J. Membrane Biol. 30, 153–173 (1976)

Influence of Membrane Potential on the Sodium-Dependent Uptake of Gamma-Aminobutyric Acid by Presynaptic Nerve Terminals: Experimental Observations and Theoretical Considerations

M. P. Blaustein and A. Christie King

Department of Physiology and Biophysics, Washington University Medical School, St. Louis, Missouri 63110

Received 29 March 1976; revised 4 June 1976

Summary. Sodium, potassium and veratridine were tested for their effects on the uptake of gamma-aminobutyric acid (GABA) by pinched-off presynaptic nerve terminals (synaptosomes). As noted by previous investigators, the uptake from media containing 1 μM GABA ("high-affinity" uptake) is markedly Na-dependent; the uptake averaged 65 pmoles/mg synaptosome protein × min, with $[Na]_o = 145$ mM and $[K]_o = 5$ mM, and declined by about 90% when the external Na concentration ($[Na]_o$) was reduced to 13 mM (Na replaced by Li). The relationship between $[Na]_o$ and GABA uptake was sigmoid, suggesting that two or more Na^+ ions may be required to activate the uptake of one GABA molecule. Thermodynamic considerations indicate that with a Na^+/GABA stoichiometry of 2:1, the Na electrochemical gradient, alone, could provide sufficient energy to maintain a maximum steady-state GABA gradient ($[GABA]_i/[GABA]_o$) of about 10^4 across the plasma membrane of GABA-nergic terminals.

In Ca-free media with constant $[Na]_o$, GABA uptake was inhibited, without delay, by increasing $[K]_o$ or by introducing 75 μM veratridine; the effect of veratridine was blocked by 200 nM tetrodotoxin. The rapid onset (within 10 sec) of the veratridine and elevated-K effects implies that alterations in intra-terminal ion concentrations are not responsible for the inhibition. The uptake of GABA was inversely proportional to log $[K]_o$. These observations are consistent with the idea that the inhibitory effects of both veratridine and elevated $[K]_o$ may be a consequence of their depolarizing action. The data are discussed in terms of a barrier model (Hall, J.E., Mead, C.A., Szabo, G. 1973. *J. Membrane Biol.* **11**:75) which relates carrier-mediated ionic flux to membrane potential.

It is now widely recognized that when mammalian brain tissue is homogenized in isotonic sucrose, a considerable fraction of the presynaptic nerve terminals may pinch off and reseal. This view is supported by the numerous experimental observations which show that preparations enriched with these terminals ("synaptosomes"), when returned to a physiological environment, exhibit many of the functional properties of

intact cells: they respire and synthesize ATP (Bradford, 1969), accumulate and retain potassium (Bradford, 1969; Escueta & Appel, 1969) and extrude sodium (Ling & Abdel-Latif, 1968) against concentration gradients, and appear to have membrane potentials (Blaustein & Goldring, 1975). Like intact nerve terminals, they can accumulate calcium (Blaustein & Wiesmann, 1970; Blaustein, 1975), and release various transmitter substances by calcium-dependent mechanisms (Blaustein, Johnson & Needleman, 1972; DeBelleroche & Bradford, 1972; Levy, Haycock & Cotman, 1974; Blaustein, 1975), when treated with appropriate depolarizing agents.

Studies in many laboratories (e.g., *see* the reviews by Iversen, 1971 and 1973; by Kuhar, 1973; and by Bennett, Mulder & Snyder, 1974) have shown that the heterogeneous synaptosome preparations can accumulate a variety of transmitters and putative transmitters by "high-affinity" sodium-dependent uptake mechanisms. It has been suggested (e.g., Iversen, 1971), that these uptake mechanisms may play an important role in helping to terminate the action of those transmitters which, in contradistinction to acetylcholine, are not hydrolyzed. Furthermore, the reaccumulation of these substances (and of choline, which is apparently taken up by a similar process; *c.f.*, Mulder, Yamamura, Kuhar & Snyder, 1974), may serve a very important conservation function, since the need for *de novo* synthesis of the transmitters is thereby reduced. For example, Hedqvist and Stjärne (1969) have stated that, "the major mechanism for the maintenance of a constant noradrenaline level in the neuron ... during periods of increased ... activity is not *de novo* synthesis, but rather an almost quantitative re-uptake of the noradrenaline liberated from the neurons". The high-affinity uptake mechanisms are apparently transmitter-specific; for example, noradrenergic terminals accumulate noradrenaline, but not other transmitters, while GABA-nergic terminals selectively take up GABA (*cf.* Iversen, 1971).

Although the details of these re-uptake mechanisms are not completely understood, available evidence indicates that the transmitters (and choline, the precursor of acetylcholine) can be accumulated against concentration (or electrochemical) gradients. In several instances, uptake of these substances has been shown to be markedly inhibited by the application of metabolic poisons such as 2,4-dinitrophenol or cyanide (e.g., Iversen & Neal, 1968; Marchbanks, 1968; White & Keen, 1970; Balcar & Johnston, 1975). These observations imply that metabolic energy must be utilized, either directly or indirectly, in order for the terminals to accumulate the transmitters. The uptake processes also require external Na; they are inhibited by external K depletion and by cardiac glycosides such as ouabain

(e.g., Bogdanski, Tissari & Brodie, 1968; Marchbanks, 1968; Levi, 1972; Martin & Smith, 1972; Holtz & Coyle, 1974). Since these treatments are known to deplete the normal transmembrane Na and K concentration gradients, many investigators (e.g., Bogdanski *et al.*, 1968; Martin, 1973; Holtz & Coyle, 1974) have suggested that the energy stored in the transmembrane Na gradient may be an important source of energy for the transmitter transport processes. In this respect, these processes may be very similar to the Na-dependent mechanisms involved in amino acid and sugar transport in renal and gastrointestinal tract epithelia and in certain other types of cells (*see* the review by Schultz & Curran, 1970).

During the past few years there has been an increasing awareness of the role of the membrane potential in the Na-coupled transport of sugars (Murer & Hopfer, 1974; Beck & Sacktor, 1975), amino acids (Gibb & Eddy, 1972; Reid, Gibb & Eddy, 1974; Colombini & Johnstone, 1974; Johnstone, 1975) and calcium (Blaustein, Russell & De Weer, 1974; Brinley & Mullins, 1974; Mullins & Brinley, 1975). There has, however, been only passing reference (e.g., Holtz & Coyle, 1974; Martin, 1973) to its possible role in Na-co-transport of neural transmitter substances, although there are many reports that raising the extracellular potassium concentration ($[K]_o$) inhibits transmitter re-uptake (e.g., norepinephrine: Colburn, Goodwin, Murphy, Bunney & Davis, 1968; gamma-aminobutyric acid: Martin & Smith, 1972; choline: Marchbanks, 1968). The present study focuses on this aspect of the problem. Our observations on the effects of $[K]_o$ and veratridine (a depolarizing agent: Ohta, Narahashi & Keeler, 1973; and *see* Blaustein & Goldring, 1975) in synaptosomes strongly support the view that transmitter re-uptake is influenced by *both* the Na concentration gradient *and* the membrane potential gradient; that is, transmitter re-accumulation is a function of the Na *electrochemical* gradient. The experimental results are discussed in terms of the theoretically expected contributions of the Na and voltage gradients to the fluxes and steady-state distribution of Na-co-transported solutes.

Materials and Methods

Solutions

The standard salt solution ("145 Na + 5 K") contained (in mmoles/liter): NaCl, 145; KCl, 5; $MgCl_2$, 2.6; NaH_2PO_4, 1.2; glucose, 10; mannitol, 5; and HEPES (N-2-hydroxyethylpiperazine-N′-2-ethanesulfonic acid), 20. The solution was titrated to pH 7.65 at 22 °C with Tris (hydroxymethyl) aminomethane base. In many instances, some or all of the NaCl was replaced mole-for-mole by LiCl (e.g., "85 Na + 5 K" contained 60 mM LiCl) or by KCl (e.g.,

"85 Na + 55 K" contained 55 mM KCl and 10 mM LiCl); the sum of NaCl + KCl + LiCl was always 150 mM. All salts were reagent grade; the water was demineralized and then glass-distilled.

Veratridine (K&K Laboratories, Plainview, New York) was dissolved in a few drops of 1 N HCl, and diluted with 145 Na + 5 K (re-titrated to pH 7.65 with Tris base). Tetrodotoxin ("TTX"; Calbiochem, Palo Alto, California) was directly dissolved in 145 Na + 5 K.

Tritium-labeled gamma-aminobutyric acid (GABA) and ^{14}C-mannitol were obtained from New England Nuclear (Boston, Massachusetts); nonradioactive GABA and mannitol were purchased from Sigma Chemical Co. (St. Louis, Missouri).

Preparation of Synaptosomes

Synaptosomes were prepared from whole rat brain homogenates by a slightly modified Gray and Whittaker (1962) differential and discontinuous sucrose density gradient procedure. The modification, previously described in detail (Blaustein & Ector, 1976), included two washes of the crude mitochondrial pellets ("P_2" of Gray and Whittaker) to reduce microsomal contamination (Gurd, Jones, Mahler & Moore, 1974). The washed pellets were resuspended in 0.32 M sucrose and layered onto the discontinuous sucrose gradient (Gray & Whittaker, 1962). Following a two-hour centrifugation (60,000 × g, 4 °C), the material at the 0.8–1.2 M sucrose interface (= "synaptosomes") was removed and slowly diluted with 10–12 volumes of ice-cold 145 Na + 5 K. Aliquots of this suspension were centrifuged at 9,000 × g for 4 min at 4 °C, the supernatant solution was then discarded.

Experimental Procedures

The 145 Na + 5 K-equilibrated synaptosome pellets (0.35–0.45 mg protein) were re-suspended in 0.2 ml of 145 Na + 5 K and incubated for 12 min at 30 °C in order to return them to a physiological steady-state. Additional aliquots (usually 2 ml) of appropriate incubation solutions containing ^{3}H-labeled GABA (specific activity, after dilution, about 400 Ci/mole) and ^{14}C-mannitol (specific activity, after dilution, about 4×10^{-3} Ci/mole) were added to the suspensions; the GABA concentration, after dilution, was 1 μM. The mannitol, which is not taken up by synaptosomes (Blaustein & Ector, 1975, and *unpublished data*), was used to correct for extra-synaptosomal tritium. The labeled incubation solutions frequently contained an altered alkali metal ion composition, or veratridine and/or TTX. Details regarding the final composition of the suspending media, will be given in *Results*.

The tracer-labeled suspensions were incubated at 30 °C, usually for one min (but *see Results*); GABA accumulation was terminated by vacuum filtration of the synaptosome suspension on pre-washed 0.3 μ pore diameter Millipore (Bedford, Massachusetts) cellulose acetate filters (approximately 0.15–0.2 mg protein/filter). Each filter was then rapidly washed with 2–4 ml aliquots of ice-cold 145 Na + 5 K saline containing 1 mM GABA. In agreement with the observations of Simon, Martin & Kroll (1974), we found no evidence for loss of previously-accumulated ^{3}H-GABA due to "cold-shock" (*cf.* Levi & Raiteri, 1973) under the experimental conditions reported here.

The washed filters were transfered to scintillation vials, and 12 ml of a 2:1 (v/v) toluene/Triton X-100 cocktail, containing 4 gm Omnifluor (New England Nuclear) per liter, was added. The samples were counted in a three-channel Packard (Downers Grove, Illinois) Tricarb liquid scintillation counter. The channel-ratio method was employed to separate ^{3}H and ^{14}C counts. Specific activities were computed from counts in aliquots of the incubation solutions.

In each experiment several of the 145 Na + 5 K-equilibrated synaptosome pellets were digested in 1 N NaOH, and analyzed for protein by the Lowry method (Lowry, Rosebrough, Farr & Randall, 1951); bovine serum albumin was used as a standard.

Results

Sodium-Dependent Uptake of Gamma-Aminobutyric Acid

Studies in many laboratories have documented a Na-dependent, "high-affinity" uptake of GABA by brain slice preparations and by synaptosomes (reviewed by Martin, 1976). As a starting point for the present study, we repeated and confirmed, in synaptosomes, many of the findings from Martin's laboratory (Martin & Smith, 1972; Martin, 1973). One of the critical observations, illustrated in Fig. 1, is that ^{3}H-GABA uptake from a medium containing 1 μM GABA is almost entirely dependent upon the presence of Na in the bathing medium; decreasing $[Na]_o$ (the external Na concentration) from 145 to 13 mM reduced ^{3}H-GABA uptake by nearly 90%. Although the external Na was replaced isosmotically by Li in the experiments of Fig. 1, the nature of the Na substitute is apparently inconsequential, as Martin and Smith obtained very similar results with sucrose or choline chloride substituted for NaCl.

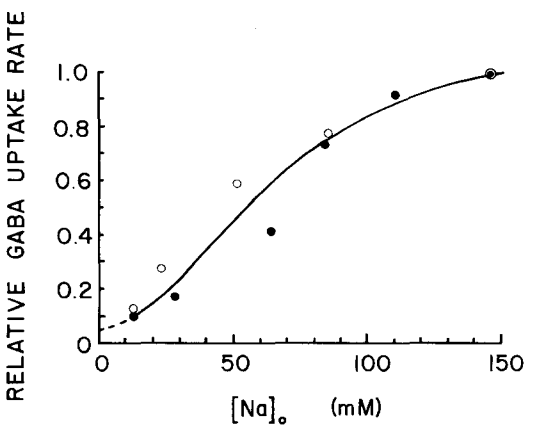

Fig. 1. Effect of external Na concentration on ^{3}H-GABA uptake. The data from two experiments (open *vs.* filled symbols) have been normalized to the uptake from the 145 Na + 5 K solutions (= 1.0), and are shown on a relative scale. Each point indicates the mean of five determinations. In one experiment the uptake from 145 Na + 5 K was 68.8 ± 4.5 (SEM) nmoles/gm protein × min, and in the second, 59.7 ± 2.5 nmoles/gm protein × min. All incubation solutions contained 5 mM K and 145 mM Na + Li; the Na concentration is indicated on the abscissa scale. The curve was drawn to fit the equation:

$$J_{GABA} = \frac{J^*_{GABA}}{1 + \left(\frac{\bar{K}_{Na}}{[Na]_o}\right)^2} + 0.05$$

where J_{GABA} is the relative GABA uptake at any $[Na]_o$; the maximum uptake, J^*_{GABA}, has a value of 1.15 on this scale. $\bar{K}_{Na}$ is the apparent mean Na^+ dissociation constant, with a value of 67 mM; the constant, 0.05 may represent GABA uptake which is Na-independent

The GABA concentration employed in these and the subsequent experiments, 1 μM, was the concentration employed by Martin and Smith (1972) for most of their initial studies; this value is lower than the apparent half-saturation concentration (presumably for the "high-affinity" uptake) of 4 μM, obtained in the presence of 95 mM Na (Martin, 1973). The low GABA concentration was used in order to minimize tissue saturation.

To obtain information about the initial rate of GABA uptake (i.e., influx), a short (1 min) incubation was employed in most experiments. Problems related to metabolic degradation of the ^{3}H-GABA (Martin & Smith, 1972) were presumably avoided by this brief incubation.

In agreement with the finding of Martin and Smith (1972), the uptake of ^{3}H-GABA from Na-rich media was linear with time for up to 10 min of incubation. The rates of GABA uptake from a solution containing 1 μM GABA were also rather similar: about 100 nmoles GABA/gm protein × min (*cf.* Figs. 4 and 6 of Martin & Smith, 1972), as compared to our averaged value (for 9 experiments) of 59.2 ± 4.6 (SEM) nmoles/gm protein × min. The difference between these values may be accounted for primarily by the fact that our synaptosomes were prepared from whole rat brain, and not from "trimmed" cerebral cortex, which is particularly rich in GABA-nergic terminals (Martin, 1976).

The sigmoid relationship between $[Na]_o$ and GABA uptake, seen in Fig. 1, is also consistent with the observations of Martin and Smith (1972). Hill plots of the data from Fig. 1 yielded lines with slopes of 1.7 and 1.9 (open and filled circles, respectively); these values are slightly smaller than the mean of 2.4 obtained by Martin (1973). Our results may indicate that at least two (Martin suggested three) Na^+ ions are required to activate the uptake of one GABA molecule (*see Discussion*).

The Effect of Potassium on GABA Uptake

Martin and Smith (1972) also observed that the uptake of GABA by synaptosomes was influenced by the K concentration in the bathing medium: the omission of K inhibited GABA uptake, as did raising $[K]_o$ above 6 mM. One obvious interpretation of the K-free effect is that the reduced GABA uptake is a consequence of the reduced Na concentration gradient, since entering Na can no longer be extruded in exchange for K via the sodium pump (*cf.* Ling & Abdel-Latif, 1968). The delayed onset of inhibition of GABA uptake observed in K-free media (Martin, 1976) is consistent with this interpretation because the ion gradient should not dissipate instantaneously.

The inhibitory effect of elevated $[K]_o$ clearly requires a different explanation, because raising $[K]_o$ at constant $[Na]_o$ should stimulate the sodium pump (e.g., Baker, Blaustein, Keynes, Manil, Shaw & Steinhardt, 1969) and help to maintain the Na and K gradients. One interpretation (*cf.* Heinz, Geck & Wilbrandt, 1972) of the data is that external K^+ can simply bind to the "carrier"[1] (presumably in place of Na^+), and can thereby either inhibit GABA binding, or reduce the "mobility" of the GABA-carrier complex. Inhibition of GABA binding appears to be ruled out, however, because the double-reciprocal plot of uptake velocity *vs.* GABA concentration indicates that increased $[K]_o$ behaves like a non-competitive inhibitor; it reduces the maximum transport rate (J_{Max}), but does not affect the apparent half-saturation for GABA (Martin, 1973). While this implies that the "mobility" of the GABA-carrier complex is reduced by high $[K]_o$, displacement of Na from carrier sites, by K, is not the only way to account for a reduced carrier "mobility"; moreover, the fact that raising $[K]_o$ appears to reduce J_{Max}, even at saturating $[Na]_o$ (Martin, 1973; Fig. 8), may indicate that K does not displace Na from the carrier in a simple competitive manner.

An alternative interpretation of the $[K]_o$ effect is that the inhibition of GABA uptake is a consequence of the K-induced (synaptosome) plasma membrane depolarization (*cf.* Blaustein & Goldring, 1975). This possibility is suggested by the fact that carriers saturated with GABA + Na^+ will have more positive net charge than unloaded carriers, and the movement of the cation-loaded (or "charged") carriers should, in part, be governed by the electric field (*cf.* Bockris & Reddy, 1970). There is now ample precedent to indicate that the carrier-mediated transport of ions in artificial lipid bilayers (e.g., Hall *et al.*, 1973, and *see* the review by Haydon & Hladky, 1972) and in natural membranes (Blaustein et al., 1974; Mullins & Brinley, 1975) can be affected by changes in the electric field in the absence of alterations in the ion concentration gradients (the sodium pump may be an exception, however: *cf.* Brinley & Mullins, 1974). The influence of elevated $[K]_o$ on ^{3}H-GABA accumulation by synaptosomes was therefore examined with this possibility in mind.

Fig. 2 shows that the uptake of ^{3}H-GABA from a medium containing 55 mM K is linear with time. This linearity, with a reduced rate of uptake

1 For the sake of convenience, the term "carrier" will be used here to denote the Na-dependent transport mechanisms exemplified by the GABA uptake system. Nevertheless, it should be recognized that these "carriers" may be fundamentally different from the lipophilic ionophorous antibiotics, such as valinomycin, which can serve as "mobile ion carriers" (*see Discussion*).

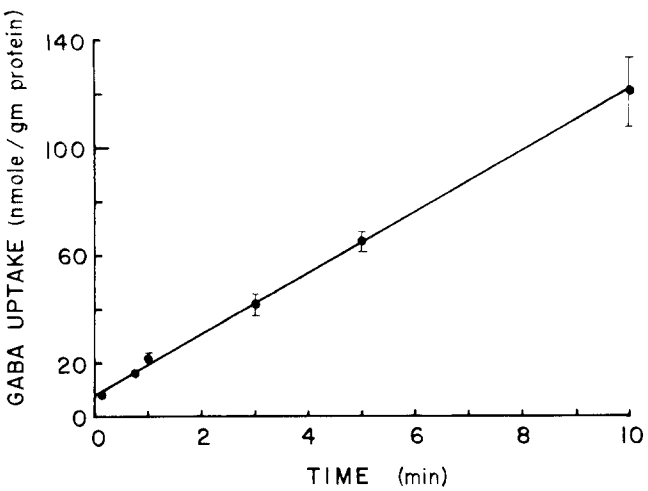

Fig. 2. Time-course of ^{3}H-GABA uptake from a medium containing 85 mM Na, 55 mM K, and 1 μM GABA. Each symbol denotes the mean of four determinations; bars indicate ±1 SE where SE extends beyond the radius of the symbol

(*cf.* Figs. 3 and 5), would appear to eliminate certain explanations for the high $[K]_o$-induced inhibition. For example, the inhibition cannot be the result of altered internal ion concentrations, because such alterations are time-dependent. Also, the inhibition cannot be the result of an increased GABA efflux without change in influx. [It should be noted that Ca was omitted from the bathing medium in all of the experiments described here, in order to minimize depolarization-triggered Ca-dependent GABA efflux (*cf.*, Levy *et al.*, 1974; Cotman, Haycock & White, 1976).]

A linear plot of the rate of ^{3}H-GABA uptake, as a function of $[K]_o$, is shown in Fig. 3; in this experiment the external Na concentration was a constant 85 mM. The shape of the curve is typical of those obtained in four such experiments. Its most prominent feature is a steep decline in GABA uptake when $[K]_o$ is increased from 5 to 15 mM, with a progressive fall in slope as $[K]_o$ is increased further. This is not the result to be expected if K displaces Na from carrier sites on a 1-for-1 basis, especially under conditions where $[Na]_o$ is close to saturation (*cf.* Fig. 1). If, however, the effect of elevated $[K]_o$ is primarily due to its influence on the membrane potential, then a graph of GABA uptake as a function of log $[K]_o$ may be more meaningful because of the proportionality between log $[K]_o$ and membrane potential (*cf.* Blaustein & Goldring, 1975). Indeed, an approximately linear relationship is obtained when data from experiments like those of Fig. 3 are plotted semilogarithmically; a similar correla-

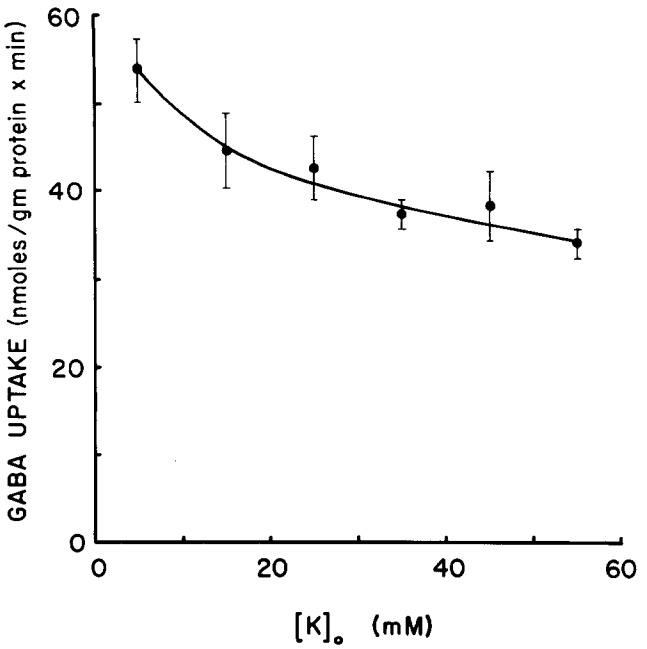

Fig. 3. Effect of external K concentration on ^{3}H-GABA uptake by synaptosomes. The terminals were incubated for 1 min at 30 °C in media containing 1 μM GABA. The Na concentration was constant (85 mM); the 5 mM K solution contained 65 mM Li, and external K was increased at the expense of Li. Each symbol denotes the mean of 4 determinations; the bars indicate ±1 SE. The curve was drawn by eye

tion between synaptosome uptake of norepinephrine and log $[K]_o$ was observed by Colburn *et al.* (1968).

Experiments with reduced $[K]_o$ provide additional support for the view that the membrane potential influences the rate of GABA uptake. As already noted, the reduction of $[K]_o$ below about 5 mM may slowly alter the transmembrane Na and K gradients (as well as depolarize the terminals) by inhibiting the plasma membrane Na pumps. However, the initial effect of reducing $[K]_o$ (especially in media with a low [Na]) may be to hyperpolarize the terminals, due to the sudden decrease in the $[K]_o/[K]_i$ ratio (where the subscript, *i*, refers to the intracellular concentration). Under these circumstances, if GABA uptake is inversely related to the magnitude of the membrane potential, uptake, should *increase* when $[K]_o$ is reduced to a low level, provided the incubation is brief. This effect is illustrated in Fig. 4: ^{3}H-GABA uptake increased from 48.5 ± 1.8 nmoles/gm protein × min to 74.3 ± 2.2 ($n = 4$) nmoles/gm protein × min when $[K]_o$ was reduced from 5 to 0.5 mM. The samples were incubated with ^{3}H-GABA (and with low $[K]_o$ in the appropriate flasks) for 1 min only; $[Na]_o$ was a constant 55 mM.

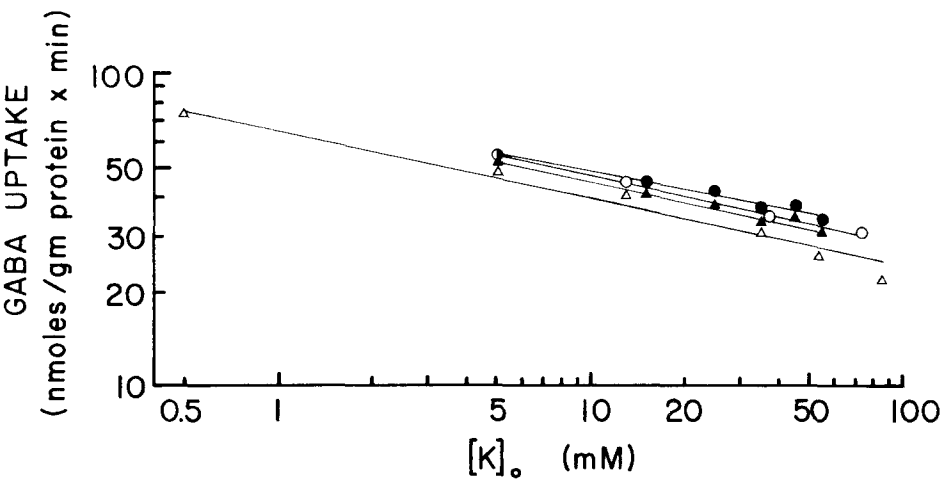

Fig. 4. Plot of log ^{3}H-GABA uptake versus log $[K]_o$. Each point is the mean of three or four determinations. Each symbol refers to a different synaptosome preparation. Filled circles and triangles: $[Na]_o = 85$ mM; the filled circles refer to data from the experiment of Fig. 3. Open circles and triangles: $[Na]_o = 55$ mM

While the aforementioned observations suggest a correlation between membrane potential and amino acid uptake, the relationship need not necessarily be linear. In fact, as noted in the *Discussion* (below), a linear correlation between the membrane potential and the *logarithm* of the solute flux is suggested by certain theoretical considerations. Consequently, the data from several experiments similar to (and including) the one of Fig. 3 are shown on a log-log plot (Fig. 4) which may be approximately equivalent to a graph of log GABA uptake *vs.* membrane potential.

The Effect of Veratridine on GABA Uptake

If the inhibitory influence of elevated $[K]_o$ on GABA uptake is primarily a consequence of its depolarizing action, it should be mimicked by other depolarizing agents which may have a different mode of action. This hypothesis was explored by testing the effect of veratridine, an agent which depolarizes nerve axons (Ohta *et al.*, 1973) and terminals (Blaustein & Goldring, 1975) by opening TTX-sensitive Na channels; veratridine has previously been shown to inhibit dopamine accumulation by synaptosomes (Holtz & Coyle, 1974).

Table 1 shows that the uptake of ^{3}H-GABA is inhibited by veratridine, and that this inhibition is largely prevented by TTX; similar results were

Table 1. Effects of veratridine and tetrodotoxin on ^{3}H-GABA uptake by synaptosomes

Incubation medium[a]	GABA uptake[b] (nmoles/gm × min)	Δ[c]
145 Na + 5 K	47.4 ± 2.3	–
145 Na + 5 K + 75 μM veratridine	24.3 ± 2.4	−23.1
145 Na + 5 K + 200 nM TTX	62.4 ± 1.3	+15.0
145 Na + 5 K + 75 μM veratridine + 200 nM TTX	50.6 ± 1.6	+ 3.2

[a] Synaptosomes were incubated with ^{3}H-GABA and drugs for 1 min at 30 °C.
[b] Each value is the mean ± SEM of 5 determinations.
[c] Increment (+) or decrement (−) due to the addition of GABA and/or TTX.

obtained in one other experiment. The greater-than-"control" uptake observed with TTX present may be accounted for if the TTX-treated synaptosomes are less "leaky" to Na and, therefore, less depolarized (*cf.* Blaustein & Goldring, 1975) than the untreated controls.

Veratridine appears to act very rapidly, as indicated by the time-course experiment of Fig. 5. In two experiments the ^{3}H-GABA uptake was measured after a 10 sec incubation, and was significantly reduced ($p < 0.05$) as compared to controls; as illustrated in Fig. 5, inhibition was quite obvious after 30 sec of incubation. Holtz and Coyle (1974) also noted that dopamine uptake was rapidly reduced by veratridine, whereas the inhibition due to ouabain was delayed in onset. These results imply that the veratridine-induced inhibition of transmitter uptake is not, primarily, a consequence of altered Na^+ and K^+ concentration gradients (resulting

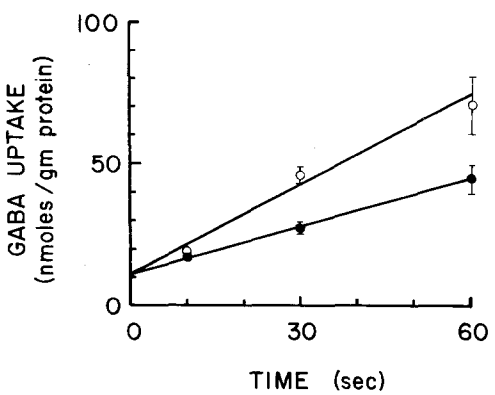

Fig. 5. Effects of veratridine and tetrodotoxin on the time-course of GABA uptake by synaptosomes. 145 Na + 5 K containing 1 μM ^{3}H-GABA and 75 μM veratridine, without (filled circles) or with (open circles) 200 nM TTX was added at zero-time. Each symbol denotes the mean of three determinations. The bars indicate ± 1 SE; errors were smaller than the circle radii at 10 sec

from the opening of Na channels by veratridine), because significant alterations would be expected to occur with some temporal delay. Furthermore, treatment with veratridine should eventually lead to an increase in $[Na]_i$ and decrease in $[K]_i$, whereas increasing $[K]_o$ (*see* above) should help to reduce $[Na]_i$ and increase $[K]_i$ by stimulating the sodium pump (e.g., Baker *et al.*, 1969). Thus, although veratridine and elevated $[K]_o$ may exert opposite effects on the intra-terminal cation concentrations, both treatments have a depolarizing action (Blaustein & Goldring, 1975); it therefore seems reasonable to attribute the inhibition of GABA uptake by both of these treatments to depolarization.

Discussion

There is now abundant evidence (*see* Introduction) that the accumulation of many transmitters and putative transmitters by nerve terminals requires a large Na concentration gradient ($[Na]_o \gg [Na]_i$) across the plasma membrane. Despite the absence of direct Na flux data, which may be exceptionally difficult to obtain in nerve terminal preparations, stoichiometric co-transport of Na and transmitter is implied by the dependence on external Na and by analogies with other apparently similar Na-dependent transport mechanisms (e.g., Schultz, Curran, Chez & Fuisz, 1967; Curran, Schultz, Chez & Fuisz, 1967). Under these circumstances the carrier-mediated net transport of uncharged solutes, such as GABA, will result in the concurrent net transfer of charge across the plasma membrane and, if the membrane resistance is sufficiently high, in alteration of the membrane potential (e.g., Rose & Schultz, 1970 and 1971; White & Armstrong, 1971; and *see* Krnjević & Schwartz, 1967). An interesting consequence, suggested to us by Dr. C. M. Rovainen (*personal communication*), is that presynaptic depolarization due to this net entry of positive charge, during the recovery period following neuronal activity, could conceivably contribute to presynaptic inhibition.

In order to evaluate appropriately the kinetics and thermodynamics of these Na-coupled co-transport mechanisms, the influence of the electric field (membrane potential) *both* on the fluxes of the loaded (charged) carriers, *and* on the equilibrium [2] distribution of the co-transported solutes

2 Both unloaded and fully-loaded carriers will be in diffusion (or translocation) equilibrium across the plasma membrane. Although the carriers at the internal and external membrane faces will also be in association-dissociation equilibrium with the carrier substrates (including Na^+) in the internal and external solutions, respectively, the Na^+ will not be distributed at equilibrium because of the action of the Na pump.

(such as GABA), must be taken into account. The latter consequences are more straightforward and have been more widely recognized; they are therefore considered first.

Theoretical Considerations

I. The Relationship between the Limiting Transmitter Concentration Gradient and the Membrane Potential

The functional advantage conferred by coupling the transport of, for example, transmitter substances to that of Na^+ ions is that energy stored in the Na^+ electrochemical gradient, through the action of the Na pump (e.g., Caldwell, 1969), can be used to drive the co-transported (or counter-transported; *cf.* Mitchell, 1970; Blaustein, 1974) substances against their respective electrochemical gradients. The energy released by the inward movement of one mole of Na^+ ions, down its electrochemical gradient, is given by the Na^+ electrochemical potential difference, $\Delta\bar{\mu}_{Na}$:

$$\Delta\bar{\mu}_{Na} = RT \ln \frac{[Na^+]_o}{[Na^+]_i} - V_m F. \tag{1}$$

V_m is the intracellular electrical potential (with respect to the external medium); F, R and T are Faraday's number, the gas constant, and absolute temperature, respectively. The external/internal Na^+ concentration ratio is $[Na^+]_o/[Na^+]_i$; the intracellular and extracellular ionic activity coefficients are assumed to be equal.

If the Na gradient is maintained by the sodium pump, and if the energy dissipated by the inward ("downhill") movement of n moles of Na^+ is harnessed to move m moles of transmitter, S, inward ("uphill"), via a co-transport mechanism, the limiting electrochemical potential difference for S, $\Delta\bar{\mu}_s$, and that for Na, $\Delta\bar{\mu}_{Na}$, will be related by (e.g., *see* Mitchell, 1970):

$$m\,\Delta\bar{\mu}_s + n\,\Delta\bar{\mu}_{Na} = 0 \tag{2}$$

where $\Delta\bar{\mu}_s$ is given by:

$$\Delta\bar{\mu}_s = RT \ln \frac{[S^z]_o}{[S^z]_i} - z\,V_m F. \tag{3}$$

The valence of the transmitter is z, and its external/internal concentration ratio is $[S^z]_o/[S^z]_i$. Substituting Eqs. (1) and (3) into (2), and re-arranging terms, yields:

$$\frac{[S^z]_i}{[S^z]_o} = \left(\frac{[Na^+]_o}{[Na^+]_i}\right)^{n/m} \exp\left[-\left(\frac{n}{m}+z\right)\frac{V_m F}{RT}\right]. \tag{4}$$

With tight coupling of S uptake to Na uptake and a minimal leak of S through other pathways (*cf.* Heinz & Geck, 1974), the maximal concentration ratio of S will be given by the right-hand expression if all of the energy for transmitter accumulation is derived from the Na^+ electrochemical gradient. Taking GABA, a zwitterion ($z = 0$ at physiological pH), as an example: if the Na^+/GABA transport stoichiometry (n/m) is 2:1, as suggested above, with typical values for intact neurons of

$$[Na^+]_o/[Na^+]_i \simeq 10 \quad \text{and} \quad V_m \simeq -60\,\text{mV},$$

the maximal expected $[GABA]_i/[GABA]_o$ ratio is about 10^4; if the stoichiometry is 3:1, as suggested by Martin (1973), the maximal $[GABA]_i/[GABA]_o$ ratio would be about 10^6.

As another example of the use of Eq. 4, consider the uptake of the acetylcholine precursor, choline ($z = +1$). Assuming that the Na^+/choline stoichiometry is 1:1, the maximal expected $[choline]_i/[choline]_o$ ratio will be about 10^3. In this case, the acetylcholine synthetic enzyme, choline acetyl-transferase, could be maintained at half-saturation with (intracellular) choline (apparent Michaelis constant, $K_{choline} < 20\,\mu M$; Potter, Glover & Saelens, 1968) when the extracellular choline concentration is less than 2×10^{-8} M.

Detailed information about the steady-state transmembrane gradients of transmitter substances or other co-transported solutes is needed in order to test quantitatively the applicability of Eq. 4. There is evidence that transmitter substances such as GABA (e.g., Elliot & van Gelder, 1958; Iversen & Kravitz; 1968; Iversen & Neal, 1968; and *see* Martin, 1976) can be accumulated against a large concentration gradient by nervous tissue. However, a considerable fraction of intracellular stores may be sequestered (e.g., in synaptic vesicles), so that the actual gradient across the plasma membrane may be overestimated by the tissue/medium ratio. On the other hand if only a small fraction of the cells in the preparation (synaptosomes or brain slices), have the specificity to accumulate the substance in question, this would lead to an underestimate of the transmembrane gradient (i.e., the left-hand term in Eq. (4)). Martin (1976) has recently reviewed the current status of this problem.

Eq. 4 is also based upon the assumption that only energy from the sodium gradient is used to concentrate the co-transported solutes. If additional energy, e.g., in the form of ATP, also powers these co-transport mechanisms, the concentration gradient obtained from Eq. 4 will be an underestimate. The question of whether or not metabolic energy directly powers Na-coupled co-transport in other tissues has not yet been complete-

ly resolved (e.g., compare Geck, Heinz & Pfeiffer, 1974, and Johnstone, 1975), and this question may be even more difficult to resolve in presynaptic terminals.

II. The Influence of Membrane Potential on the Rate of Carrier-Mediated Transmitter Uptake

As noted in the *Results* section, the most straightforward explanation for the inhibition of GABA uptake by K-rich media and by veratridine is that the influx is reduced by depolarization. If the carrier-mediated translocation step involves the movement of charged particles[3], it seems reasonable to expect that the membrane potential (i.e., the transmembrane electric field) may affect the kinetics of translocation. An important contribution in this regard is the "image force" model of Neumcke and Läuger (1969; and *see* Bockris & Reddy, 1970; Hall *et al.*, 1973; Eisenman, Krasne & Ciani, 1975), which can be used to determine the relationship between electric field and ion flux. The model is based on Eyring absolute reaction rate theory (*cf.* Laidler & Tweedale, 1971). It is assumed that there is a linear voltage gradient ("constant field," Goldman, 1943; Hodgkin & Katz, 1949) across the plasma membrane, and that the association-dissociation reactions between carriers and solutes are in equilibrium and are not affected by the membrane potential. For conditions of unequal ion concentrations in the internal and external environments, the relationship between the net flux (J) of transmitter (S), and the membrane potential (V_m) will be given by (*cf.* Hall *et al.*, 1973, Eq. 12):

$$J_{u>u_0} \propto \{[Na_n^+ S_m^z C]_o \exp[-(1-\beta)(mz+n)u] - [Na_n^+ S_m^z C]_i \exp[\beta(mz+n)u]\} \tag{5}$$

$$J_{u<u_0} \propto \{[Na_n^+ S_m^z C]_o \exp[-\beta(mz+n)u] - [Na_n^+ S_m^z C]_i \exp[(1-\beta)(mz+n)u]\} \tag{5'}$$

where influx is taken as the positive direction, and where $mz+n$ is the net transported charge, and $u = V_m F/RT$; u_0 is the membrane potential at which the net carrier flux is zero. The exponential term, β, indicates the

3 For simplicity, it will be assumed that the free carriers are electrically neutral, and that the charge is conferred by the carrier "substrates" (Na^+ and transmitters bearing net charge). This assumption is not critical to the main arguments, so long as the net transport of the solutes involves net movement of charge: since there is no net movement of carrier out of the plane of the membrane, the loaded carrier may be neutral, and the free carrier may bear a net charge.

fraction of the membrane potential which contributes to the potential energy barrier across which the loaded carriers must pass. With a symmetrical (triangular) barrier, corresponding to a single jump Eyring rate process with the barrier peak half-way across the membrane, β will have a value of 0.5. If the peak of the barrier is significantly flattened, corresponding to a multi-jump process, β will be less than 0.5 because the highest part of the barrier will then be nearer the internal (Eq. (5)) or external (Eq. (5′)) surface of the membrane (*cf.* Hall *et al.*, 1973).

The terms $[Na_n^+ S_m^z C]_o$ and $[Na_n^+ S_m^z C]_i$ refer to the concentrations of fully-loaded carriers facing, respectively, the external and internal aqueous phases; furthermore, these carrier concentrations should be proportional to $[S^z]_o^m \cdot [Na^+]_o^n$ and $[S^z]_i^m \cdot [Na^+]_i^n$, respectively. It is readily apparent that the net fluxes (J's) in expressions (5) and (5′) represent the difference between an influx term ($J_{in} \propto [\ ]_o \exp -[\ ]$) and an efflux term ($J_{out} \propto [\ ]_i \exp [\ ]$). For neutral molecules such as GABA ($z = 0$), expression (5′) can be replaced by:

$$J_{u<u_0} \propto [Na]_o^n \exp[-\beta n u] - [Na]_i^n \exp[(1-\beta) n u] \tag{6}$$

where u_0 is equal to $V_{Na} F/RT$, and V_{Na} is the sodium equilibrium (Nernst) potential. In this case the influx of transmitter, J_{in} will be given by:

$$J_{in} \propto \left\{ [Na^+]_o^n \exp -\beta n \frac{V_m F}{RT} \right\} \tag{6a}$$

or, at constant $[Na]_o$,

$$\log J_{in} \propto -\beta n \frac{V_m F}{RT}. \tag{6b}$$

Furthermore, if the membrane potential, V_m, is approximately equal to the equilibrium (Nernst) potential for potassium ions (an approximation which may be valid with a low $[Na]_o$ and elevated $[K]_o$ (*cf.* Blaustein & Goldring, 1975)), then assuming $[K]_i$ constant:

$$V_m \propto \frac{RT}{F} \log [K]_o.$$

Thus, Eq. (6*b*) can be approximated by:

$$\log J_{in} \propto \{-\beta n \log [K]_o\}. \tag{6c}$$

The main feature of these expressions is that the logarithm of the flux is proportional to the membrane potential or to the logarithm of $[K]_o$. This is the relationship illustrated in Fig. 4; the slopes of the regression lines,

determined by the method of least squares, were -0.18 and -0.21 for $[Na]_o = 85$ mM (filled circles and triangles, respectively), and -0.21 and -0.24 for $[Na]_o = 55$ mM (open circles and triangles, respectively). If the Na^+/GABA stoichiometry is 2:1 (n = 2), these results indicate that β may have a value of about 0.09–0.12; in other words, the loaded carriers may "see" only a small fraction of the membrane potential. The slopes may be underestimated if the membrane potential is not strictly proportional to $\log [K]_o$ at low values of $[K]_o$, due to the contributions of other ions (*cf.* Blaustein & Goldring, 1975).

The values for β, calculated from the data in Fig. 4 are all smaller than the value of 0.28 obtained by Hall *et al.* (1973) from determinations of the current carried by the K^+-nonactin complex across a phosphatidyl ethanolamine bilayer (note that our "β" corresponds to the "n" of Hall *et al.*). Hall *et al.* approximated the K^+-nonactin translocation by a two-jump Eyring rate process; they represented the energy barrier by a trapezoid.

This model system is certainly less complex than the Na-co-transport systems of biological membranes, such as the GABA uptake mechanism with which we are concerned. Evidence is rapidly accumulating (*cf.* Bretscher & Raff, 1975; Rothstein, Cabantchik & Knauf, 1976) to support the view that "carrier" transport in higher organisms is mediated by a family of high molecular weight proteins which are firmly embedded in the plasma membrane and may span the hydrophobic region. These "carriers" may be contrasted with the low molecular weight, lipid-soluble ionophores, exemplified by nonactin, which can simply shuttle across the hydrophobic region of a natural or artificial membrane bearing a shielded ion. In light of these differences, there is no *a priori* reason to expect similar values for β in the natural and model systems. For example, membrane asymmetries may affect the current-voltage (or ion flux-voltage) relationship (Hall & Latorre, 1976); furthermore, it would not be surprising to find a distorted membrane potential profile and/or surface charge distribution in the micro-environment around the large carrier proteins. Measurements of β might also be distorted if the association-dissociation reactions between the carriers and the transported solutes are influenced by the membrane potential.

Regrettably, the available data do not provide a sufficiently rigorous test of the model represented by expressions (5) and (5′) to decide whether or not this formalism is applicable to Na-co-transport systems such as the GABA uptake mechanism. Despite the spread of $[K]_o$ values (*see* Fig. 4), the exponential term in expression 6*a* may only vary over a limited range –

especially if β is very small and if V_m is not strictly proportional to log $[K]_o$; under these circumstances plots of log J versus log $[K]_o$ (or V_m) and of J versus log $[K]_o$ may both be linear, as we have observed (*see Results*).

Since the efflux expression in (5′) includes a $1-\beta$ term in the exponent (because, with $u<u_0$, the critical barrier peak is located near the external surface of the membrane; *cf.* Hall *et al.*, 1973), efflux should have a steeper dependence on V_m (or log $[K]_o$) than influx, according to the model. It may be possible to test this prediction if the depolarization-triggered Ca-dependent transmitter release (which presumably does not involve Na-co-transport) can be adequately inhibited with Ca-free media (*cf.* Cotman, Haycock & White, 1976). In fact, Redburn, Shelton & Cotman (1976) have recently described a Ca-independent GABA efflux which is enhanced by increasing $[K]_o$.

In conclusion, the data reported here are consistent with the idea that the membrane potential influences the rate of GABA uptake, as well as its steady-state distribution in GABA nergic nerve terminals. However, additional experiments will be required in order to characterize more completely the shape of the energy barrier for this transport process.

Note Added in Proof. Geck and Heinz (1976) have also recently examined some theoretical aspects of the influence of the membrane potential on the kinetics of co-transport systems. They, too, have based their analysis on the barrier model of Hall *et al.* (1973).

We are indebted to Prof. P. Läuger for fruitful discussions, to Drs. P. De Weer, N.C. Kendrick and R.F. Rakowski for their helpful comments concerning a preliminary draft of the manuscript, to G. Jerman for secretarial assistance, and to S. McConnell for preparing the graphs. Supported by U.S. Public Health Service NIH grant NS-08442.

References

Baker, P.F., Blaustein, M.P., Keynes, R.D., Manil, J., Shaw, T.I., Steinhardt, R. 1969. The ouabain-sensitive fluxes of sodium and potassium in squid giant axons. *J. Physiol.* (*London*) **200**:459

Balcar, V.J., Johnston, G.A.R. 1975. High affinity uptake of L-glutamine in rat brain slices. *J. Neurochem.* **24**:875

Beck, J.C., Sacktor, B. 1975. Energetics of the Na^+-dependent transport of D-glucose in renal brush border membrane vesicles. *J. Biol. Chem.* **250**:8674

Bennett, J.P., Jr., Mulder, A.H., Snyder, S.H. 1974. Neurochemical correlates of synaptically active amino acids. *Life Sci.* **15**:1045

Blaustein, M.P. 1974. The interrelationship between sodium ions and calcium fluxes across cell membranes. *Rev. Physiol. Biochem. Pharmacol.* **70**:33

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

Blaustein, M.P., Ector, A.C. 1975. Barbiturate inhibition of calcium uptake by depolarized nerve terminals *in vitro*. *Molec. Pharmacol.* **11**:369

Blaustein, M.P., Ector, A.C. 1976. Carrier-mediated sodium-dependent and calcium-dependent calcium efflux from pinched-off presynaptic nerve terminals (synaptosomes) *in vitro*. *Biochim. Biophys. Acta* **419**:295

Blaustein, M.P., Goldring, J.M. 1975. Membrane potentials in pinched-off presynaptic nerve terminals monitored with a fluorescent probe: evidence that synaptosomes have potassium diffusion potentials. *J. Physiol. (London)* **247**:589

Blaustein, M.P., Johnson, E.M., Jr., Needleman, P. 1972. Calcium-dependent norepinephrine release from presynaptic nerve endings *in vitro*. *Proc. Nat. Acad. Sci. USA* **69**:2237

Blaustein, M.P., Russell, J.M., De Weer, P. 1974. Calcium efflux from internally-dialyzed squid axons: the influence of external and internal cations. *J. Supramolec. Struct.* **2**:558

Blaustein, M.P., Wiesmann, W.P. 1970. Potassium ions and calcium fluxes in isolated nerve terminals. *In:* Cholinergic Mechanisms in the CNS, E. Heilbronn and A.P. Winter, editors. p. 291. Research Institute of National Defense, Stockholm

Bockris, J.O'M., Reddy, A.K.N. 1970. Modern Electrochemistry. Vol. 1. p. 387–398 and Vol. 2. p. 879–894. Plenum Press, New York

Bogdanski, D.F., Tissari, A., Brodie, B.B. 1968. Role of sodium, potassium, ouabain and reserpine in uptake, storage and metabolism of biogenic amines in synaptosomes. *Life Sci.* **7**:419 (Part I)

Bradford, H.F. 1969. Respiration *in vitro* of synaptosomes from mammalian cerebral cortex. *J. Neurochem.* **16**:675

Bretscher, M.S., Raff, M.C. 1975. Mammalian plasma membranes. *Nature (London)* **258**:43

Brinley, F.J., Jr., Mullins, L.J. 1974. Effects of membrane potential on sodium and potassium fluxes in squid axons. *Ann. N.Y. Acad. Sci.* **242**:406

Caldwell, P.C. 1969. Energy relationships and the active transport of ions. *Curr. Top. Bioenerg.* **3**:251

Colburn, R.W., Goodwin, F.K., Murphy, D.L., Bunney, W.E., Jr., Davis, J.M. 1968. Quantitative studies of norepinephrine uptake by synaptosomes. *Biochem. Pharmacol.* **17**:957

Colombini, M., Johnstone, R.M. 1974. Na^+-gradient-stimulated AIB transport in membrane vesicles from Ehrlich ascites cells. *J. Membrane Biol.* **18**:315

Cotman, C.W., Haycock, J.W., White, W.F. 1976. Stimulus-secretion coupling processes in brain: Analysis of noradrenaline and gamma-aminobutyric acid release. *J. Physiol. (London)* **254**:475

Curran, P.F., Schultz, S.C., Chez, R.A., Fuisz, R.E. 1967. Kinetic relations of the Na-amino acid interaction at the mucosal border of intestine. *J. Gen. Physiol.* **50**:1261

DeBelleroche, J.S., Bradford, H.F. 1972. The stimulus-induced release of acetylcholine from synaptosome beds and its calcium dependence. *J. Neurochem.* **19**:1817

Eisenman, G., Krasne, S., Ciani, S. 1975. The kinetic and equilibrium components of selective ionic permeability mediated by nactin- and valinomycin-type carriers having systematically varied degrees of methylation. *Ann. N.Y. Acad. Sci.* **264**:34

Elliott, K.A.C., van Gelder, N.M. 1958. Occlusion and metabolism of γ-aminobutyric acid by brain tissue. *J. Neurochem.* **3**:28

Escueta, A.V., Appel, S.H. 1969. Biochemical studies of synaptosomes *in vitro*. II. Potassium transport. *Biochemistry* **8**:725

Geck, P., Heinz, E. 1976. Coupling in secondary transport. Effect of electrical potentials on the kinetics of ion linked co-transport. *Biochim. Biophys. Acta* **443**:49

Geck, P., Heinz, E., Pfeiffer, B. 1974. Evidence against direct coupling between amino acid transport and ATP hydrolysis. *Biochim. Biophys. Acta* **339**:419

Gibb, L.E., Eddy, A.A. 1972. An electrogenic sodium pump as a possible factor leading to the concentration of amino acids by mouse ascites-tumour cells with reversed sodium ion concentration gradients. *Biochem. J.* **129**:979

Goldman, D.E. 1943. Potential, impedance and rectification in membranes. *J. Gen. Physiol.* **27**:37

Gray, E. G., Whittaker, V. P. 1962. The isolation of nerve endings from brain: An electron-microscopic study of cell fragments derived by homogenization and centrifugation. *J. Anat.* **96**:79

Gurd, J. W., Jones, L. R., Mahler, H. R., Moore, W. J. 1974. Isolation and partial characterization of rat brain synaptic plasma membranes. *J. Neurochem.* **22**:281

Hall, J. E., Latorre, R. 1976. Nonactin-K^+ complex as a probe for membrane asymmetry. *Biophys. J.* **16**:99

Hall, J. E., Mead, C. A., Szabo, G. 1973. A barrier model for current flow in lipid bilayer membranes. *J. Membrane Biol.* **11**:75

Haydon, D. A., Hladky, S. B. 1972. Ion transport across thin membranes: A critical discussion of mechanisms in selected systems. *Q. Rev. Biophys.* **5**:187

Hedqvist, P., Stjärne, L. 1969. The relative role of recapture and of de novo synthesis for the maintenance of neurotransmitter homeostasis in noradrenergic nerves. *Acta Physiol. Scand.* **76**:270

Heinz, E., Geck, P. 1974. The efficiency of energetic coupling between Na^+ flow and amino acid transport in Ehrlich cells—a revised assessment. *Biochim. Biophys. Acta* **339**:426

Heinz, E., Geck, P., Wilbrandt, W. 1972. Coupling in secondary active transport. Activation of transport by co-transport and/or counter-transport with the fluxes of other solutes. *Biochim. Biophys. Acta* **255**:442

Hodgkin, A. L., Katz, B. 1949. The effect of sodium ions on the electrical activity of the giant axon of the squid. *J. Physiol. (London)* **108**:37

Holtz, R. W., Coyle, J. T. 1974. The effects of various salts, temperature, and the alkaloids veratridine and batrachotoxin on the uptake of [^{3}H] dopamine into synaptosomes from rat striatum. *Molec. Pharmacol.* **10**:746

Iversen, L. L. 1971. Role of transmitter uptake mechanisms in synaptic transmission. *Br. J. Pharmacol.* **41**:571

Iversen, L. L. 1973. Catecholamine uptake processes. *Br. Med. Bull.* **29**:130

Iversen, L. L., Kravitz, E. A. 1968. The metabolism of γ-aminobutyric acid (GABA) in the lobster nervous system—uptake of GABA in nerve-muscle preparations. *J. Neurochem.* **15**:609

Iversen, L. L., Neal, M. J. 1968. The uptake of [^{3}H] GABA by slices of rat cerebral cortex. *J. Neurochem.* **15**:1141

Johnstone, R. M. 1975. Reversed transport of amino acids in Ehrlich cells. *Biochim. Biophys. Acta* **413**:252

Krnjević, K., Schwartz, S. 1967. Some properties of unresponsive cells in the cerebral cortex. *Exp. Brain Res.* **3**:306

Kuhar, J. M. 1973. Neurotransmitter uptake: A tool in identifying transmitter-specific pathways. *Life Sci.* **13**:1623

Laidler, K. J., Tweedale, A. 1971. The current status of Eyring's rate theory. *In:* Chemical Dynamics, Papers in Honor of Henry Eyring. J. O. Hirschfelder and D. Henderson, editors. *Adv. Chem. Phys.* **21**:113

Levi, G. 1972. Transport systems for GABA and for other amino acids in incubated chick brain tissue during development. *Arch. Biochem. Biophys.* **151**:8

Levi, G., Raiteri, M. 1973. GABA and glutamate uptake by subcellular fractions enriched in synaptosomes: critical evaluation of some δ methodological aspects. *Brain Res.* **57**:165

Levy, W. B., Haycock, J. W., Cotman, C. W. 1974. Effects of polyvalent cations on stimulus-coupled secretion of [^{14}C]-λ-aminobutryic acid from isolated brain synaptosomes. *Molec. Pharmacol.* **10**:438

Ling, C. M., Abdel-Latif, A. A. 1968. Studies on sodium transport in brain nerve-ending particles. *J. Neurochem.* **15**:721

Lowry, O. M., Rosebrough, N. J., Farr, A. L., Randall, R. J. 1951. Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* **193**:265

Marchbanks, R.M. 1968. The uptake of [^{14}C] choline into synaptosomes *in vitro*. *Biochem. J.* **110**:533

Martin, D.L. 1973. Kinetics of the sodium-dependent transport of gamma-aminobutyric acid by synaptosomes. *J. Neurochem.* **21**:345

Martin, D.L. 1976. Carrier-mediated transport and removal of GABA from synaptic regions. *In:* GABA in Nervous System Function. E. Roberts, T.N. Chase and D.B. Tower, editors. p. 347. Raven Press, New York

Martin, D.L., Smith, A.A., III. 1972. Ions and the transport of gamma-aminobutyric acid by synaptosomes. *J. Neurochem.* **19**:841

Mitchell, P. 1970. Reversible coupling between transport and chemical reactions. *In:* Membranes and Ion Transport. Vol. 1, p. 192. E.E. Bittar, editor. Wiley-Interscience, London

Mulder, A.H., Yamamura, H.I., Kuhar, M.J., Snyder, S.H. 1974. Release of acetylcholine from hippocampal slices by potassium depolarization: Dependence on high affinity choline uptake. *Brain Res.* **70**:372

Mullins, L.J., Brinley, F.J., Jr. 1975. Sensitivity of calcium efflux from squid axons to changes in membrane potential. *J. Gen. Physiol.* **65**:135

Murer, H., Hopfer, U. 1974. Demonstration of electrogenic Na^+-dependent D-glucose transport in intestinal brush border membranes. *Proc. Nat. Acad. Sci. USA* **71**:484

Neumcke, B., Läuger, P. 1969. Nonlinear electrical effects in lipid bilayer membranes. II. Integration of the generalized Nernst-Planck equation. *Biophys. J.* **9**:1160

Ohta, M., Narahashi, T., Keeler, R.F. 1973. Effects of veratrum alkaloids on membrane potential and conductance of squid and crayfish giant axons. *J. Pharmacol. Exp. Ther.* **184**: 143

Potter, L.T., Glover, V.A.S., Saelens, J.K. 1968. Choline acetyltransferase from rat brain. *J. Biol. Chem.* **243**:3864

Redburn, D.A., Shelton, D., Cotman, C.W. 1976. Calcium-dependent release of exogenously loaded γ-amino-[U-^{14}C] butyrate from synaptosomes: Time course of stimulation by potassium, veratridine and the calcium ionophore, A23187. *J. Neurochem.* **26**:297

Reid, M., Gibb, L.E., Eddy, A.A. 1974. Ionophore-mediated coupling between ion fluxes and amino acid absorption in mouse ascites-tumour cells. *Biochem. J.* **140**:383

Rose, R.C., Schultz, S.G. 1970. Alanine and glucose effects on the intracellular electrical potential of rabbit ileum. *Biochim. Biophys. Acta* **211**:376

Rose, R.C., Schultz, S.G. 1971. Studies on the electrical potential profile across rabbit ileum. Effects of sugars and amino acids on transmural and transmucosal electrical potential differences. *J. Gen. Physiol.* **57**:639

Rothstein, A., Cabantchik, Z.I., Knauf, P. 1976. Mechanism of anion transport in red blood cells: Role of membrane proteins. *Fed. Proc.* **35**:3

Schultz, S.G., Curran, P.F. 1970. Coupled transport of sodium and organic solutes. *Physiol. Rev.* **50**:637

Schultz, S.G., Curran, P.F., Chez, R.A., Fuisz, R.E. 1967. Alanine and sodium fluxes across mucosal border of rabbit ileum. *J. Gen. Physiol.* **50**:1241

Simon, J.R., Martin, D.L., Kroll, M. 1974. Sodium-dependent efflux and exchange of GABA in synaptosomes. *J. Neurochem.* **23**:981

White, J.F., Armstrong, W.McD. 1971. Effect of transported solutes on membrane potentials in bullfrog small intestine. *Am. J. Physiol.* **221**:194

White, T.D., Keen, P. 1970. The role of internal and external Na^+ and K^+ on the uptake of ^{3}H-norepinephrine by synaptosomes prepared from rat brain. *Biochim. Biophys. Acta* **196**:285