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COMMENTARY

THE NEUROTRANSMITTER AMINO ACID TRANSPORT SYSTEMS

A FRESH OUTLOOK ON AN OLD PROBLEM*

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It has become increasingly clear during the past 20 years that two widely distributed amino acids, glutamate and aspartate, are not only involved in a number of diverse metabolic reactions but also contribute to the functional control of the mammalian central nervous system by acting as neurotransmitters [1]. In addition to glutamate and aspartate, at least two other amino acids, α-aminobutyric acid (GABA) and glycine, are endowed with the same important property. However, GABA, in contrast to the other three amino acids, has no other known function in the CNS except that of a neurotransmitter. It is also well established that, whereas the acidic amino acids cause neuronal depolarization with consequent excitation, the two neutral amino acids have variable effects on membrane potential but consistently depress excitability [1, 2].

During the past 15 years several excellent and authoritative reviews have been written on amino acid neurotransmitters [1–11]. Nevertheless, there are at least two reasons why a fresh perspective on the development and current standing of the field may be appropriate. First, most reviews have treated the problem from the standpoint of the classical stimulus–secretion coupling theory [12] which relies on the assumption that amino acid neurotransmitters are sequestered in intrasynaptic vesicles and preferentially released from them during nerve stimulation. Since evidence for the existence of such vesicles is, at best, tentative ([13] but see Refs. 14 and 15 to the contrary), this approach has led to a certain bias in selection and interpretation of experimental results. Second, there has been increasing evidence that high-affinity, sodium-dependent amino acid transport systems on presynaptic nerve membranes can operate in both inward and outward directions [16–21]. This may provide an alternative yet parallel route, in addition to release from vesicles, whereby these substances can be expelled rapidly from the terminals in concentrations sufficient to elicit effects at postynaptic sites. The object of this commentary is to summarize information on how such systems are constructed and how they may operate *in vivo*. Although the emphasis will be placed on neuronal transport systems, and the discussion will utilize, to a large extent, information accumulated *in vitro*, predominantly on slices and preparations of nerve ending particles (synaptosomes), some attention will be paid to glial cell uptake of amino acids, since the latter may represent an important part of the overall neurotransmitter "traffic" and metabolism.

General properties of plasma-membrane amino acid transporters

It has long been known that intracellular concentrations of GABA, aspartate and glutamate in the mammalian CNS are much higher than those in the external environment [2, 4, 5, 7, 22, 23]. This observation led to an early hypothesis [3, 17] that uptake of these amino acids is carrier-mediated and occurs via an "active" process, traditionally defined as the net transmembrane movement of a chemical species against its own prevailing electrochemical potential gradient [24]. Detailed characterization of the active, high-affinity amino acid transporters resulted from concerted efforts in a number of laboratories (see Refs. 1–11, for review), and the properties of these systems which emerged in the middle 1980s can be summarized as follows:

(1) They exhibit Michaelis–Menten kinetics.
(2) They lead to accumulation of amino acids within the cell at concentrations 3–4 orders of magnitude higher than outside.
(3) They utilize energy although the process they mediate does not directly involve ATP splitting.
(4) They require sodium ions.

Delineation of these properties resulted in the postulate [17–21] that uptake of amino acid neurotransmitters is coupled to a second and different energy-dependent transport process, in this case that of Na^+, and *per se* does not require a direct input of ATP. The driving force for amino acid transport is

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hence provided by the simultaneous downhill movement of sodium ions which are maintained in a quasi steady-state, far from equilibrium, by the operation of the ATP-linked Na^+/K^+ pump. When the pump is operating, a transmembrane electrochemical gradient of amino acid can be maintained, and its final steady-state will reflect the size of the sodium gradient. The transmembrane electrochemical potential has two components: the chemical potential (which depends on the concentration, or activity, ratios) and membrane electrical potential. The latter can affect the transmembrane activity ratio of the ion and its mobility in the membrane as well as the mobility of the carrier, if the carrier is charged in one of its forms. Since mammalian cells maintain a membrane potential negative inside with respect to outside, additional driving forces for amino acid influx will be provided if a loaded carrier is positively charged or a free carrier is negatively charged. If both occur on the same system, energy obtained from the membrane potential will be doubled. It is also self-evident that increasing the number of positive charges on the loaded carrier or of negative charges on the free carrier will also increase the magnitude of the driving force. In formal terms these considerations can be written as follows:

$$\text{amino acid}^x_o + n\text{Na}^+_o \rightarrow \text{amino acid}^x_i + n\text{Na}^+_i$$

$$\frac{[\text{amino acid}^x]_i}{[\text{amino acid}^x]_o} = \frac{[\text{Na}^+]_o}{[\text{Na}^+]_i} e^{-\frac{(n+x)}{RT}FE}$$

or

$$\ln \frac{[\text{amino acid}^x]_i}{[\text{amino acid}^x]_o} = \ln \frac{[\text{Na}^+]_o}{[\text{Na}^+]_i} - \frac{n+x}{RT} FE$$

There are several predictions which stem from this model [24]:

(1) The rates of unidirectional fluxes of both amino acid and sodium should be functions of the concentrations of the two substrates on the side of the membrane from which transport is measured.

(2) Amino acid and sodium should translocate in some stoichiometric ratio.

(3) When the Na^+/K^+ pump is working, the steady-state accumulation ratio of amino ($[\text{amino acid}]_i/[\text{amino acid}]_o$) should depend on the transmembrane chemical potential gradient of Na^+ and the electrical membrane potential, E.

(4) At equilibrium, substrate exchange occurs with the same substrate specificity as active transport.

Characteristics of GABA and acidic amino acid transport systems

The two most important and most extensively studied high-affinity amino acid transport systems in mammalian brain are those for the inhibitory neurotransmitter, GABA, and the excitatory transmitters, glutamate and aspartate. The first is specific for GABA and a few related compounds such as hydroxy-GABA [25], diaminobutyric acid [26–29], *cis*-3-aminocyclohexane carboxylic acid [30, 31], isoguvacine [32, 33] and nipecotic acid [11, 30, 34] but not glycine, taurine or acidic amino acids [35]. The second translocates L- and D-aspartate, L-glutamate and other acidic amino acids such as cysteate, cysteine-sulfinate and threo-3-hydroxy-D,L-aspartate [35–40] but not D-glutamate, kainate, *N*-methyl-D-aspartate or neutral amino acids [36]. The values of Michaelis constants for uptake measured at 120–140 mM $[Na^+]_o$ and 3–5 mM $[K^+]_o$ are somewhat dependent on the type of experimental material used but are not markedly different for the two uptake systems. In synaptosomes, a preparation which contains predominantly transporters of neuronal origin, they range from 4 to 20 μM for GABA and from 10 to 40 μM for acidic amino acids (Table 1, [41–52]). In the same system the maximal rates of transport (V_{max}) are also rather similar: 1–4 nmol/min/mg protein for GABA and 2–8 nmol/min/mg protein for aspartate and glutamate at 25 ± 2°. These velocities are most likely underestimates of the true values because they are based on the assumption that all cells, or their fragments, present in the preparation are involved in the accumulation process. It should also be stressed that all these values were obtained by measuring transport in one direction only, from the exterior of the cell towards the interior. At present it is not feasible to evaluate the kinetic properties of amino acid transport in the opposite direction, i.e. from inside to outside. There are no procedures currently available to deplete cells or synaptosomes, completely, of amino acids and sodium, whereas vesicle preparations, which in principle could be used for this purpose, are never 100% "inside-out" and are very often "leaky" to ions.

Both GABA and acidic amino acid transports are influenced markedly by alterations in the concentrations of external sodium ions: a decrease in $[Na^+]_o$ leads to an increase in the K_m and a decrease in the V_{max} for uptake [21, 42, 53]. Although this response to the removal of sodium is a common property of both transporters, the sensitivity of the two systems to this cation is different. In the case of GABA, the concentration of sodium required to decrease the rate of uptake by 50% is 60–70 mM ([18, 42] and Table 1), whereas in the case of acidic amino acids, it is 14–18 mM [35, 46, 50, 54]. When sodium is replaced completely by lithium, aspartate uptake occurs at 20% of the control (i.e. at high $[Na^+]_o$) rate, glutamate at 6%, and GABA at only 1–2% [36, 55]. This means that the sodium binding sites on the various carriers must have different properties.

Analyses of the rates of uptake at various external sodium concentrations have provided estimates for the number of Na^+ cotransported with each amino acid molecule. The values range from 2–3 for GABA [11, 18, 53, 56, 57] to 1–2 for acidic amino acids [11, 38, 48, 50, 54, 58]. However, in one study only [38] were the simultaneous movements of sodium and glutamate evaluated with the radioactive tracer technique, and this yielded a stoichiometry of 2 Na^+ for each amino acid molecule taken up.

In addition to their effects on amino acid uptake, sodium ions also influence the efflux process. Release of GABA from synaptosomes that is evoked by depolarization was found to be dependent on internal $[Na^+]$ [59, 60], whereas GABA-promoted GABA efflux from isolated horizontal cells of the toad retina was shown to be accompanied by a simultaneous

Table 1. Representative values of kinetic constants for neurotransmitter amino acid–Na^+ cotransport in neuronal systems

Transport system (preparation)	Amino acid K_m (μM)	Na^+ (mM)	Stoichiometry (Na^+: amino acid)	V_{max}	Temperature (°)	Ref.
GABA						
Slices	2.2			0.115*	25	41
Synaptosomes	4.0		2–3	1.1†	27	42
	6.25			1.2†	25	43
	5.7	70	2	1.0†	25	Unpublished
		67	2		30	18
	6.19			1.0†		44
Synaptic vesicles	4.0			0.75†	25	45
Aspartate						
Slices (D-)	13.7	14		0.29*	25	46
(L-)	15.6	14		0.1*	25	46
Homogenate (L-)	16.9					47
						21 and
Synaptosomes (D-)	14.6	16	2	4.0†	25	Unpublished
Glutamate (L-)						
Homogenate	25	28	1		37	48
Isolated nerve cells	3			2–8†	37	49
Cultured neurons	33–40		2	6–10†	37	50
Nerve cell line	50		2	6.3†	37	38
Synaptosomes	36				25	51
Synaptic vesicles	3.0			4.2†	25	52

* $\mu mol \times min^{-1} \times g\ wet\ weight^{-1}$.
† $nmol \times min^{-1} \times mg\ protein^{-1}$.

outward movement of $^{24}Na^+$ [61]. A decrease in the concentration of Na^+ in the medium enhances amino acid efflux [62], which in synaptosomes is especially prominent for the acidic amino acids [40].

The rates of uptake of neurotransmitter amino acids are also affected by external potassium ions [18, 21, 56]. At concentrations of the latter lower than 3 mM, transport is inhibited. This phenomenon is most likely due to accumulation of Na^+ inside, and a consequent reduction in its concentration gradient, since sodium which enters with the amino acid can no longer be extruded in exchange for K^+ via the Na^+/K^+ pump. A delayed onset of inhibition of GABA uptake observed by Martin and Smith [56] in potassium-free medium is consistent with this interpretation since ion gradients do not dissipate instantaneously. A rise in external potassium above 6 mM also results in a progressive non-competitive inhibition of amino acid uptake: it reduces the V_{max} but does not affect the K_m [18, 21]. Since the rate of GABA transport at 55 mM external potassium and 85 mM external sodium (a nearly saturating concentration) has been shown to be linear with time [18], it was postulated that inhibition could neither result from altered ion concentration (because these would be time dependent) nor be the consequence of an increased efflux without a change of influx. This behavior indicates that increased $[K^+]_o$ affects both the inward and outward rate of amino acid transport. Moreover, both the velocity of GABA uptake [18] and of its efflux [59] were found to be linear functions of the logarithm of $[K^+]_o$ which suggests that the effects of increased potassium ion concentrations may be related to the depolarization they cause, i.e. alteration in membrane potential, and are not simply induced by replacement of K^+ with Na^+ (see also Ref. 63).

In addition to sodium and potassium, chloride ion has also been found to influence transport kinetics of GABA and acidic amino acid transmitters [64]. In general, a decrease in chloride concentration in the external medium reduces the rate of amino acid uptake (but see Ref. 42 for lack of chloride effect), whereas it is without marked effect on the rate of efflux [64, 65]. Although the latter observation may be true for GABA, recent reports seem to suggest that low $[Cl^-]_o$ can stimulate aspartate release [66]. It is interesting that other halogen ions, such as bromide, can restore up to 80% of the control rates of amino acid uptake [64]. Thus, although there may, in some cases, be a more specific requirement for chloride (as for example for GABA uptake by synaptosomal membrane vesicles [11]), the simplest interpretation of the available data is that simultaneous movement of this anion through another transport system preserves electrical neutrality during amino acid–Na^+ cotransport and helps to maintain the electrochemical gradient of sodium.

In addition to net transport, the high-affinity sodium-dependent transport systems for GABA and acidic amino acids mediate an exchange of external for internal amino acid [11, 27, 40, 41, 67–70]. In slices, cells and synaptosomes, the properties of this reaction are remarkably similar to those of uptake in their substrate specificities, values for kinetic constants, and ionic requirements [27, 40, 61, 69, 70]. However, in vesicles formed from synaptosomal membranes, GABA exchange, in contrast to uptake, has been found to be independent of external chloride concentration [11]. Although elaborate kinetic schemes were offered to account for this difference (the reader is referred to the original publications to evaluate their merits), the simplest explanation is that exchanges which do not lead to any net gain in

either intracellular amino acid or sodium may not require compensatory ion movements to sustain their operation. The observations [11, 40] that exchange, but not net uptake, is unaffected by alterations in $[K^+]_o$ is consistent with this suggestion.

It is interesting that exchange of external for internal GABA has been shown to be stimulated by manipulations which increase internal calcium levels [71] and inhibited by addition of verapamil [72]. These unexpected findings raise the exciting possibility that activity of the amino acid transporters may be regulated by alterations in the intracellular level of calcium ions.

Energetics of neurotransmitter amino acid transport

One of the predictions of the cotransport model is that the steady-state gradients of neurotransmitter amino acids ($[\text{amino acid}]_i/[\text{amino acid}]_o$) reflect the electrochemical gradient of sodium ions (i.e. the Na^+ concentration gradient plus the membrane electrical potential). The observed accumulation ratios represent an equilibrium between ion and substrate gradients, and analysis of such systems allows determination of stoichiometry for the number of Na^+ cotransported. The thermodynamic condition for such evaluation is that there is no net flux of either substance via the transporter, or an equivalent one, that the driving forces for Na^+ and amino acid fluxes are balanced. Moreover, for amino acid gradients to reflect the electrochemical potential of sodium ions faithfully, the amino acid must have access to the interior only via the Na^+-dependent carrier. Hence, the interpretation of the results is complicated by the existence of "leaks" which allow some uncoupling of the driving forces. Such leaks may either represent other pathways in the membrane through which the amino acid may move or may be due to intrinsic properties of the transporters which themselves are not "tightly" coupled. In the presence of such leaks, the observed accumulation ratios will represent a kinetic rather than thermodynamic steady-state and the calculated n values will represent lower limits on the true stoichiometry.

With these reservations in mind, systematic thermodynamic studies of both GABA and D-aspartate transport were undertaken in preparations of rat brain synaptosomes [20, 21]. Experiments were performed at concentrations of amino acids which were low relative to those at which carrier saturation occurs. Under such conditions, the net flow of ions via the carrier is moderate and therefore, does not itself impose a load on the system and thus possibly distort the steady-state electrical potential otherwise maintained in the absence of carrier function. Hence, low substrate levels would tend to maximize the magnitude of substrate gradients that can be established. Moreover, low concentrations of amino acid will reduce markedly contributions from low-affinity, carrier-mediated transport (i.e. external leaks).

Analyses of the maximum accumulation ratios of GABA and D-aspartate at various concentrations of K^+ (at a constant $[Na^+]_o$) and Na^+ (at a constant $[K^+]_o$) in the medium showed them to be dependent, as predicted, on both the transmembrane electrical potential (i.e. $\log [K^+]_i/[K^+]_o$ since in synaptosomes the membrane potential is a K^+ diffusion potential [73, 74]) and the sodium concentration gradient. The slopes of the plots of $\log [\text{amino acid}]_i/[\text{amino acid}]_o$ against either the $\log [K^+]_i/[K^+]_o$ or the $\log [Na^+]_o/[Na^+]_i$ were, within the limits of experimental error, equal to 2. These findings prompted a suggestion that both GABA and the acidic amino acid neurotransmitters are moved from outside to inside with a net charge of +2 and that these charges are provided by the simultaneous transfer of sodium ions. It is interesting to note that diaminobutyric acid, which differs from GABA by the presence of an extra amino group (and hence a positive charge at neutral pH), is taken up by synaptosomes on the GABA carrier in a cotransport with only one Na^+ [29]. This led to the speculation that one of the sodium binding sites on the transport protein must be in close proximity to the substrate binding site.

The above investigations also showed that the dependencies of GABA and D-aspartate uptake on the Na^+ and K^+ concentration gradients were essentially identical, although at physiological pH values the former is electrically neutral while the latter is negatively charged and, moreover, transported as an anion (aspartate^-). It came, therefore, as no surprise that aspartate uptake was accompanied by an alkalinization of the external medium which suggests that H^+ is taken up (or OH^- is ejected) with this amino acid to neutralize its negative charge at physiological pH. Since neither synaptosomes [74] nor neurons [75] maintain any significant pH gradient across the plasma membrane, proton movements themselves will have very little effect on the energetics.

It was mentioned in the preceeding section that, in preparations of synaptosomal vesicles, uptake of GABA also shows a dependence on external chloride, whereas that of glutamate requires internal potassium [11]. However, in synaptosomes, a 14-fold decrease in external chloride (from 140 to 10 mM) reduced the GABA concentration gradient by a factor of only 2 [20] which is much too small to support the postulate of energetic coupling between the GABA and the chloride transport. Similarly, in order to reconcile the observed second power dependence of the gradient of acidic amino acids on membrane potential with the postulated outward movement of K^+ [11], 3 Na^+ should move from outside to inside with each amino acid molecule taken up. Although precise measurements of intrasynaptosomal sodium are difficult, it is unlikely that the measured stoichiometry of 2 for D-aspartate Na^+-cotransport was so grossly underestimated. Moreover, the stoichiometry of 3 Na^+_{in}:1 K^+_{out} would raise the $[\text{aspartate}]_i/[\text{aspartate}]_o$ with respect to that for GABA by the magnitude of the sodium-gradient. This, however, was not confirmed experimentally in synaptosomal preparations where the final accumulation ratios for the two amino acids are equal, within the limits of experimental error. It is, moreover, interesting to point out that, since both K^+ and Cl^- are close to thermodynamic equilibrium across the synaptosomal membrane [73, 76], they cannot contribute appreciably to the driving force for amino acid accumulation. All these arguments suggest that the dependence of GABA transport on external chloride and glutamate uptake on internal potassium observed in vesicle preparations may be linked to

some conformational changes in the carrier that require specifically one or the other ion and that such phenomena may occur in vesicles but not in synaptosomes.

A model for amino acid neurotransmitter–Na^+ cotransport

A model for amino acid neurotransmitter–Na^+ cotransport outlined in this section is an extension of previous schemes originally proposed by Weinstein *et al.* [16] and later expanded by Martin [17] and by Blaustein and coworkers [18, 19]. The essential features of the model can be summarized in four major points:

(1) The true substrate of the cotransporter is amino acid × $2\,Na^+$, i.e. for productive substrate translocation one molecule of amino acid and two molecules of sodium must bind to the carrier. The stoichiometry of one amino acid per each carrier molecule is based on the findings that the rate of uptake at infinite sodium concentration is hyperbolically related to amino acid concentration (i.e. at a constant and high external Na^+, transport follows Michaelis–Menten kinetics with respect to amino acid concentration) [19, 21, 42]. The stoichiometry of $2\,Na^+$ per amino acid molecule is supported by three lines of evidence: (i) the relationships between the rate of GABA [18, 42] or aspartate [54] influx and $[Na^+]_o$ are sigmoid; (ii) $2\ ^{22}Na^+$ are transported from outside to inside with each glutamate taken up by a cerebellar nerve cell line [39]; and (iii) the accumulation ratios of amino acids ($[\text{amino acid}]_i/[\text{amino acid}]_o$) are proportional to the square of the chemical Na^+ gradient [20, 21].

(2) The driving force for amino acid uptake is provided by a combination of the transmembrane electrical potential and the Na^+ concentration gradient (see the preceding section for the relevant arguments). This finding suggests that transport is reversible and "symmetric" in the sense that it also mediates exit of amino acid–Na^+ complexes. The latter suggestion is further supported by the observations which show that depolarization stimulates efflux through the same, sodium-dependent system [19, 59, 60]. Also consistent with the postulated reversibility of the system is the finding that thiol reagents affect the inward and the outward movements of GABA across the synaptosomal membrane equally [77].

(3) The transporters mediate an exchange reaction which is dependent on external and internal sodium. The existence of such exchange provides evidence that the same transport system can move amino acids in both directions across the plasma membrane and strengthens the argument of reversibility discussed above.

(4) The transport cycle involves movements of net charges across the plasma membrane: uptake is inhibited while influx is stimulated by depolarization of synaptosomes (see above). (A corollary is that the movement of charged species should depolarize the terminals.) As pointed out earlier, this dependence of amino acid–Na^+ cotransport on membrane potential means that either the unloaded carrier is negatively charged, and therefore the rate-limiting step in the overall transport cycle is the return of the empty carrier, or the fully loaded carrier is positively charged and thus the rate-limiting step is the inward movement of this form of the carrier. If the first alternative is true, then the effect of transmembrane electrical potential, negative inside, would be to accelerate the reorientation of unloaded carrier from inside to outside, thereby increasing the number of binding sites available on the external surface. Two lines of experimental evidence suggest that this possibility may hold true. First, the rate of transport of an amino acid–Na^+ complex is strongly enhanced by either the same or a closely related amino acid added to the opposite ("trans") side of the membrane, i.e. in that solution towards which the flux is directed [11, 27, 40, 41, 67–71]. This suggests that the unloaded carrier moves more slowly than the loaded one. Second, the rate of amino acid exchange has been found to be independent of membrane potential in plasma membrane vesicles and only slightly (about 20–30%) decreased in synaptosomes. If the fully loaded carrier had a net charge of +2, a decline in the rate of exchange would be expected upon depolarization since the rate of influx would be diminished, while the distribution of the unloaded carrier (net charge of 0) would not be affected by a decrease in membrane potential. On the other hand, if the unloaded carrier had a charge of −2, the fully loaded species would be electrically neutral. In such a situation, during productive translocation of the amino acid–Na^+ complex, the energetic contribution from the transmembrane electrical potential would be due to outward movement of the negatively charged substrate binding site of the unloaded carrier.

It has been suggested recently by Stein [78], on the basis of elegant theoretical considerations, that the effectiveness of cotransporters in moving substrate molecules from an environment in which their concentrations are low into one in which they are high, can be influenced markedly, as far as the rate is concerned, by the order in which the ion and the substrate bind to the carrier (for a detailed discussion of the importance of the order of binding in the design of the cotransport systems the reader is referred to the original publication). Hence, in order to propose a realistic model for neurotransmitter amino acid transport, a knowledge of the order of binding would be important. This has been recognized previously, and attempts have been made by two groups of investigators to throw some light on the problem. Wheeler and Hollingsworth [53, 58] suggested, on the basis of experimental studies on synaptosomes and subsequent computer fitting, that the order in which GABA and glutamate associate with their respective transporters on the external surface of the plasma membrane is Na-Na-amino acid. By contrast, Nelson and Blaustein [19] postulated an opposite sequence of addition. This was based on three lines of experimental evidence: (i) in the absence of Na^+ in the medium, external GABA inhibits GABA efflux which suggests that trapping of the carrier in a complex with GABA on the external surface of the membrane prevents the former from cycling [19]; (ii) the maximum rate of GABA uptake, not only the K_m for transport, is affected by a decrease in $[Na^+]$ in the medium. If both Na^+ were to bind to the carrier before GABA,

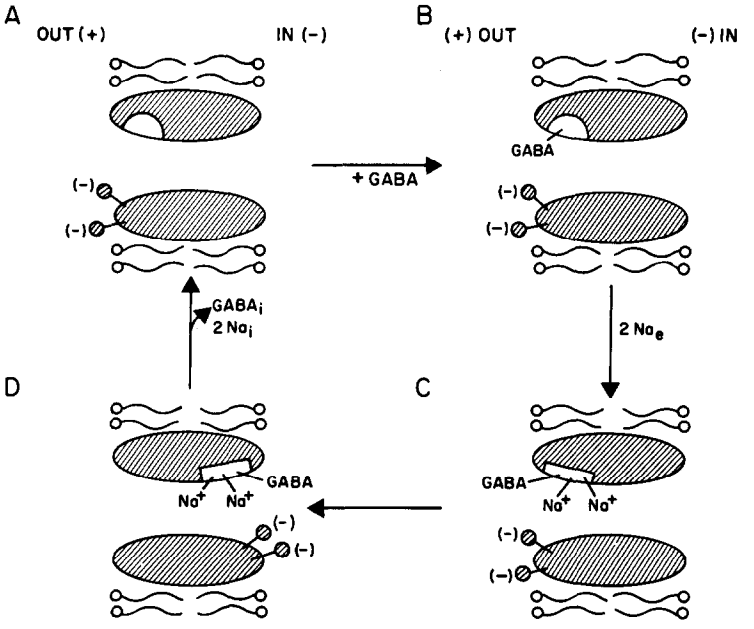

Fig. 1. A model for GABA transport across the synaptosomal membrane. The shaded areas depict the carrier molecule with a "space" to allow transport of the 2 Na^+–amino acid complex. This neither implies that the transporter is a dimer nor that it has a "channel" within its structure. The negative charges on the transporter may represent carboxyl residues but this remains to be established.

the V_{max} should be independent of $[Na^+]_o$ [19, 42]; and (iii) in the absence of external GABA, external sodium has no effect on GABA efflux. Although in both models only the free and the fully loaded carriers (i.e. 2 Na^+: 1 amino acid) were allowed to cross the membrane, Nelson and Blaustein were unable to fit their own results and those from other laboratories either to the sequence proposed by Wheeler or to the model based on random binding. By contrast, a good fit to the experimental observations was obtained with the order GABA-Na-Na which led these authors to suggest that the latter sequence of addition is most consistent with the data available to-date on synaptosomal transport of this amino acid. It should be added that, in their fitting procedures, both groups of investigators have considered the influx pathway only; hence, the order of dissociation of Na^+ and GABA on the internal surface of the plasma membrane and the efflux portion of the transport cycle were not a subject of their evaluation.

The information summarized in this section has been used to construct a pictorial model presented in Fig. 1. Since GABA has been the most extensively studied amino acid neurotransmitter, the figure utilizes it as the "representative". However, it is to be recognized that acidic amino acid transmitters, after appropriate compensation for their net negative charge at physiological pH, use an analogous mechanism to cross the plasma membrane at nerve terminals.

Neurotransmitter amino acid transport in glial cells

In 1967, Krnjević and Schwartz [79] noted that large doses of iontophoretically applied GABA were able to depolarize unresponsive, high-resistance (presumably glial) cells in the pericruciate cortex of cats. They suggested, on the basis of these observations, that glia may remove amino acids from the extracellular space through stimulation of an active electrogenic process. This suggestion was amply substantiated by subsequent studies from numerous laboratories which demonstrated that GABA, glutamate and aspartate are taken up with high affinity and at high velocity in Na^+-dependent processes by glial cells from the CNS (for review, see Refs. 80–82). Although lack of space does not permit detailed discussion of the properties of these transporters, there are some points which are of crucial importance for our understanding of the overall amino acid "traffic" in the brain *in vivo*. First, the kinetic constants for glial uptake of amino acid transmitters do not seem to differ markedly from those in synaptosomes, albeit they do depend on the age of the culture and source of the cells. In general, longer culturing increases the K_m and decreases the V_{max} for uptake [49], while 3- to 10-fold differences in the values for kinetic constants are seen in astrocytes cultured from various regions of the brain [50]. Moreover, the V_{max} for synaptosomal GABA transport is higher than the respective value in glial cells, whereas the opposite is true for acidic amino acids. Second, the stoichiometry for Na^+:amino acid cotransport has been found to be either 1 [50, 83] or 2 [84]. The K_m for sodium has been reported as 15–18 mM in cultured astrocytes [50, 83], whereas no saturation has been seen even at 140 mM NaCl in cultured C6 cells transporting D-aspartate [84]. Third, in C6 cells, the maximum accumulation ratio of D-aspartate increases as the second power of both the Na^+ concentration gradient and the membrane electrical potential which suggests a cotransport with 2 Na^+ [84]. However, the maximum aspartate gradients at

physiological sodium and potassium concentrations in the medium never exceeded a value of 3–4 × 10^3 which was two orders of magnitude less than the combined driving forces. If a similar excess of driving forces over accumulation ratio exists in glial cells *in vivo*, then their high-affinity transport systems for amino acid neurotransmitters would operate predominantly in the direction of net uptake. Fourth, in agreement with the postulate above, it was observed that the rate of efflux of amino acid neurotransmitters from glial cells was very poorly enhanced by increase in external $[K^+]$ [84–86] and that concentrations of K_o^+ higher than 40–50 mM were necessary to evoke net release [50, 84–88]. Fifth, unlike synaptosomes, glial cells under conditions used for uptake studies did not appear to exhibit any substantial exchange-diffusion [84, 85, 89, 90], again consistent with the suggestion that they mediate primarily net amino acid accumulation. This brief bird's eye view of glial amino acid transport indicates that there are substantial differences in the properties of the transporters in the two major populations of cerebral cells.

Are the amino acid neurotransmitter transport systems dependent on calcium?

It is generally believed that vesicular (i.e. stimulus–secretion coupled) release of neurotransmitters, including the amino acids, is obligatorily linked to an increase in internal concentration of calcium ions, whereas efflux which occurs through membrane-bound transporters is not. Although a detailed discussion of the calcium dependence of neurotransmitter amino acid release is far beyond the scope of the present commentary, it is important to list observations that are inconsistent with the simplistic view advanced above. First, synaptosomal exchange of GABA, which is a carrier-mediated reaction, has been shown to be enhanced by Ca^{2+} [71, 72]. Second, efflux and exchange of GABA from glial cells, which do not contain any neurotransmitter-storing compartments, have been shown to exhibit dependence on the presence of this cation [89, 91]. Third, the decrease in cytoplasmic acetylcholine which occurs upon stimulation of Torpedo electric organ or synaptosomes is strictly Ca^{2+} dependent [92]. These observations suggest that transporter-mediated processes may also be affected by alterations of intracelluar calcium concentrations (see also Ref. 93 for a similar suggestion) and raise a measure of concern in relation to interpretations of *all* experimental findings in the frame of a single dogma.

On the function of the high-affinity, Na^+*-dependent amino acid transport systems in the CNS* in vivo

The high-affinity transport systems for neutral and acidic amino acid neurotransmitters have at least three important functions:

(1) To terminate neurotransmission.

(2) To maintain constant and low levels of amino acids in the external environment.

(3) To conserve the molecules and reduce the need for their *de novo* synthesis.

It follows from the description of the transporters given in the preceding sections that these functions can be performed equally well by the neural and the glial uptake systems. It should be mentioned that it has been argued [28, 44] that, at concentrations of amino acid transmitters which approximate those in the external environment in the brain under physiological conditions (i.e. GABA < 1 μM; aspartate, 2–3 μM; glutamate 3–4 μM), the synaptosomal transporters mediate predominantly exchange and not net uptake. However, our own studies show consistently (see, for example, Ref. 94) that, if isolated synaptosomes are able to maintain high $[K^+]_i$ and low $[Na^+]_i$ (i.e. high transmembrane concentration gradients for these two cations), they are also competent in scavenging GABA, aspartate and glutamate down to micromolar levels.

There is one important difference *in vitro* between neural and glial uptake systems which, if maintained *in vivo*, may provide an important clue as to how the function of the two types of cells complement each other. In synaptosomes, the gradients of amino acid transmitters appear to equal, within the limits of experimental error (a factor of 2–3), the magnitude of the driving forces which fuel their uptake (i.e. the transmembrane electrical potential plus the sodium concentration gradient). Such a situation may also hold true in neurons *in vivo*, even if a substantial proportion of these amino acids were to be sequestered in the intrasynaptic vesicles, because at the very high (20–80 mM [22, 23]) levels at which these molecules are present inside the cell their free cytosolic concentrations must be in the millimolar range. This means that during an action potential, when neurons depolarize subsequent to an influx of Na^+, the magnitude of the driving forces for amino acid accumulation is reduced markedly and the transporters will operate in the reverse direction, i.e. in the direction of net release. (To appreciate the magnitude of the amino acid release, the following calculation may prove helpful. If one takes the velocity of the amino acid release from depolarized terminals to be equal to the V_{max} of their uptake into synaptosomes, i.e. about 5 nmol/min/mg protein at 25°, or 10 nmol/min/mg protein at 37°, and assume that 50% of the endings contain the transporter of interest, one arrives at the figure of 20 nmol/min/mg protein. In addition, if one accepts that the volume of the synaptic cleft is about 1% of that of a synaptosome, i.e. 1% of 4 μl/mg protein or 0.04 μl/mg protein, the above velocity translates into the rate of 500 mM/min or about 10 μM/msec. Hence, during an action potential with a duration of 3 msec, 30 μM amino acid could be released into the cleft which is within the range of values for the K_d for amino acids at postynaptic receptors.) This suggestion is consistent with observations [18, 21, 59, 63] that, in synaptosomes, influx of GABA is inhibited while efflux is stimulated by depolarization. It is also supported by the finding that K^+-stimulated release of neurotransmitter amino acids occurs predominantly from the cytosolic pool [95]. This rapid efflux of transmitters from the nerve terminals via the transporters may help to maintain concentrations of these molecules in the cleft at levels sufficiently high to elicit effects at postynaptic sites. It should also be pointed out that, as soon as the terminals are repolarized, the transport systems will switch to operating

in the direction of net uptake and the amino acids will thus be cleared rapidly from the cleft into the terminal.

In contrast to the behavior of synaptosomes, it has been shown that in C6 cells, an established astroglial cell line, the combined driving forces exceed, by a large amount, the amino acid concentration gradients [84]. Because of this excess in the amount of energy available to drive amino acid influx, it has been suggested that in glial cells the amino acid transport systems operate in the direction of net uptake, even under conditions of somewhat lowered $[Na^+]_o$ and increased $[K^+]_o$ i.e., an ideal situation for serving as the primary scavengers for amino acid during and immediately after neuronal activity. Moreover, the excess of energy in the driving forces over the amino acid accumulation ratio would ensure that even during partial glial depolarization these cells would not release their content of amino acid transmitter into the environment. The latter suggestion is consistent with the relative refractoriness of the glial cell release to an increase in $[K^+]_o$ reported by several authors [50, 84–88]. These differences in the patterns of response exhibited by the two main types of brain cells may form a basis for the interaction between neurons and glia in carrying out their physiological functions: while the former are involved in both the enhancement and termination of neurotransmission, the role of the latter is predominantly in clearing and scavenging neurotransmitters released into the synaptic cleft during neuronal activity.

REFERENCES

1. K. Krnjević, *Physiol. Rev.* **54**, 418 (1974).
2. D. R. Curtis and G. A. R. Johnston, *Ergebn. Physiol.* **69**, 97 (1974).
3. L. L. Iversen, *Br. J. Pharmac.* **41**, 571 (1971).
4. F. V. DeFeudis, *A. Rev. Pharmac.* **15**, 105 (1975).
5. E. Roberts, T. N. Chase and D. B. Tower (Eds.), *GABA in Nervous System Function*. Raven Press, New York (1976).
6. J. L. Johnson, *Prog. Neurobiol.* **10**, 155 (1978).
7. F. Fonnum (Ed.), *Amino Acids as Chemical Transmitters*. Plenum Press, New York (1978).
8. G. E. Fagg and J. D. Lane, *Neuroscience* **4**, 1015 (1979).
9. G. Di Chiara and G. L. Gessa (Eds.), *Adv. Biochem. Psychopharmac.* **27**, 1 (1981).
10. J. C. Watkins and R. H. Evans, *A. Rev. Pharmac. Toxic.* **21**, 165 (1981).
11. B. I. Kanner, *Biochim. biophys. Acta* **726**, 293 (1983).
12. R. P. Rubin, *Pharmac. Rev.* **22**, 389 (1970).
13. S. Naito and T. Ueda, *J. Neurochem.* **44**, 99 (1985).
14. J. L. Mangan and V. P. Whittaker, *Biochem. J.* **98**, 128 (1966).
15. J. S. De Belleroche and H. F. Bradford, *J. Neurochem.* **21**, 441 (1973).
16. H. Weinstein, S. Varon, D. R. Muhleman and E. Roberts, *Biochem. Pharmac.* **14**, 273 (1965).
17. D. L. Martin, in *GABA in Nervous System Function* (Eds. E. Roberts, T. N. Chase and D. B. Tower), p. 347. Raven Press, New York (1976).
18. M. P. Blaustein and A. C. King, *J. membr. Biol.* **30**, 153 (1976).
19. M. T. Nelson and M. P. Blaustein, *J. membr. Biol.* **69**, 213 (1982).
20. A. Pastuszko, D. F. Wilson and M. Erecińska, *J. biol. Chem.* **257**, 7514 (1982).
21. M. Erecińska, D. Wantorsky and D. F. Wilson, *J. biol. Chem.* **258**, 9069 (1983).
22. F. Fonnum and F. Walberg, *Brain Res.* **54**, 115 (1973).
23. S. K. Berger, J. G. Carter and O. H. Lowry, *J. Neurochem.* **28**, 149 (1977).
24. R. K. Crane, *Rev. Physiol. Biochem. Pharmac.* **78**, 101 (1977).
25. R. J. Hitzemann and H. H. Loh, *J. Neurochem.* **30**, 471 (1978).
26. J. R. Simon and D. L. Martin, *Archs Biochem. Biophys.* **167**, 348 (1973).
27. J. R. Simon, D. L. Martin and M. Kroll, *J. Neurochem.* **23**, 981 (1984).
28. G. Levi, M. Banay-Schwartz and M. Raiteri, in *Amino Acids as Chemical Transmitters* (Ed. F. Fonnum), p. 327. Plenum Press, New York (1978).
29. M. Erecińska, M. B. Troeger and Th. A. Alston, *J. Neurochem.* **46**, 1452 (1986).
30. G. P. Jones and M. J. Neal, *Nature, Lond.* **264**, 281 (1976).
31. M. J. Neal and N. G. Bowery, *Brain Res.* **138**, 169 (1977).
32. P. Krogsgaard-Larsen and G. A. R. Johnston, *J. Neurochem,* **25**, 797 (1975).
33. W. F. White and S. R. Snodgrass, *J. Neurochem.* **40**, 1701 (1983).
34. J. C. Szerb, *J. Neurochem.* **39**, 850 (1982).
35. V. J. Balcar and G. A. R. Johnston, *J. Neurochem.* **20**, 529 (1973).
36. V. J. Balcar and G. A. R. Johnston, *J. Neurochem.* **19**, 2657 (1972).
37. P. J. Roberts and J. C. Watkins, *Brain Res.* **85**, 120 (1975).
38. W. B. Stallcup, K. Buloch and E. E. Baetge, *J. Neurochem.* **32**, 57 (1979).
39. H. Iwata, S. Yamagani, H. Mizuo and A. Baba, *J. Neurochem.* **38**, 1268 (1982).
40. M. Erecińska and M. B. Troeger, *Fedn Eur. Biochem. Soc. Lett.* **199**, 95 (1986).
41. L. L. Iversen and M. J. Neal, *J. Neurochem.* **15**, 1141 (1968).
42. D. L. Martin, *J. Neurochem.* **21**, 345 (1973).
43. A. Pastuszko, D. F. Wilson and M. Erecińska, *Proc. natn. Acad. Sci. U.S.A* **78**, 1242 (1981).
44. G-B. Halversson, I. Karlsson and A. Sellström, *Life Sci.* **37**, 209 (1985).
45. B. I. Kanner, *Biochemistry* **17**, 1207 (1978).
46. L. P. Davies and G. A. R. Johnston, *J. Neurochem.* **26**, 1007 (1976).
47. S. H. Snyder, A. B. Young, J. P. Bennet and A. H. Mulder, *Fedn Proc.* **32**, 2039 (1973).
48. J. P. Bennett, W. J. Logan and S. H. Snyder, *J. Neurochem.* **21**, 1533 (1973).
49. R. D. Gordon and R. Balázs, *J. Neurochem.* **540**, 1090 (1983).
50. J. Drejer, O. M. Larsson and A. Schousboe, *Expl Brain Res.* **47**, 259 (1982).
51. F. A. Henn, M. N. Goldstein and A. Hamberger, *Nature, Lond.* **243**, 663 (1974).
52. B. I. Kanner and I. Sharon, *Biochemistry* **17**, 3949 (1978).
53. D. D. Wheeler and R. G. Hollingsworth, *J. Neurosci. Res.* **4**, 266 (1979).
54. N. A. Peterson and E. Raghupathy, *J. Neurochem.* **19**, 1423 (1972).
55. N. A. Paterson and E. Raghupathy, *Biochem. Pharmac.* **23**, 2491 (1974).
56. D. L. Martin and A. A. Smith, III, *J. Neurochem.* **19**, 841 (1982).
57. P. Kontro, *Neurochem. Res.* **7**, 1391 (1982).
58. D. D. Wheeler and R. G. Hollingsworth, *J. Neurochem.* **30**, 1311 (1978).

59. J. W. Haycock, W. B. Levy, L. A. Denner and C. W. Cotman, *J. Neurochem.* **30**, 1113 (1978).
60. M. E. Sandoval, *J. Neurochem.* **35**, 915 (1980).
61. E. A. Schwartz, *J. Physiol., Lond.* **323**, 211 (1982).
62. J. S. De Belleroche and H. F. Bradford, *J. Neurochem.* **19**, 585 (1972).
63. T. S. Sihra, T. G. Scott and D. G. Nicholls, *J. Neurochem.* **43**, 1624 (1984).
64. M. J. Kuhar and M. A. Zarbin, *J. Neurochem.* **31**, 251 (1978).
65. J. Cunningham and M. J. Neal, *Br. J. Pharmac.* **73**, 655 (1981).
66. L. U. Naalsund and F. Fonnum, *J. Neurochem.* **47**, 687 (1986).
67. D. M. Crnic, J. P. Hammerstad and R. W. P. Cutler, *J. Neurochem.* **20**, 203 (1973).
68. G. Levi, A. Bertollini, J. Chen and M. Raiteri, *J. Pharmac. exp. Ther.* **188**, 429 (1974).
69. M. Raiteri, R. Federico, A Coletti and G. Levi, *J. Neurochem.* **24**, 1243 (1975).
70. R. Roskoski, Jr., *J. Neurochem.* **31**, 493 (1978).
71. G. Levi, P. J. Roberts and M. Raiteri, *Neurochem. Res.* **1**, 409 (1976).
72. P. J. Norris, D. K. Dhaliwal, D. P. Druce and H. F. Bradford, *J. Neurochem.* **40**, 514 (1983).
73. M. P. Blaustein and J. M. Goldring, *J. Physiol., Lond.* **247**, 589 (1975).
74. C. J. Deutsch, U. Rafalowka, C. Drown and I. A. Silver, *J. Neurochem.* **36**, 2063 (1981).
75. I. A. Silver, *Ciba Fdn Symp.* **56**, 49 (1978).
76. R. M. Marchbanks, *J. Neurochem.* **25**, 463 (1975).
77. M. B. Troeger, D. F. Wilson and M. Erecińska, *Fedn Eur. Biochem. Soc. Lett.* **171**, 303 (1984).
78. W. D. Stein, *Am. J. Physiol.* **250**, C523 (1986).
79. K. Krnjević and S. Schwartz, *Expl Brain. Res.* **3**, (1967).
80. L. Hertz, *Prog. Neurobiol.* **13**, 277 (1979).
81. A. Schousboe, *Int. Rev. Neurobiol.* **22**, 1 (1981).
82. M. Erecińska and I. A. Silver, *Brain Res.* **369**, 193 (1986).
83. A. Schousboe, G. Svenneby and L. Hertz, *J. Neurochem.* **29**, 999 (1977).
84. M. Erecińska, M. B. Troeger, D. F. Wilson and I. A. Silver. *Brain Res.* **369**, 203 (1986).
85. N. G. Bowery, D. A. Brown and S. Marsh, *J. Physiol., Lond.* **293**, 75 (1979).
86. L. Hertz, P. H. Wu and A. Schousboe, *Neurochem. Res.* **3**, 313 (1978).
87. M. C. W. Minchin and L. L. Iversen, *J. Neurochem.* **23**, 533 (1974).
88. V. Gallo, M. T. Ciotti, A. Coletti, F. Aloisi and G. Levi, *Proc. natn. Acad. Sci. U.S.A.* **79**, 7919 (1982).
89. M. C. W. Minchin, *J. Neurochem.* **24**, 571 (1975).
90. L. Hertz, A. Schousboe, N. Boechler, S. Mukerji and S. Federoff, *Neurochem. Res.* **3**, 1 (1978).
91. P. V. Sarthy, *J. Neurosci.* **12**, 2494 (1983).
92. M. Israel, Y. Dunant and R. Manaranche, *Prog. Neurobiol.* **13**, 237 (1979).
93. O. Vargas, M. C. D. de Lorenzo, M. C. Saldate and F. Orrego, *J. Neurochem.* **28**, 165 (1977).
94. M. Hauptmann, D. Nelson, D. F. Wilson and M. Erecińska, *Brain.Res.* **304**, 23 (1984).
95. J. S. De Belleroche and H. F. Bradford, *J. Neurochem.* **29**, 335 (1977).