J. Neurochem.* **28**, 87–93.

Baughman R. W. and Gilbert C. D. (1981) Aspartate and glutamate as possible neurotransmitters in the visual cortex. *J. Neurosci.* **1**, 427–439.

Baudry M., Seman R., Smith E. W., and Lynch G. (1983) Regulation by calcium ions of glutamate receptor binding in hippocampal slices. *Eur. J. Pharmacol.* **90**, 161–166.

Berl S. and Clarke D. D. (1978) Metabolic compartmentation of the glutamate-glutamine system; glial contribution, in *Amino Acids as Chemical Transmitters* (Fonnum F., ed), pp. 691–708. Plenum Press, New York.

Bennet J. P. Jr., Logan W. J., and Snyder S. W. (1973) Amino acids as central nervous transmitters. The influence of ions, amino acid analogues and ontogeny on transport systems for L-glutamic and L-aspartic acids and glycine into central ner-

vous synaptosomes of the rat. *J. Neurochem.* **21**, 1533–1550.

Berl S., Lajtha A., and Waelsch H. (1961) Amino acid and protein metabolism. VI. Cerebral compartments of glutamic acid metabolism. *J. Neurochem.* **7**, 186–197.

Berl S., Takagaki G., Clarke D. D., and Waelsch H. (1962) Metabolic compartments *in vivo:* ammonia and glutamic acid metabolism in brain and liver. *J. Biol. Chem.* **237**, 2562–2569.

Blaustein M. P. (1975) Effects of potassium, veratridine and scorpion venom on calcium accumulation and transmitter release by nerve terminals *in vivo*. *J. Physiol. (Lond.)* **247**, 617–653.

Bowey, N. G., Brown D. A., and Marsh S. (1979) γ-Aminobutyric acid efflux from sympathetic glial cells: effect of depolarizing agents. *J. Physiol. (Lond.)*, **293**, 75–90.

Bradford H. F. and Ward H. K. (1976) On glutaminase in mammalian synaptosomes. *Brain. Res.* **110**, 115–125.

Bradford, H. F., Ward H. K., and Tomas A. J. (1978) Glutamine—a major substrate for nerve endings. *J. Neurochem.* **30**, 1453–1459.

Canzek V. and Reubi J. C. (1980) The effect of cochlear nerve lesion on the release of glutamate, aspartate and GABA from the cochlear nucleus *in vitro*. *Exp. Brain Res.* **38**, 437–441.

Clark, R. M., and Collins G. G. S. (1975) The spontaneous and potassium-evoked release of endogenous amino acids from the mammalian visual cortex. *J. Physiol. (Lond.)* **246**, 16–17.

Clark R. M. and Collins G. G. S. (1976) The release of endogenous amino acids from the rat visual cortex. *J. Physiol. (Lond.)*, **262**, 383–400.

Clarke D. D., Ronan E. J., Dicker E., and Tirri L. (1975) Ethanol and its relation to amino acid metabolism in brain, in *Metabolic Compartmentation and Neurotransmission* (Berl S., Clarke D. D., and Schneider D., eds), pp. 449–460. Plenum Press, New York.

Collins G. G. S. (1980) Release of endogenous amino acid neurotransmitter candidates from rat olfactory cortex slices: possible regulatory mechanisms and the effects of pentobarbitone. *Brain Res.* **190**, 517–528.

Collins G. G. S. and Probett G. A. (1981) Aspartate and not glutamate is the likely transmitter of the rat lateral olfactory tract fibres. *Brain Res.* **201**, 231–234.

Cotman C. W., Foster A., and Lanthorn T. (1981) An overview of glutamate as a neurotransmitter, in *Glutamate as a Neurotransmitter* (DiChiara G. and Gessa G. L., eds), pp. 1–27. Raven Press, New York.

Coyle J. T. (1983) Neurotoxic action of kainic acid. *J. Neurochem.* **41**, 1–11.

Crawford I. L. and Connor J. D. (1973) Localization and release of glutamic acid in relation to the hippocampal mossy fibre pathway. *Nature* **244**, 442–443.

Cremer J. E., Heath D. F., Patel A. J., Balazs R., and Cavanagh J. B. (1975) An experimental model of CNS changes associated with chronic liver disease: portocaval anastomosis in the rat, in *Metabolic Compartmentation and Neurotransmission* (Berl S., Clarke D. D., and Schneider D., eds), pp. 461–478. Plenum Press, New York.

Cuenod M., Beaudet A., Canzek V., Streit P., and Reubi J. C. (1981) Glutamergic pathways in the pigeon and in the rat brain. *Adv. Biochem. Psychopharmacol.* **27**, 57–68.

Curtis D. R. and Johnston G. A. R. (1974) Amino acid transmitters in the mammalian central nervous system. *Ergebn. Physiol.* **69**, 94–188.

Curtis D. R. and Watkins J. C. (1961) The chemical excitation of spinal neurons by certain amino acids. *J. Physiol. (Lond.)* **166**, 1–14.

Davidoff R. A., Graham L. T., Shank R. P., Werman R., and Aprison M. H. (1967) Changes in amino acid concentrations associated with loss of spinal interneurons. *J. Neurochem.* **14**, 1025–1031.

Davies L. P. and Johnston G. A. R. (1976) Uptake and release of D- and L-aspartate by rat brain slices. *J. Neurochem.* **26**, 1007–1014.

de Barry J., Langley O. K., Vincendon G., and Gombos G. (1982) L-glutamate and L-glutamine uptake in adult rat cerebellum: an autoradiographic study. *Neuroscience* **7**, 1289–1297.

de Belleroche J. S. and Bradford H. F. (1972) Metabolism of beds of mammalian cortical synaptosomes: response to depolarizing influences. *J. Neurochem.* **19**, 525–602.

de Belleroche J. S. and Bradford H. F. (1973) Amino acids in synaptic vesicles from mammalian cerebral cortex: a reappraisal. *J. Neurochem.* **21**, 441–451.

diChiara G., and Gessa G. L. (1981) *Glutamate as a Neurotransmitter.* Raven Press, New York,

Dolly J. O., Tse C. K., Spokes J. W., and Diniz C. R. (1978) Effects of β-bungarotoxin and *Tityus* toxin on uptake and release of neurotransmitters. *Biochem. Soc. Trans.* **6**, 652–654.

Duce I. R. and Keen P. (1983) Selective uptake of [^{3}H]glutamine and [^{3}H]glutamate into neurons and satellite cells of dorsal root ganglia *in vitro*. *Neuroscience* **8**, 861–866.

Ellory J. C., Jones S. E. M., and Young J. D. (1981) Glycine transport in human erythrocytes. *J. Physiol. (Lond.)* **320**, 403–422.

Engelsen B. and Fonnum F. (1982) Effects of hypoglycemia on transmitter and non-transmitter glutamate. *Acta Neurol. Scand. (Suppl. 90)* **65**, 63–64.

Fagg G. E., Foster A. C., Mena E. E., and Cotman C. W. (1982) Chloride and calcium ions reveal a pharmacological distinct population of L-glutamate binding sites in synaptic membranes: correspondence between biochemical and electrophysiological data. *J. Neurosci.* **2**, 958–965.

Fonnum F. (1968) The distribution of glutamate decarboxylase and aspartate transaminase in subcellular fractions of rat and guinea-pig brain. *Biochem. J.* **106**, 401–412.

Fonnum F. (Ed.) (1978) *Amino Acids as Chemical Transmitters, NATO Advanced Study Institutes Series, Series A, Life Sciences; Vol. 16*. Plenum Press, New York.

Fonnum F. (1981) The turnover of transmitter amino acids with special reference to GABA, in *Central Neurotransmitter Turnover*. (Pycock C. J. and Taberner P. V., eds), pp. 105–124. University Park Press, Baltimore.

Fonnum F. and Walaas I. (1978) The effect of intrahippocampal kainic acid injections and surgical lesions on neurotransmitters in hippocampus and septum. *J. Neurochem.* **31**, 1173–1181

Fonnum F., and Walberg F. (1973) An estimation of the concentration of γ-aminobutyric acid and glutamate decarboxylase in the inhibitory Purkinje axon terminals of the cat. *Brain Res.* **54**, 115–127.

Fonnum F., Lund-Karlsen R., Malthe-Sørenssen D., Skrede K. K., and Walaas I. (1979) Localization of neurotransmitters, particularly glutamate, in hippocampus, septum, nucleus accumbens and superior colliculus. *Progr. Brain Res.* **51**, 167–191.

Fonnum F., Lund-Karlsen R., Malthe-Sørenssen D., Sterri S., and Walaas I. (1980) High affinity transport systems and their role in transmitter action, in *The Cell Surface and Neuronal Function*, (Cotman C. W., Poste G., and Nicholson G. L., eds), pp. 455–504. Elsevier/North-Holland Biomedical Press, Amsterdam.

Fonnum F., Storm-Mathisen J., and Divac I. (1981*a*) Biochemical evidence for glutamate as neurotransmitter in the cortico-striatal and cortico-thalamic fibres in rat brain. *Neuroscience* **6**, 863–875.

Fonnum F., Søreide A., Kvale I., Walker J., and Walaas I. (1981*b*) Glutamate in corticofugal fibres in glutamate as a neurotransmitter in *Advances in Biochemical Psychopharmacology, Vol. 27* (Di Chiara G. and Gessa G. L., eds), pp. 29–41. Raven Press, New York.

Godukhin O. V., Zharikova A. D., and Novoselov V. I. (1980) The release of labeled L-glutamic acid from rat neostriatum

in vivo following stimulation of frontal cortex. *Neuroscience* **5**, 2151–2154.

Graham L. T. Jr. and Aprison M. H. (1969) Distribution of some enzymes associated with the metabolism of aspartate, glutamate, γ-aminobutyrate and glutamine in cat spinal cord. *J. Neurochem.* **16**, 559–566.

Haldeman S. and McLennan H. (1972) The antagonistic action of glutamic acid diethylester towards amino acid-induced and synaptic excitation of central neurones. *Brain Res.* **45**, 393–400.

Haldeman S. and McLennan H. (1973) The action of two inhibitors of glutamic acid uptake upon amino acid-induced and synaptic excitations of thalamic neurones. *Brain Res.* **63**, 123–129.

Hamberger A., Chiang G. H., Nylén E. S., Scheff S. W., and Cotman C. W. (1979*a*) Glutamate as a CNS transmitter. I. Evaluation of glucose and glutamine as precursors for the synthesis of preferentially released glutamate. *Brain Res.* **168**, 513–530.

Hamberger A., Chiang G. H., Sandoval E., and Cotman C. W. (1979*b*) Glutamate as a CNS transmitter. II. Regulation of synthesis in the releasable pool. *Brain Res.* **168**, 531–541.

Hassler R., Haug P., Nitsch C., Kim J. S., and Paik K. (1982) Effect of motor and premotor cortex ablation on concentrations of amino acids, monoamines, and acetylcholine and on the ultrastructure in rat striatum. A confirmation of glutamate as the specific cortico-striatal transmitter. *J. Neurochem.* **38**, 1087–1098.

Henn F. A. and Hamberger A. (1971) Glial cell function: uptake of transmitter substances. *Proc. Natl. Acad. Sci. USA* **68**, 2686–2690.

Hertz L., Bock E., and Schousboe A. (1979) GFA content, glutamate uptake and activity of glutamate metabolizing enzymes in differentating mouse astrocytes in primary cultures. *Dev. Neurosci.* **1**, 226–238.

Hökfelt T. and Ljungdahl A. (1972) Autoradiographic identification of cerebral and cerebellar cortical neurons accumulating labelled gamma-aminobutyric acid. *Exp. Brain Res.* **14**, 354–362.

Hudson D. B., Valcana T., Bean G., and Timiras P. S. (1976) Glutamic acid: a strong candidate as the neurotransmitter of the cerebellar granule cell. *Neurochem. Res.* **1**, 83–92.

Jasper H. H. and Koyama I. (1969) Rate of release of amino acids from the cerebral cortex in the cat as affected by brainstem and thalamic stimulation. *Can. J. Physiol. Pharmacol.* **47**, 887–905.

Johnson J. L. (1972) An analysis of the activities with the interconversion of α-ketoglutarate and glutamate: correlations with free glutamate levels in 20 specific regions in the nervous system. *Brain Res.* **45**, 205–215.

Johnston G. A. R., Lodge D., Bornstein J. C., and Curtis D. R. (1980) Potentiation of L-glutamate and L-aspartate excitation of cat spinal neurons by the stereoisomers of *threo*-3-hydroxyaspartate. *J. Neurochem.* **34**, 241–243.

Kane E. S. (1979) Central transport and distribution of labelled glutamic and aspartic acids to the cochlear nucleus in cats: an autoradiography study. *Neuroscience* **4**, 729–745.

Kontro P., Marnela K. M., and Oja S. S. (1980) Free amino acids in the synaptosome and synaptic vesicle fractions of different bovine brain area. *Brain Res.* **184**, 129–141.

Korf J. and Venema K. (1983) Amino acids in the substantia nigra of rats with striatal lesions produced by kainic acid. *J. Neurochem.* **40**, 1171–1173.

Krebs H. A. (1935) Metabolism of amino acids. IV. Synthesis of glutamine from glutamic acid and ammonia, and the enzymic hydrolysis of glutamine in animal tissue. *Biochem. J.* **29**, 1951–1969.

Kvamme E. and Olsen B. E. (1980) Substrate mediated regulation of phosphate-activated glutaminase in nervous tissue. *Brain Res.* **181**, 228–233.

Lambert J. D. C., Flatman J. A., and Engberg J. (1981) Actions of excitatory amino acids on membrane conductance and potential in motoneurones, in *Glutamate as a Neurotransmitter* (Di Chiara G. and Gessa G. L., eds), pp. 205–216. Raven Press, New York.

Levi G., Roberts P. J., and Raiteri M. (1976) Release and exchange of neurotransmitters in synaptosomes: effects of the ionophore A23187 and of ouabain. *Neurochem. Res.* **1**, 409–416.

Levi G., Gordon R. D., Gallo V., Wilkin G. P., and Balazs R. (1982) Putative acidic amino acid transmitters in the cerebellum. *Brain Res.* **239**, 425–445.

Logan W. J. and Snyder S. H. (1971) Unique high affinity uptake systems for glycine, glutamic and aspartic acids in central nervous tissue of the rat. *Nature* **234**, 297–299.

Lucas D. R. and Newhouse J. P. (1957) The toxic effect of sodium L-glutamate on the inner layers of the retina. *Ann. Am. Ophthalmol.* **58**, 193–200.

Lund-Karlsen R. and Fonnum F. (1976) The toxic effect of sodium glutamate on rat retina: changes in putative transmitters and their corresponding enzymes *J. Neurochem.* **27**, 1437–1441.

Lund-Karlsen R. and Fonnum F. (1978) Evidence for glutamate as a neurotransmitter in the corticofugal fibres to the dorsal lateral geniculate body and the superior colliculus in rats. *Brain Res.* **151**, 457–467.

Machiyama Y., Balazs R., Hammond B. J., Julian T., and Richer D. (1970) The metabolism of GABA and glucose in potassium stimulated brain tissue *in vitro*. *Biochem J.* **116**, 469–482.

Malthe-Sørenssen D., Skrede K. K., and Fonnum F. (1979) Calcium dependent release of D-[^{3}-H]aspartate evoked by selective electrical stimulation of excitatory afferent fibres to hippocampal pyramidal cells *in vitro*. *Neuroscience* **4**, 1255–1263.

Malthe-Sørenssen D., Skrede K. K., and Fonnum F. (1980) Release of [^{3}H]-D-aspartate from dorso-lateral septum after electrical stimulation of fimbria *in vitro*. *Neuroscience* **5**, 127–133.

Mangan S. L. and Whittaker V. P. (1966) The distribution of free amino acids in subcellular fractions of guinea-pig brain. *Biochem. J.* **98**, 128–137.

Marc R. E. and Lam D. M.W. (1981) Uptake of aspartic and glutamic acid by photoreceptors in goldfish retina. *Proc. Natl Acad. Sci. USA* **78**, 7185–7189.

McBean G. J. and Roberts P. J. (1981) Glutamate-preferring receptors regulate the release of D-[^{3}H]aspartate from rat hippocampal slices. *Nature* **291**, 593–594.

McGeer P. L., McGeer E. G., Scherer U., and Singh K. (1977) A glutamergic corticostriatal path? *Brain Res.* **128**, 369–373.

McLennan H. (1981) On the nature of the receptors for various excitatory amino acids in the mammalian central nervous system. *Adv. Biochem. Psychopharmacol.* **27**, 283–262.

Meister A. (1979) Biochemistry of glutamate, glutamine and glutathione, in *Advances in Biochemistry and Physiology: Glutamic Acid* (Keler L. J. Jr., Garattini S., Kane M. R., Reynolds W. A., Wurtman R. J. eds), pp. 64–84. Raven Press, New York.

Michaelis E. W., Michaelis M. L., Stormann T. M., Chittenden W. L., and Grubbs R. D. (1983) Purification and molecular characterization of the brain synaptic membrane glutamate-binding protein. *J. Neurochem.* **40**, 1742–1753.

Minchin M. C. W. and Beart P. M. (1974) Compartmentation of amino acid metabolism in the rat dorsal ganglia: a metabolic and autoradiographic study. *Brain Res.* **83**, 437–449.

Minchin M. C. W. and Fonnum F. (1979) The metabolism of GABA and other amino acids in rat substantia nigra slices following lesions of the striato-nigral pathway. *J. Neurochem.* **32**, 203–210.

Nadler J. V. and Smith E. M. (1981) Perforant path lesion depletes glutamate content of fascia dentata synaptosomes. *Neurosci. Lett.* **3**, 275–280.

Nadler J. V., White W. F., Vaca K. W., Redburn D. A., and Cotman C. W. (1977) Characterization of putative amino

acid transmitter release from slices of rat dentate gyrus. *J. Neurochem.* **29**, 179–290.
Nadler J. V., White W. F., Vaca K. W., Perry B. W., and Cotman C. W. (1978) Biochemical correlates of transmission mediated by glutamate and aspartate. *J. Neurochem.* **31**, 147–155.
Naito S. and Ueda T. (1983) Adenosine triphosphate-dependent uptake of glutamate into protein 1-associated vesicles. *J. Biol. Chem.* **258**, 696–699.
Nitsch C., Kim J.-K., Shimada Y., and Okada Y. (1979) Effects of hippocampus extirpation in the rat on glutamate levels in the target structures of hippocampal efferents. *Neurosci. Lett.* **11**, 295–299.
Norenberg M. D. and Martinez-Hernandez, A. (1979) Fine structural localization of glutamine synthetase in astrocytes of rat brain. *Brain Res.* **161**, 303–310.
Norris P. J., Smith C. C. T., de Belleroche J., Bradford H. F., Mantle P. G., Thomas A. J., and Penny R. H. C. (1980) Actions of tremorgenic fungal toxins on neurotransmitter release. *J. Neurochem.* **34**, 33–43.
Oldendorf W. H. (1971) Brain uptake of radiolabelled amino acids, amines, and hexoses after arterial injection. *Am. J. Physiol.* **221**, 1629–1639.
Oldendorf W. H. and Szabo J. (1976) Amino acid assignment to one of three blood-brain barrier amino acid carriers. *Am. J. Physiol.* **230**, 94–98.
Olney J. W., Rhee V., and Ho O. L. (1974) Kainic acid: A powerful neurotoxic analogue of glutamate. *Brain Res.* **77**, 507–512.
Ottersen O. P. and Storm-Mathisen J. (1983) Neurons containing or accumulating transmitter amino acids, in *Handbook of Chemical Neuroanatomy, Vol 2* (Bjørklund A. and Hökfelt T., eds) (in press).
Potashner S. J. (1978) The spontaneous and electrically evoked release from slices of guinea-pig cerebral cortex of endogenous amino acids labelled via metabolism of D-[U-^{14}] glucose. *J. Neurochem.* **31**, 177–186.
Pratt O. E. (1976) The transport of metabolizable substances into the living brain. *Adv. Exp. Biol. Med.* **69**, 55–75.
Raiteri M. and Levi G. (1973) Depletion of synaptosomal neurotransmitter pool by sudden cooling. *Nature* **243**, 180–182.
Rassin D. L. (1972) Amino acids as putative transmitters: failure to bind to synaptic vesicles of guinea-pig cerebral cortex. *J. Neurochem.* **19**, 139–148.
Rea M. A., McBride W. J., and Rhode B. (1980) Regional and synaptosomal levels of amino acid neurotransmitters in 3-acetylpyridine deafferented rat cerebellum. *J. Neurochem.* **34**, 1106–1108.
Reijnierse G. L. A., Veldstra H., and van den Berg C. J. (1975) Subcellular localization of γ-aminobutyrate transaminase and glutamate dehydrogenase in adult rat brain. *Biochem. J.* **152**, 469–475.
Reubi J. C. (1980) Comparative study of the release of glutamate and GABA, newly synthesized from glutamine in various regions of the central nervous system. *Neuroscience* **5**, 2145–2150.
Reubi J. C. and Cuenod M. (1979) Glutamate release *in vitro* from cortico-striatal terminals. *Brain Res.* **176**, 185–188.
Reubi J. C., Toggenburger G., and Cuenod M. (1980) Asparagine as precursor for transmitter aspartate in corticostriatal fibres. *J. Neurochem.* **35**, 1015–1017.
Roberts E. and Frankel D. (1950) γ-Aminobutyric acid in brain: its formation from glutamic acid. *J. Biol. Chem.* **187**, 55–63.
Roberts P. J. and Watkins J. C. (1975) Structural requirements for the inhibition of L-glutamate uptake by glia and nerve endings. *Brain Res.* **85**, 120–125.
Roberts P. J., Storm-Mathisen J., and Johnston G. A. R. (1981) *Glutamate Transmitter in the Central Nervous System.* John Wiley & Sons, Chichester.
Rohde B. H., Rea M. A., Simon J. R., and McBride W. J. (1979) Effects of X-irradiation-induced loss of cerebellar granule cells on synaptosomal levels and the high affinity uptake of amino acids. *J. Neurochem.* **32**, 1431–1435.
Rowlands G. J. and Roberts P. J. (1980) Specific calcium-dependent release of endogenous glutamate from rat striatum is reduced by destruction of the cortico-striatal tract. *Exp. Brain Res.* **39**, 239–240.
Rustioni A. and Cuenod M. (1982) Selective retrograde transport of D-aspartate in spinal interneurons and cortical neurons of rats. *Brain Res.* **236**, 143–155.
Rustioni A., Spreafico R., Cheema S., and Cuenod M. (1982) Selective retrograde labelling of somatosensory cortical neurons after ^{3}H-D-aspartate injections in the ventrobasal complex of rats and cats. *Neuroscience (Suppl.)* **7**, S183.
Sandoval M. E. and Cotman C. W. (1978) Evalution of glutamate as a neurotransmitter of cerebellar fibres. *Neuroscience* **3**, 199–206.
Schon F. and Kelly J. S. (1974) Autoradiographic localization of [^{3}H]-GABA and [^{3}H]-glutamate over satellite glial cells. *Brain Res.* **66**, 275–288.
Sellström A. and Hamberger A. (1977) Potassium-stimulated γ-aminobutyric acid release from neurons and glia. *Brain Res.* **119**, 189–198.
Shank R. P. and Campbell G. Lem. (1982) Glutamine and alpha-ketoglutarate uptake and metabolism by nerve terminal enriched material from mouse cerebellum. *Neurochem. Res.* **7**, 601–616.
Shank R. P. and Campbell G. Lem. (1983) Amino acid uptake, content and metabolism by neuronal and glial enriched cellular fractions from mouse cerebellum. *J. Neurosci.* (in press).
Sharif N. A. and Roberts P. J. (1981) L-Aspartate binding sites in rat cerebellum: a comparison of the binding of L-[^{3}H]aspartate and L-[^{3}H]glutamate to synaptic membranes. *Brain Res.* **211**, 293–303.
Skrede K. K. and Malthe-Sørenssen D. (1981) Differential release of D-[^{3}H]aspartate and [^{14}C]-γ-aminobutyric acid following activation of commissural fibres in a longitudinal slice preparation of guinea-pig hippocampus. *Neurosci. Lett.* **21**, 71–76.
Smith C. C. T., Bradford H. F., Thompson E. J., and MacDermot J. (1980) Actions of β-bungarotoxin on amino acid transmitter release. *J. Neurochem.* **34**, 487–494.
Søreide A. and Fonnum F. (1980) High affinity uptake of D-[^{3}H]aspartate in the barrel subfield of the mouse somatic sensory cortex. *Brain Res.* **201**, 727–730.
Spencer H. J. (1976) Antagonism of cortical excitation of striatal neurons by glutamic acid diethylester: evidence for glutamic acid as an excitatory transmitter in the rat striatum. *Brain Res.* **102**, 91–101.
Stallcup W. B., Bulloch K., and Baetge E. E. (1979) Coupled transport of glutamate and sodium in cerebellar nerve cell line. *J. Neurochem.* **32**, 57–65.
Stone T. W. (1979) Amino acids as neurotransmitters of corticofugal neurons in the rat: a comparison of glutamate and aspartate. *Br. J. Pharmacol.* **67**, 545–551.
Storm-Mathisen J. (1977) Glutamic acid and excitatory nerve endings: reduction of glutamic acid uptake after axotomy. *Brain Res.* **120**, 379–386.
Storm-Mathisen J. and Iversen L. L. (1979) Uptake of [^{3}H]-glutamic acid in excitatory nerve endings. Light and electron-microscopic observations in the hippocampal formation of the rat. *Neuroscience* **4**, 1237–1253.
Storm-Mathisen J. and Woxen-Opsahl M. (1978) Aspartate and/or glutamate may be transmitters in hippocampal efferents to septum and hypothalamus. *Neurosci. Lett.* **9**, 65–70.
Storm-Mathisen J., Leknes A. K., Bore A. T., Vaaland J. L., Edminson P., Haug F. M.-S., and Ottersen O. P. (1983) Glutamate and GABA: first visualization in neurones by immunocytochemistry. *Nature* **301**, 517–520.
Streit P. (1980) Selective retrograde labeling indicating the trans-

mitter of neuronal pathways. *J. Comp. Neurol.* **191**, 429–463.

Talman W. T., Perrone M. H., and Reis D. J. (1980) Evidence of glutamate as a neurotransmitter of baroreceptor afferent nerve fibres. *Science* **209**, 813–815.

Thangnipon W., Taxt T., Brodal P., and Storm-Mathisen J. (1983) The corticopontine projection: axotomy-induced loss of high affinity L-glutamate and D-aspartate uptake, but not of gamma-aminobutyrate uptake, glutamate decarboxylase or choline acetyltransferase, in the pontine nuclei. *Neuroscience* **8**, 449–457.

Thanki C. M., Sugden D., Thomas N. J., and Bradford H. F. (1983) *In vivo* release from cerebral cortex of [^{14}C]glutamate synthesized from [U-^{14}C]glutamine. *J. Neurochem.* **41**, 611–617.

Toggenburger G., Felix D., Cuenod M., and Henke H. (1982) *In vitro* release of endogenous beta-alanine, GABA and glutamate, and electrophysiological effect of beta-alanine in pigeon optic tectum. *J. Neurochem.* **39**, 176–183.

Usherwood P. N. R. (1981) Glutamate synapses and receptors in insect muscle, in *Glutamate as a Neurotransmitter* (DiChiara G. and Gessal G. L., eds), pp 183–192. Raven Press, New York.

van den Berg C. J. (1973) A model of compartmentation in mouse brain based on glucose and acetate metabolism, in *Metabolic Compartmentation in the Brain* (Balazs R. and Cremer J. E., eds), pp. 137–166. Macmillan, New York.

van den Berg C. J., Matheson D. F., Ronda G., Reijnierse G. L. A., Blokhuis G. G. D., Kroon M. C., Clarke D. D., and Garfinkel D. (1975) A model of glutamate metabolism in brain: a biochemical analysis of a heterogenous structure, in *Metabolic Compartmentation and Neurotransmission* (Berl S., Clarke D. D., and Schneider D., eds), pp. 709–723. Plenum Press, New York.

Vincent S. R. and McGeer E. G. (1980) A comparison of sodium dependent glutamate binding with high affinity glutamate uptake in rat striatum. *Brain Res.* **184**, 99–105.

Walaas I. (1981) Biochemical evidence for overlapping neocortical and allocortical glutamate projections to the nucleus accumbens and rostral caudatoputamen in the rat brain. *Neuroscience* **6**, 399–406.

Walaas I. and Fonnum F. (1979) The effects of surgical and chemical lesions on neurotransmitter candidates in the nucleus accumbens of the rat. *Neuroscience* **4**, 209–216.

Walaas I. and Fonnum F. (1980) Biochemical evidence for glutamate as a transmitter in hippocampal efferents to the basal forebrain and hypothalamus in rat brain. *Neuroscience* **5**, 1691–1698.

Walker J. and Fonnum F. (1983) Regional cortical glutamergic and aspartergic projections to the amygdala and thalamus of the rat. *Brain Res.* **267**, 371–374.

Waniewski R. A. and Martin D. L. (1983) Selective inhibition of glial versus neuronal uptake of L-glutamic acid by SITS. *Brain Res.* **268**, 390–394.

Ward H. W., Thanki C. M., Peterson D. W., and Bradford H. F. (1982) Brain glutaminase activity in relation to transmitter glutamate biosynthesis. *Biochem. Soc. Trans.* **10**, 369–370.

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

Watkins J. C. (1981) Pharmacology of excitatory amino acid receptors, in *Glutamate: Transmitter in the Central Nervous System* (Roberts P. J., Storm-Mathisen J., and Johnston G. A. R., eds), pp. 1–24. John Wiley & Sons, Chichester.

Weil-Malherbe H. (1950) Significance of glutamic acid for the metabolism of nervous tissue. *Physiol. Rev.* **30**, 549–568.

Wenthold R. J. (1978) Glutamic acid and aspartic acid in subdivisions of the cochlear nucleus after auditory nerve lesion. *Brain Res.* **143**, 544–548.

Wenthold R. J. (1979) Release of endogenous glutamic acid, aspartic acid and GABA from cochlear nucleus slices. *Brain Res.* **162**, 338–343.

Wenthold R. J. (1980) Glutaminase and aspartate amino transferase decrease in cochlear nucleus after lesion of the auditory. *Brain Res.* **190**, 283–297.

Wheeler D. D. and Hollingsworth R. G. (1978) A model of high affinity glutamic acid transport by cortical synaptosomes from the Long-Evans rat. *J. Neurochem.* **30**, 1311–1319.

Wieraszko A. and Lynch G. (1979) Stimulation dependent release of possible transmitter substances from hippocampal slices studied with localized perfusion. *Brain Res.* **160**, 372–376.

Wiklund L., Toggenburger G., and Cuenod M. (1982) Aspartate: possible neurotransmitter in cerebellar climbing fibers. *Science* **216**, 78–80.

Wilkins G. P., Garthwaite J., and Balazs R. (1982) Putative amino acid transmitters in the cerebellum. II. Electron microscopic localization of transport sites. *Brain Res.* **244**, 69–80.

Yoneda Y., Roberts E., and Dietz G. W. Jr. (1982) A new synaptosomal biosynthetic pathway of glutamate and GABA from ornithine and its negative feedback inhibition by GABA. *J. Neurochem.* **38**, 1686–1694.

Young A. B., Oster-Granite M. L., Herndon R. M., and Snyder S. H. (1974) Glutamic acid: selective depletion by viral induced granule cell loss in hamster cerebellum. *Brain Res.* **73**, 1–13.

Young A. B., Bromberg M. B., and Penny, J. B. (1981) Decreased glutamate in subcortical areas deafferented by sensorimotor cortex ablation in the cat. *J. Neurosci.* **1**, 241–249.