Na-DEPENDENT TRANSPORT OF γ-AMINOBUTYRIC ACID IN SUBCELLULAR BRAIN PARTICLES[1]

S. VARON AND W. WILBRANDT

Department of Genetics, Stanford University, Palo Alto, California, and Department of Pharmacology, University of Berne, Switzerland

γ-Aminobutyric acid (GABA) is of interest to the neuroscientists on a number of grounds [11]. Although extensively present in lower organisms and in plants, in the vertebrates GABA can be found only in the central nervous system (CNS). It is formed within the CNS by decarboxylation of glutamic acid and is metabolized to succinic acid by transamination (with α-ketoglutaric acid) and dehydrogenation. The presence of GABA and of the three GABA-related enzymes thus confers on the vertebrate CNS the unique ability to bypass the Krebs cycle pathway for the conversion of α-ketoglutarate to succinate. It has been calculated that as much as 40% of the energetic metabolism of brain could go through this special GABA shunt. A neurophysiological role of GABA in the vertebrate CNS is suggested by its demonstrable inhibitory effects on the evoked electrical activity of cerebral and cerebellar cortex, spinal cord, and other parts of the CNS. Furthermore, in the crustacean peripheral nervous system, GABA has been shown to mimic the stimulation of inhibitory fibers; the finding [9] that GABA is present in such fibers, but not in motor or sensory ones, has raised the question of whether this compound acts as an inhibitory neurotransmitter or, more generally, as an inhibitory neuromodulator. Indeed, GABA presents [20] a striking number of analogies, both at the biochemical and the functional level, with the catecholamines, the importance of which in the modulation of CNS activity is well established, even though only partially understood.

More recent findings indicate the existence of mechanisms for intracellular transport of GABA in brain. Particulate matter sedimented from brain homogenates retains a sizable portion of the

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total GABA content in brain, as first reported by Elliott and van Gelder [5]. Sano and Roberts [14] showed that such particles, in contrast with those from other tissues, at 0°C can "bind" radioactive GABA from the medium provided Na ions are present. Subsequent investigations, summarized in a recent symposium [22, 28], gave evidence that exogenous GABA is actually transported into brain particles at 0°C and at higher temperatures, and that in both cases Na ions are involved. This Na-dependence of GABA transport parallels that reported for other transport systems both at the cellular [2–4, 7, 16, 23–26, 29] and the subcellular [1] levels. The aim of the present paper is a further analysis of the available data on GABA transport[2] and a comparison with the other Na-dependent systems. It will be shown that the hypothesis [3, 4, 23–26] of an equilibrating carrier mechanism, coupling the transport of substrate and Na-ions through a common carrier and supported by an energy-dependent extrusion of Na, adequately fits the GABA system and can in fact offer a suggestive basis for a relationship between the metabolic and neurophysiological properties of GABA in the vertebrate CNS.

THE GABA SYSTEM AT 0°C [18–20, 27]

A 10% mouse brain homogenate in 0.25 *M* sucrose yields, upon centrifugation between 1,500 and 15,000 *g*, a highly heterogeneous pellet which comprises mitochondria, microsomes, and pinched-off nerve endings (synaptosomes). All three types of particles retain endogenous GABA, while no appreciable amounts of GABA are found to associate with the membranous strands and myelin material of the pellet. The pellet also contains some free GABA in the entrained sucrose supernatant.

When the pellet is resuspended in a buffered NaCl medium containing a small amount of ^{14}C-GABA, incubation at 0°C results in a rapid accumulation of radioactivity by the particles (reaching a maximum within 30 minutes) which is accompanied by a relatively smaller increase of the particulate GABA. Part of the acquired radioactivity remains in equilibrium with the medium and can be diluted out by adding an excess of nonradioactive free GABA to the system. Both

[2] These data were obtained in Dr. E. Roberts' laboratory at the City of Hope Medical Center, California, more than two years ago. They are reported and discussed elsewhere [17-22, 27, 28]. The additional analysis given here, and the conclusions drawn, reflect only the personal opinions of the present authors.

the radioactivity and the GABA present in this rapidly equilibrating pool are promptly released from the particles after their transfer to a Na-free medium. The radioactivity remaining with the particles and most of their endogenous GABA constitute a second pool, which decreases in size and increases in specific activity with prolonged incubation in Na-containing media. Such changes occur much more slowly than those affecting the first pool. They become barely noticeable in Na-free media. Evidence from electron microscopy studies indicates that both pools are associated with the same particles and are in fact present in all three types of membrane-enclosed particles occurring in the system. The following model has been suggested.

1. The membranes which enclose mitochondria, microsomes, and nerve-ending particles from brain are impermeable to free GABA.
2. These membranes contain sites which are capable of binding GABA only in the presence of Na-ions. Exposure of the particles to a Na-containing medium thus results in the acquisition of exogenous GABA and the formation of a rapidly equilibrating pool.
3. The binding sites are mobile carriers and cross the membrane (or the barriers within the membrane) at a rate which, at 0°C, is considerably slower than that at which they bind or exchange GABA. Because of such carrier movements, radioactivity enters the internal pool and internal GABA moves out, with a net progressive loss of particulate GABA owing to the higher GABA concentration inside than outside the barrier.

According to the model, an equilibrating carrier mechanism is responsible for the slow changes in the internal GABA pool while the outer, rapidly equilibrating, pool merely reflects the binding of free external GABA by the Na-activated carriers present at any time outside the barrier. The measurable size of this rapidly equilibrating pool implies a relatively high number of carrier molecules as compared with estimates for other carrier systems [6].

THE GABA SYSTEM AT 29°C [17, 20, 22]

The same particles, suspended in buffered NaCl medium containing ^{14}C-GABA and incubated at 0°C for a brief period to allow tracer accumulation, show a markedly different behavior when further incubated at 29°C under a constant air flow. Three major processes take place: massive release of particulate GABA, uptake of free GABA into some particles, and active metabolic degradation of GABA within the same particles.

1. Release of particulate GABA occurs at a much faster rate at 29° than at 0°C. This can be directly demonstrated for microsomes by use of pure microsomal preparations (which, at 0°C, have been shown [18, 19] to behave with respect to GABA in the same way as the heterogeneous suspension discussed thus far). It is also clearly observable in the standard heterogeneous suspensions under such conditions as a nitrogen atmosphere, where uptake and metabolism of GABA are inhibited; the complete removal of particulate GABA that can be achieved with a sufficiently long incubation demonstrates that this release process is not confined to the microsomal constituents of the suspension, but involves all its GABA-containing particles. Under these inhibitory conditions, all the GABA lost by the particles is recovered in the medium; therefore, a plot of particulate GABA levels versus time yields a time curve for the release process. One of the most striking experimental findings was that such a particulate GABA time curve is identical to those obtainable under air or pure oxygen, where both uptake and metabolism of GABA take place, suggesting that these two processes occur in such a way as to balance each other out. This is borne out by independent evidence indicating (see below) that the GABA newly taken up is metabolized very rapidly and does not contribute significantly to the particulate GABA levels. It has therefore been concluded that essentially the same massive release occurs under both air and nitrogen and is in both cases depicted by the particulate GABA time curve.

2. At 0°C, the slow but progressive release of particulate GABA results in a corresponding increase in the free GABA levels of the medium. In contrast with this pattern, at 29°C the GABA content of the medium undergoes a considerable drop in the initial 30 to 60 minutes of incubation, and remains constant thereafter. This decrease has been shown not to be due to metabolism of free GABA within the suspending fluid and can therefore only result from the uptake of free GABA by the particles. In fact, uptake of GABA by the particles must occur at a much greater extent and for a longer period than indicated by the GABA depletion in the medium since, as discussed in the preceding paragraph, a considerable release of GABA is taking place throughout the incubation. The uptake of GABA does not result in any observable increase of the particulate GABA levels but is accompanied by an accumulation in the particles of GABA metabolites. This finding, that the newly taken-up GABA is immediately made available to metabolic degradation, demonstrates

that the uptake is not due to an increased binding capacity of the particles and, furthermore, that it takes place in particles which have the ability to metabolize GABA (see below). The uptake of free GABA by such particles does not occur in Na-free media and is inhibited by cardiac glycosides, dinitrophenol, lack of oxygen, and by specific inhibitors of GABA metabolism (such as aminooxyacetic acid); in the last case, addition of another energy source such as pyruvate restores the ability of the particles to take up GABA. Thus, GABA uptake at 29°C is a process which is both Na-dependent and energy-dependent and which draws normally the required energy from the metabolic degradation of GABA itself.

3. Evidence for active GABA metabolism is provided by the rapid and progressive decrease of the total GABA content in the system. No GABA metabolism has been found to occur in the medium (after removal of the particles) or in the microsomal particles. Mitochondria, on the other hand, are clearly indicated as GABA metabolizing particles by their reported content of GABA-transaminase [13] and the observed involvement of Krebs cycle steps in the metabolic degradation of GABA taking place in the system [17]. It is possible, although less likely [13], that mitochondria-containing nerve-ending particles also contribute to GABA metabolism. Inhibition of GABA metabolism can be achieved by incubating the particles in the absence of oxygen (nitrogen flow) or in the presence of aminooxyacetic acid, a known specific inhibitor of GABA-transaminase. It is also observed under all conditions (see above) where uptake of free GABA is inhibited. This, and the immediate metabolic degradation undergone by the newly taken-up GABA (see above), strongly suggest that most, if not all, of the GABA degraded in the system is made available to the metabolic sites by the uptake process.

The main features of the 29°C GABA system are shown diagrammatically in Fig. 1. The particles are regarded as two functionally distinct classes (A, B) linked through the suspending fluid (S). Only one class (A) is the site of active GABA metabolism and takes up free GABA from the medium. The other class (B) rapidly releases its GABA content into the suspending fluid and thus makes it available to uptake and metabolism by the A particles. Owing to the cyclic nature of the sequence metabolism → energy → uptake → metabolism, interference at any level will bring about the interruption of both metabolism and uptake.

A better understanding of the system and an exact kinetic study

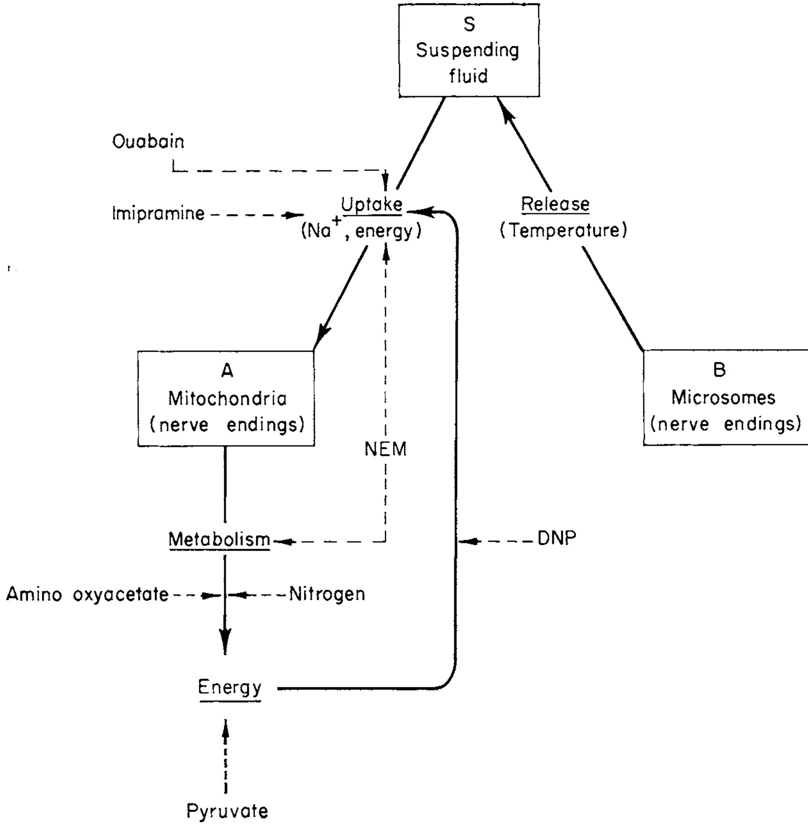


FIG. 1. Schematic representation of the interrelationships between particles A and particles B.

of the two types of GABA movement involved will obviously have to await additional data on the separate behavior of the different types of particles, as well as on sodium distribution in the particles. On the basis of the available evidence, however, a number of additional considerations can be discussed.

The Release of GABA from the B Particles (29°C)

This process is considerably faster than the release occurring at 0°C. It is, on the other hand, only slightly, if at all, slowed down in Na-free media. It is not affected by the occurrence or absence of energy-yielding metabolism in the A particles. It is also unaffected

by the concentration of free GABA in the medium (which increases with time under nitrogen, decreases to a low constant level under air).

Based upon the particulate GABA time curve, a kinetic analysis can be made, as summarized in Fig. 2. It is found that a double reciprocal plot of GABA decrement in the particles ($p_0 - p$) versus time (t) yields a straight line, the equation for which can be written

$$\frac{1}{p_0 - p} = \alpha + \beta \frac{1}{t} \tag{1}$$

where $\alpha = 1/p_0$ and the slope β characterizes the release at 29°C. From the previous equation, one obtains:

$$\frac{dp}{dt} = -kp^2 \quad \left(\text{where } k = -\frac{1}{\beta p_0^2}\right) \tag{2}$$

The validity of this treatment has been verified by integrating Eq. (2) into

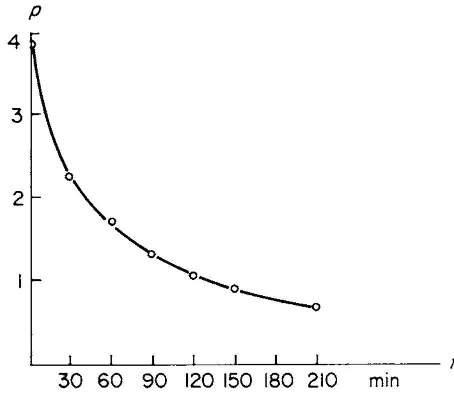
$$\frac{1}{p} = \frac{1}{p_0} + kt \tag{3}$$

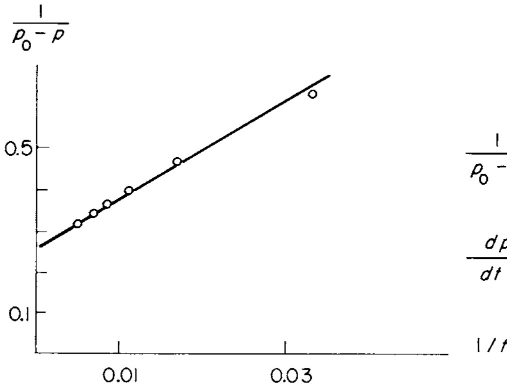
and plotting against time the reciprocals of the various experimental values for p. The rigorous linearity of this plot is shown in Fig. 2.

Equation (2) states that the rate at which GABA is released is a second-order function of the GABA left in the particles. This rules out diffusion as the underlying mechanism for the release. It is also hard to reconcile with the interpretation that the GABA release results from a temperature-enhanced breakdown of the B particles. Possible mechanisms compatible with this second-order relationship include a carrier mechanism operating under low saturation conditions and with complexes involving two substrate molecules per carrier. Under the same conditions, the above-mentioned irrelevance of the external GABA concentration would be consistent with a carrier mechanism in view of the large difference between the GABA concentrations inside and outside the particles.

The Uptake of GABA into the A Particles (29°C)

The rapidity with which the GABA newly entering the particles is metabolized and the attribution to the releasing B class of all GABA-containing particles suggest that under standard conditions





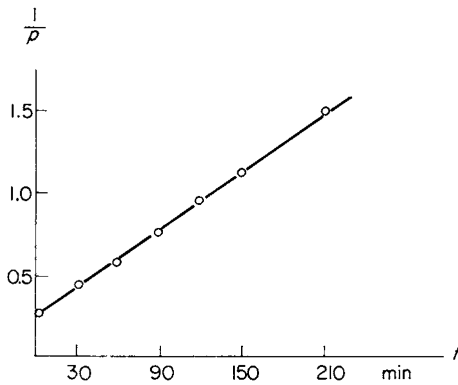


Fig. 2. Kinetic analysis of GABA release at 29°C from particles B.

the free GABA inside the A particles is brought down to, and maintained at, negligible concentrations by its metabolic degradation. A GABA gradient would thus obtain between external and internal free GABA which could be the driving force for the GABA uptake into the A particles. In fact, it was observed that increasing GABA concentrations in the medium result in increasing rates of disappearance from the medium. The Na-dependence of the uptake process, among other considerations, rules out a passive diffusion of GABA along the metabolically maintained GABA gradient. It is therefore tempting to interpret the GABA uptake into A particles as a downhill transport mediated by the same equilibrating carrier mechanism that has been postulated in the 0°C system.

The issue is, however, complicated by other features of the process. The sensitivity to ouabain, for instance, can hardly be explained as a direct inhibition of metabolic processes by cardiac glycoside (for which no examples have ever been cited), and the possibility that ouabain interferes with the Na-activated binding ability of the carrier is ruled out by the demonstrable lack of such an effect at 0°C. Moreover, there are experimental conditions under which the GABA uptake results in an accumulation of GABA in the particles and, provided the particulate GABA is in a free form, the system appears to behave as a GABA uphill transport. This is the case, for example, where GABA uptake takes place in spite of specifically blocked GABA metabolism (with the support of pyruvate). Without additional features, then, an equilibrating carrier system cannot account for all the observed properties of the GABA uptake at 29°C. However, the involvement of Na-ions and the sensitivity to cardiac glycosides allow for an interpretation of the whole process in terms of a downhill equilibrating carrier mechanism *for a mixed Na-GABA carrier complex* sustained by the simultanous action of a Na-pump. Such an interpretation is suggested by the comparison of the GABA system with other Na-dependent transport systems, to be discussed in the following section.

Na-DEPENDENT "UPHILL" TRANSPORT SYSTEMS

Since the 1953 report [15] that cardiac glycosides inhibit sodium and potassium uphill transport in red blood cells, similar observations have been made in many other cell types [8]; in fact, no cell type appears to have been tested thus far with negative results. This widespread inhibitory effect of cardiac glycosides on Na^+ and K^+ transport

is characterized by two features: It is mainly affecting the uphill movement of the ions and it does not derive from interference with the energy-yielding metabolism. In recent years, a number of other transport systems concerning sugars and amino acids have also been found to be inhibited by cardiac glycosides; the suggestion occasionally has been made that cardiac glycosides have a general action on pumping systems rather than a specific one for the Na-K transport. A very strong point against such a suggestion is, however, that in all the cases where cardiac glycoside inhibition was observed, the transport was found to be Na-dependent. This fact supports the alternative interpretation, that in these cases the glycoside inhibitory action is directly exerted on a sodium-potassium pump linked in some manner to the substrate transport. If this interpretation is accepted, the question may be asked whether the relationship to the sodium transport is by way of a direct coupling between sodium pump and substrate pump or whether the role of the sodium pump is rather to maintain a sodium gradient. In the case of iodide transport, this question has been studied by Iff [7] by following the uptake of radioactive iodide into thyroid slices, continuously perfused directly underneath a scintillation counter. In an initial phase, the iodide uptake was inhibited by using a lithium- instead of a sodium-containing medium. Later, ouabain was added and a sufficient time was allowed for the glycoside action to develop fully. Thus, a condition was reached under which the sodium pump was certainly blocked, but a sodium accumulation had been prevented through the absence of sodium in the external medium. Then, lithium was replaced by sodium without removing the glycoside. The restored sodium gradient reactivated the iodide pump temporarily even though the sodium pump still was, and remained, blocked. The answer therefore was that it is not necessary for iodide uphill transport that the sodium pump be actually running, but that its functioning is required for the maintenance of a sodium gradient.

One possibility for a sodium gradient to do transport work would be to have the Na-transport linked to a second transport system by means of a common carrier. In systems of this kind, uphill transport may be induced in various ways [30]. Examples are listed in Table I. Among them the best-known case is that of countertransport [12], which has been observed in a number of cell types and with different substrates. Cotransport in systems involving ternary complexes also has been reported, and recent interpretations of amino

Table I. *Carrier Systems: Induced Uphill Movements of Substrate S* $(S_1 = S_2)$

Carriers	Substrates	Complexes	Primary asymmetry	Induced gradients	Induced movement: Absolute direction	Induced movement: Relative direction
C	S R	CS, CR	$R_1 > R_2$	$CR_1 > CR_2$ ↓		Counter-transport
				$C_2 > C_1 \rightarrow CS_2 > CS_1$	$S_2 \rightarrow S_1$	
C	S R	CS, CSS	$R_1 > R_2$	$\rightarrow CSR_1 > CSR_2$	$S_1 \rightarrow S_2$	Cotransport
		CR, CRR		$CR_1 > CR_2 \rightarrow CRS_1 > CRS_2$ ↓	$S_1 \rightarrow S_2$	
		CSR		$CRR_1 > CRR_2$ ↓		
		CRS		$C_2 > C_1$; $CS_2 > CS_1$	$S_2 \rightarrow S_1$	Counter-transport
				$CSS_2 > CSS_1$	$S_2 \rightarrow S_1$	
C	S	CS	$C_1 > C_2$	$\rightarrow CS_1 > CS_2$	$S_1 \rightarrow S_2$	Cotransport
AB	S	AS	$B_1 > B_2$	$\rightarrow BS_1 > BS_2$	$S_1 \rightarrow S_2$	Cotransport
		BS		$AB_1 > AB_2$ ↓		
		AB		$A_2 > A_1$; $AS_2 > AS_1$	$S_2 \rightarrow S_1$	Counter-transport

acid transport into single cells [23–26] and of glucose absorption from the intestine [2–4, 16] have assumed mixed complexes involving one carrier molecule and different ratios of sodium ions and amino acid or sugar molecules. Of particular interest for the GABA system is the successful analysis of glycine transport into avian red cells by Vidaver [23–26]. He interprets the amino acid uptake into the cells as a downhill movement of a carrier complex with 1 molecule of amino acid and 2 sodium ions. This downhill movement becomes in effect an uphill movement of the amino acid because the extrusion of sodium by the sodium pump results in the maintenance of a steep gradient for the mixed complex. Since the sodium pump is blocked by cardiac glycosides, the uphill amino acid movement also stops in the presence of this inhibitor. Vidaver showed that changing the driving force for the Na-movement either by establishing a Donnan equilibrium or by reversing the sodium gradient (using the hemolysis method of Straub), the movement of the amino acid could be changed at will, and even reversed, in accordance with prediction. From the observation that a Lineweaver-Burk plot of uptake rate versus sodium concentration fails to give a straight line, whereas a similar plot versus the square of sodium concentration is linear, he concluded that two sodium ions per molecule of amino acid are involved in the transport complex. This was confirmed in experiments in which the transport rate was varied: transport increase of one molecule of glycine was accompanied by an increment of two sodium ions. A somewhat similar analysis, using short-circuit current as a measure of Na-transport, was carried out in the case of glucose absorption from the intestine [2, 16].

According to the Lineweaver-Burk analysis, the effect of Na on the apparent carrier parameters appeared to be on the K_m in Vidaver's experiments on amino acid transport in pigeon red cells [23–26], as well as in Crane's experiments on intestinal glucose absorption [3, 4]. Experiments on glucose absorption by Schultz and Zalusky [16], however, indicated an effect on V_{max}. Whether, in a mixed complex system as discussed, the effect of sodium should be expected on the apparent K_m or the apparent V_{max} of the carrier depends on the type of binding. Table II shows two different possibilities for 1:1 and 2:1 Na-substrate complexes with carrier (C). "Independent" binding implies binding sites for Na (R) and for substrate (S) without interdependence (meaning that S reacts equally well with C and with CR or CR_2). If, then, of

all possible complexes only CRS (case 1) or CRRS (case 2) is able to move across the membrane, the effect of R is on the apparent V_{max}. On the other hand, an effect on the apparent K_m emerges if S only, or preferentially, binds to CR (case 3) or CRR (case 5) rather than to C (owing to spatial reasons or an allosteric effect of R on C). Theoretical possibilities also exist that S has to bind before R (cases 4 and 7) or in between the binding of the first and the second R (case 6), yielding an effect of R both on K_m *and* V_{max}.

In view of the successful interpretation of these cellular systems in terms of mixed complexes, in view furthermore of the close chemical relationship between GABA and α-amino acids, it appears possible that a similar mechanism is involved in the Na-dependent, ouabain-sensitive uptake of GABA into the A particles (brain mitochondria). As illustrated by the scheme given in Fig. 3, the particular conditions obtaining in a mixed complex system result in two characteristic features: (*a*) The net movement of S and R (in this case GABA and Na) must occur in a molar ratio fixed by the type of complex (in Vidaver's experiments 1:2); and (*b*) the unidirectional fluxes are functions of the concentrations for both S and R. These functions also depend on the type of complex involved and, for different types, are identical with the terms listed under V_s in Table II. The difference of the fluxes $M_{1\rightarrow 2}$ and $M_{2\rightarrow 1}$ (the V_s terms with subscript 1 and 2 respectively) is the net rate in the general case where $S_2 \neq 0$.

To test the mixed complex hypothesis for the GABA system, the analysis of a somewhat puzzling observation was found suitable. During the initial 30 to 60 minutes of 29°C incubation, the uptake of GABA into the A particles exceeds the amounts of GABA released from particles B, so that the free GABA levels of the suspending fluid decrease considerably. After this time a constant low level is maintained in the medium, which is interpreted as the attainment of a steady state between B release and A uptake. In the experiment depicted in Fig. 4A [21] this steady state was disturbed, or its establishment prevented, by an addition of GABA to the external medium after 5 or 35 minutes of incubation at 29°C. The resulting increase in the external GABA concentration is transient and after some time a steady state is again established. In this new steady state, however, the GABA level in the medium is higher than in the control experiments (without the addition). The difference is related to the total amount of free GABA in the manner illustrated in Fig. 4B: The

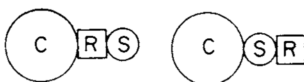
TABLE II. *Apparent Carrier Transport Parameters for Transport of Substrate S*

Types of complexes:

A. Independent binding:

Dicomplex

B. Dependent binding:



Dicomplexes

General conditions:

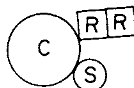
1. Equilibrium between substrate and carrier
2. Only fully saturated S-containing compound moves

	Carrier forms									Moving complex	Dissociation constants		
	C	CS	CR	CRS	CRR	CRRS	CSR	CRSR	CSRR				
1	+	+	+	+						C R S		$K_{R1} = \frac{C \times R}{CR}$	$K_{S1} = \frac{C \times S}{CS}$
2	+	+	+	+	+	+				C R R S	ditto	$K_{R2} = \frac{CR \times R}{CRR}$	
3	+		+	+						C R S	ditto		$K_{S2} = \frac{CR \times S}{CRS}$
4	+	+					+			C S R	ditto	$K_{R3} = \frac{CS \times R}{CSR}$	
5	+		+		+	+				C R R S	ditto		$K_{S3} = \frac{CRR \times S}{CRRS}$
6	+		+	+				+		C R S R	ditto	$K_{R4} = \frac{CRS \times R}{CRSR}$	
7	+	+					+		+	C S R R	ditto	$K_{R5} = \frac{CSR \times R}{CSRR}$	

Exclusively in Pluricomplexes with Carrier C and Substrate R

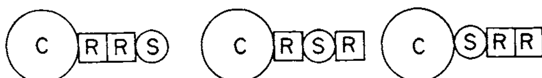
Types of complexes:

A. Independent binding:



Tricomplex

B. Dependent binding:



Tricomplexes

General conditions:

3. $S_2 = R_2 = 0$
   $S_1 = S \qquad R_1 = R$
   D = mobility of the moving complex containing S

Relative concentrations	V_S	Factors of the parameters[a] F_V	F_K
$R' = \frac{R}{K_{R1}}$ $\quad S' = \frac{S}{K_{S1}}$	$C_tD\,\frac{R'}{R'+1} \times \frac{S}{S'+1}$	$\frac{R'}{R'+1}\uparrow$	$1 \rightarrow$
ditto $\quad R'' = \frac{R}{K_{R2}}$	$C_tD\,\frac{R\,R''}{R'R''+R'+1} \times \frac{S'}{S'+1}$	$\frac{R'R''}{R'R''+R'+1}\uparrow$	$1 \rightarrow$
ditto $\quad S'' = \frac{S}{K_{S2}}$	$C_tD\,\frac{S''}{S'+\frac{R'+1}{R'}}$	$1 \rightarrow$	$1+\frac{1}{R'}\downarrow$
ditto $\quad R''' = \frac{R}{K_{R3}}$	$C_tD\,\frac{R'''}{R'''+1} \times \frac{S'}{S'+\frac{1}{R'''+1}}$	$\frac{R'''}{R'''+1}\uparrow$	$\frac{1}{R'''+1}\downarrow$
ditto $\quad S''' = \frac{S}{K_{S3}}$	$C_tD\,\frac{S'''}{S'''+\frac{R'R''+R'+1}{R'R''}}$	$1 \rightarrow$	$\frac{R'R''+R'+1}{R'R''}\downarrow$
ditto $\quad R'''' = \frac{R}{K_{R4}}$	$C_tD\,\frac{R''''}{R''''+1} \times \frac{S''}{S''+\frac{1/R'+1}{R''''+1}}$	$\frac{R''''}{R''''+1}\uparrow$	$\frac{1/R'+1}{R''''+1}\downarrow$
ditto $\quad R''''' = \frac{R}{K_{R5}}$	$C_tD\,\frac{R'R''}{R'R''+R'+1} \times \frac{S'}{S'+\frac{1}{R'R''\times R'\times 1}}$	$\frac{R'R''}{R'R''+R'+1}\uparrow$	$\frac{1}{R'R''+R'+1}\downarrow$

[a] Arrow indicates direction of parameter with rising R.

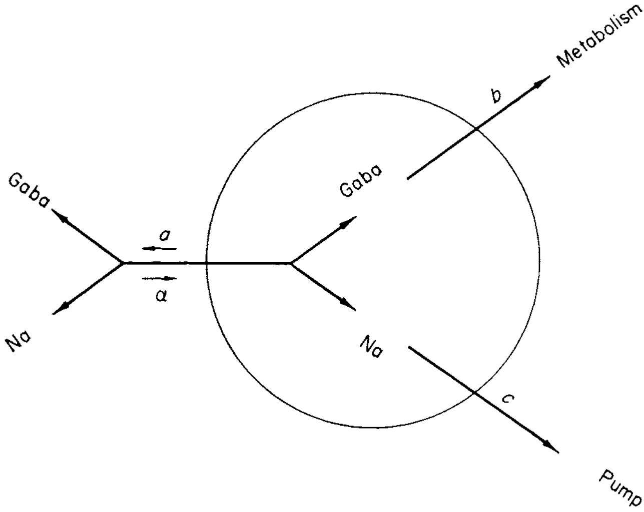


FIG. 3. Schematic representation of the movement of GABA and of sodium into and out of particles A.

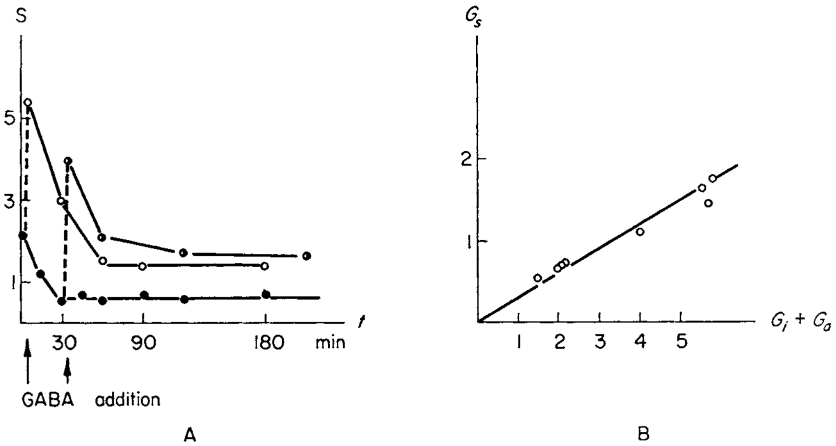


FIG. 4. A. Change of GABA concentration in the medium (S) with time. Full circles: spontaneous time course; open and half-open circles: time course in experiments with addition of GABA after 5 and 35 minutes, respectively. B. Steady-state level of GABA concentration in the medium (GS) as a function of the sum of initial GABA concentration in the medium, G_i, and concentration increment after the addition, G_a.

steady-state level proved to be a linear function of the sum $G_i + G_a$, these symbols indicating respectively the external GABA concentration at time 0 and its increment after the addition of GABA.

Figure 5 shows an analysis of the steady state with respect to over-all GABA movements from particles B through the medium into

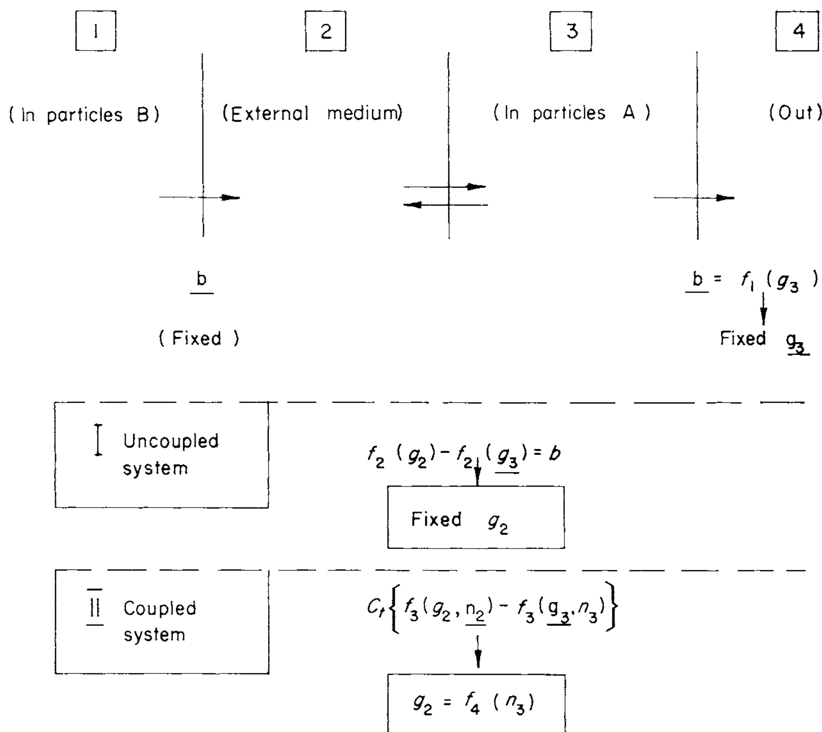


FIG. 5. Analysis of steady-state condition with respect to the GABA concentration in the medium (g_2) for an uncoupled system and a coupled system (GABA transport coupled to sodium transport by common carrier).

particles A and finally into metabolism. The coupled system (with mixed complexes) is compared with an uncoupled system in which GABA moves into particles A either by diffusion or by a carrier system not involving mixed complexes. Since the movement out of particles B has been shown to be independent of external conditions, its rate appears to control the steady state: both the movement into particles A and the rate of GABA metabolism in the particles A

must equal this rate b. In the case of transport into particles A by any uncoupled system involving only GABA itself (and possibly a carrier), regardless of whether this system follows linear kinetics or some type of carrier kinetics, there is one and only one possible steady-state concentration of GABA in the external medium. This is no longer so, however, if the transport into A particles depends on both the GABA and the sodium concentrations in the manner discussed, and if it is assumed that the external concentration of Na is fixed due to the large external volume and that the internal concentration of GABA is also fixed due to the condition that GABA metabolism equals b. In this case, the postulate that the rate of transport into particles A equals b can be met by any number of pairs for the external concentration of GABA and the internal concentration of sodium, and the external GABA concentration in the steady state will increase if the internal Na-concentration rises. One possibility for such a rise would be the operation of the sodium pump at maximum rate, in which case the increased sodium uptake into the particles during the time after GABA addition could not be compensated by increased removal through the pump. There are other possibilities for a sodium increase. Their discussion, however, would not be fruitful in view of the complete lack of additional data.

DISCUSSION

The experiments described here demonstrate the existence of sodium-dependent and cardiac glycoside-inhibited transport of GABA across membranes of subcellular particles. This finding in itself is of some interest since there are not many examples in which transport mechanisms known to operate across the cell membrane in cellular systems were found in subcellular membranes as well. Even morphological comparability of cellular and subcellular membranes is not universally accepted, as current discussions on the structure of the mitochondrial membrane show. Subcellular sodium-dependent transport of amino acid has been demonstrated across the membrane of cell nuclei [1], but the question of cardiac glycoside inhibition has not been tested in this case.

The relationship to observations in cellular systems is strengthened by the fact that the hypothesis of mixed transport complexes, which has been applied to cellular systems with considerable success, has also been helpful in the interpretation of the otherwise surprising and unexpected observation that GABA addition leads to higher

steady-state GABA levels in the medium. If the interpretation offered here is correct it also implies the existence of sodium pumps in subcellular membranes, which to our knowledge, has not been described so far. Thus the mechanisms operating in cellular transport of amino acids and in subcellular transport of GABA seem to be quite closely related.

A special feature of the subcellular system discussed here is that the sodium dependence is a common element in the observations at 0° and at 29°C. It therefore appears possible that the systems involved operate with the same carrier reacting both with sodium and GABA. This results in an equilibrating transport at 0°C and in a potentially uphill system at higher temperature. Actually such relationships would appear to be a natural consequence of the concept of mixed complex downhill movement leading to an uphill transfer of one of the components. The additional feature introduced at higher temperature, then, would be the energy-yielding, metabolic breakdown of GABA and the utilization of this energy for the sodium pump.

As to the possible physiological bearing of the observations reviewed and the interpretations offered here, it seems clear that the experimental conditions differ widely from the biological situation: In the experiment particles originating from all parts of the brain, including glia and nerve cells as well as nerve fibers, are mixed. Therefore, no direct analogy can be assumed with respect to biological conditions. Nevertheless, a few possibilities may be discussed briefly.

In a general way the inter- or intracellular translocation of GABA between sites of formation, storage, function, and removal may depend on the local concentrations of sodium ions. To name one specific although speculative possibility, the entry of sodium into a presynaptic nerve ending during excitation might trigger GABA depletion by allowing mitochondria to take up and metabolize GABA. The potentially uphill system in such a case would be used for accelerated rather than for uphill movement. Likewise sodium might trigger ejection of GABA from an inhibitory nerve ending across the cell membrane in order to translocate it to a neighboring structure for inhibitory action.

Whereas in these cases sodium would be used to promote translocation of GABA, the coupling between GABA and sodium might also be conceived to operate in the opposite sense, namely, by translocation of sodium induced by GABA. The general feature of inhibitory action, according to neurophysiological analysis, appears to be an increase

in the ion conductance of the cell membrane. With respect to the sodium exchange across the membrane, the mixed carrier complex formed in the presence of GABA could act as a "sodium shunt." This might well be one of the means by which neurophysiological inhibition can be achieved.

SUMMARY

Sodium-dependent, cardiac glycoside-inhibited, uphill transport systems at the cellular level are discussed in terms of recently suggested interpretations postulating downhill movement of substrate-sodium-carrier complexes in conjunction with the operation of a sodium pump. A subcellular transport system, demonstrated in brain particles for γ-aminobutyric acid and having in common with these systems Na-dependence and cardiac glycoside sensitivity, is reviewed and discussed in terms of the same hypothesis. It is shown that the mixed complex mechanism is compatible with all available experimental data and offers interesting neurophysiological implications.

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