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Modulation of Neurotransmitter Transport by the Activity of the Action Potential Sodium Ion Channel in Membrane Vesicles from Rat Brain†

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ABSTRACT: (1) Transport of the neurotransmitters γ-aminobutyric acid (GABA) and L-glutamic acid was measured in isolated membrane vesicles derived from rat brain, with an artificially imposed electrochemical gradient of sodium ions (out > in) as a major driving force. Both transport processes were strongly inhibited by the alkaloid neurotoxin veratridine. Tetrodotoxin, which by itself had a slight stimulatory effect, completely reversed the inhibition by veratridine. (2) The degree of inhibition of neurotransmitter transport by veratridine was strongly dependent on the nature of the internal cation. With internal Tris or lithium ions inhibition by the neurotoxin was relatively poor as compared to the case when internal potassium was used. The parameter was not dependent on the nature of either the internal or the external anion. (3) The membrane vesicles catalyzed the uptake of $^{22}Na^+$ which was 3-10-fold enhanced by veratridine. This effect was completely reversed by tetrodotoxin. The veratridine-stimulated sodium ion uptake obeyed Michaelis-Menten kinetics [apparent K_m = 11 mM, V_{max} = 150 nmol/(min mg of protein)]. Vesicles which had been allowed to accumulate $^{22}Na^+$ rapidly lost their radioactivity upon dilution into sodium-containing media, provided nigericin was present during dilution. (4) Veratridine-dependent sodium ion accumulation was also highly dependent on the nature of the internal cation, Tris and lithium being relatively poor in comparison with potassium. The nature of either the internal or the external anion was not important. (5) The concentration dependence of the inhibiting effect of veratridine on GABA transport paralleled that of its stimulatory effect on $^{22}Na^+$ uptake (the half-maximal effects ranged from 20 to 30 μM). A similar parallel was found between the reversal of the effects of veratridine on both processes by tetrodotoxin (half-maximal effects between 10 and 20 nM). (6) It is concluded that in the isolated membrane vesicles functionality of the action potential Na^+ channels is preserved and that, in rat brain, the sodium-coupled neurotransmitter high-affinity uptake systems for GABA and L-glutamic acid in the vesicles originate predominantly from the presynaptic plasma membrane.

High-affinity sodium-dependent uptake systems for a wide range of neurotransmitters have been implicated in termination of transmitter action on postsynaptic receptors (Iversen, 1971) as well as in maintaining constant levels of transmitters in the neurons (Hedgvist & Stjärne, 1969). These uptake systems have been identified in several types of brain preparations such as synaptosomes (Iversen, 1971, 1973; Kuhar, 1973; Bennett et al., 1974). Various observations have led to the proposal that ion gradients, generated primarily by devices such as the $(Na^+ + K^+)$-ATPase, are the immediate driving force for neurotransmitter accumulation (Bogdanski et al., 1968; Martin & Smith, 1972; Holtz & Coyle, 1974). Recently, this idea has been supported more directly by using membrane vesicles isolated after osmotic shock of synaptosomal preparations

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(Kanner, 1978; Kanner & Sharon, 1978). Thus, active accumulation of GABA[1] (Kanner, 1978) or L-glutamic acid (Kanner & Sharon, 1978) has been demonstrated with artificially imposed ion gradients present as the sole sources of energy. The major driving force is the electrochemical gradient for sodium ions (out > in), but additional driving forces such as a chloride ion gradient (out > in) in the case of GABA (Kanner, 1978) or a potassium ion gradient (in > out) in the case of L-glutamic acid (Kanner & Sharon, 1978) also play a role.

The movement of sodium ions into excitable cells via the sodium ion channel plays a central role in the generation and conduction of the action potential (Hodgkin, 1963; Cole, 1968). The neurotoxins aconitine, veratridine, grayanotoxin, and batrochotoxin cause repetitive action potentials and persistent depolarization of nerve terminals, apparently by opening the channel (Herzog et al., 1964; Ulbricht, 1969; Seyama & Narahashi, 1973; Albuquerque et al., 1971; Catterall, 1977). Tetrodotoxin, a specific inhibitor of the sodium ion current during the action potential, blocks the channel (Narahashi et al., 1964). Also, in synaptosomes, a preparation which retains many properties of the intact nerve cells, functional sodium channels have been detected. Thus, veratridine induced alterations in the membrane potential which were blocked by TTX (Blaustein & Goldring, 1975). Furthermore, veratridine enhanced sodium fluxes by a process inhibited by TTX (Li & White, 1977). The effects of veratridine and TTX on synaptosomal noradrenaline (White, 1977) or GABA (Blaustein & King, 1976) transport have also been taken as evidence that the latter processes are coupled to sodium transport.

In view of the above observations, it was of considerable interest whether the functionality of the sodium channel is preserved in the membrane vesicles obtained from synaptosomal fractions after osmotic shock. If the functional sodium-coupled neutotransmitter transport systems in the membrane vesicles indeed originate from nerve ending membranes, the opening and closing of the sodium ion channels would alter the electrochemical potential gradient for sodium ions.

In this communication we provide direct evidence for the presence of functional sodium channels which are located in the membrane of those vesicles which are capable of accumulating neurotransmitters. In addition, the ion dependence of the activity of the channel has been explored.

Experimental Procedure

Methods

Preparation of Membrane Vesicles. Membrane vesicles were prepared as described (Kanner, 1978) except that adult female rats (200–300 g) were used as a starting material and 20 mL of homogenization medium was used per brain. Although qualitatively similar results were obtained with 14-day-old rats, the effects of veratridine were found more pronounced and less variable by using adult rat brains as a starting material. After Ficoll gradient centrifugation the 12–16% interface was collected in addition to the 8–12% interface. After osmotic shock membrane vesicles were isolated, from both fractions separately, as described (Kanner, 1978). With regard to $^{22}Na^+$ flux experiments, the specific activity of the two types of membrane vesicles were similar and they behaved identically with regard to all parameters used. For neurotransmitter transport all experiments were performed with the vesicles derived from the 8–12% interface. Neurotransmitter transport in vesicles derived from adult rats was very similar to that in vesicles from 14-day-old rats. Protein was determined according to the Lowry method (Lowry et al., 1951).

Transport Assays. Transport of GABA (Kanner, 1978) and of L-glutamate (Kanner & Sharon, 1978) was measured as described. Transport of $^{22}Na^+$ was measured as follows. Membrane vesicles were rapidly thawed at 37 °C and loaded as described (Kanner, 1978). The composition of the loading medium was 0.1 M potassium phosphate (pH 6.8) and 1 mM $MgSO_4$, unless stated otherwise in the legends to the figures and tables. The loaded vesicles were resuspended in the loading medium at a protein concentration of 10–15 mg/mL. Of the latter suspension, 10 μL was added to 190 μL of 0.1 M choline chloride and 0.2–0.4 μCi of $^{22}Na^+$ (263 Ci/g). Since no lag in the action of veratridine or TTX was detected, all inhibitors, toxins, and ionophores were usually added to the choline chloride containing medium only. After incubation at various times at room temperature (20–23 °C), reactions were terminated by addition of 2 mL of ice-cold 0.1 M choline chloride and the reaction mixtures were filtered through membrane filters (Schleicher and Schuell, 0.45-μm pore size). The filters were then washed once with 2 mL of the choline chloride solution. Stopping the reaction, filtration, and washing took ~15 s. The washed filters were dried and counted by using liquid scintillation counting. All experimental values were corrected for by subtracting zero-time values; in the case of zero-time measurements, membrane vesicles were added after stopping the solution. For veratridine-dependent sodium transport, values of transport in the absence of veratridine were subtracted from those in its presence.

Materials

[2,3-$^3H_2$]GABA was obtained from New England Nuclear, and L-[G-^{3}H]glutamic acid was from Amersham. $^{22}Na^+$ was from either of these sources. Valinomycin, CCCP, veratridine, and tetrodotoxin were purchased from Sigma Chemical Co. Grayanotoxin I and nigericin were generously donated by Professor T. Narahashi from Northwestern University and Dr. R. J. Hosley from Eli Lilly, respectively. DiS-C_3-(5) was graciously supplied by Dr. A. Waggoner, Amherst College. Veratridine and grayanotoxin were dissolved in dimethyl sulfoxide (Me_2SO). Tetrodotoxin was dissolved in water. No effects were obtained in control experiments performed with Me_2SO or with citrate (present with the tetrodotoxin). All other materials were of the highest purity commercially available.

Results

The data presented in Figure 1A illustrate the uptake of GABA into the membrane vesicles under optimal conditions (Kanner, 1978); both a sodium ion gradient (out > in) as well as a chloride ion gradient (out > in) serve as driving forces. The uptake is inhibited by veratridine (25 μM), and the inhibition increases progressively with time up to 50% (Figure 1A). In addition, it is shown that TTX (1 μM), which by itself has only a small stimulatory effect, almost completely reverses the inhibition caused by veratridine. When the predominant driving force for transport is the sodium ion gradient, TTX reversible inhibition by veratridine is somewhat larger (Figure 1 B). On the other hand, with the chloride ion gradient (out > in) as the major driving force, this inhibition is much smaller (Figure 1C). In some experiments no inhibition at all was observed under this condition (see also Table I). These data

[1] Abbreviations used: GABA, γ-aminobutyric acid; TTX, tetrodotoxin; ATPase, adenosine triphosphatase; CCCP, carbonyl cyanide *m*-chlorophenylhydrazone; Tris, tris(hydroxymethyl)aminomethane; Tricine, *N*-tris(hydroxymethyl)methylglycine; Mes, 2-(*N*-morpholino)ethanesulfonic acid; diS-C_3-(5), 3,3′-dipropylthiodicarbocyanine.

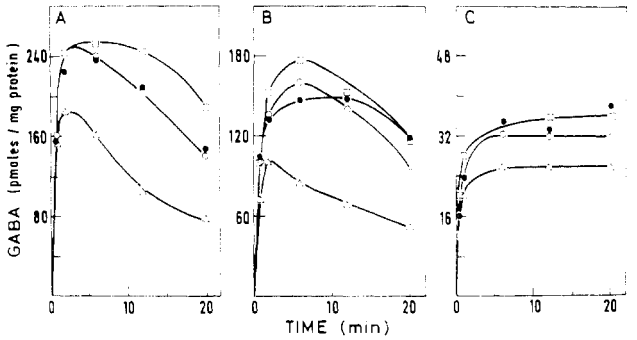

FIGURE 1: Effects of veratridine and TTX on GABA transport. Transport was measured as described under Methods. The vesicles were loaded with 0.1 M potassium phosphate and 1 mM $MgSO_4$ (panel A), 90 mM potassium chloride, 10 mM potassium phosphate, and 1 mM $MgSO_4$ (panel B), or 100 mM sodium phosphate and 1 mM $MgSO_4$ (panel C). The external medium contained 0.1 M NaCl + 0.56 μM [^{3}H]GABA (0.4 μCi). The amount of membrane protein used per assay was 37.4 (A), 35.4 (B), or 45 μg (C). Additions: none (○); veratridine, 25 μM (△); TTX, 1 μM (□); veratridine, 25 μM; and TTX, 1 μM (●).

Table I: Effect of the Composition of the Loading Medium on Veratridine-Sensitive GABA Transport[a]

	GABA transport (pmol/mg)		
loading medium	control	veratridine	TTX
potassium phosphate	210	119	224
lithium phosphate	72	56	72
Tris-phosphate	93	84	119
sodium phosphate	25	23	28

[a] GABA transport was measured at 6 min as described under Methods. Membrane vesicles were loaded with the indicated media at 0.1 M concentration, containing in addition 1 mM $MgSO_4$. Veratridine at 25 μM and TTX at 1 μM were present where indicated.

suggest that functional sodium ion channels are present in the same vesicles catalyzing GABA transport and that their opening results in an inhibition of GABA transport. When higher veratridine concentrations are used, inhibitions of 70–80% are routinely observed (see also Figure 6, lower left panel). The residual GABA transport at high veratridine concentrations is most likely due to the concentration gradient of chloride (out > in), which has been shown to be a driving force for transport in addition to the sodium gradient (out > in) (Kanner, 1978). In the presence of either valinomycin or CCCP, the percentage of inhibition by veratridine was rather similar to that in the absence of the respective ionophores (data not shown). Thus, it appears that the flow of ions, to preserve electroneutrality during dissipation of the sodium ion gradient, is not a rate-limiting factor in the latter process.

The effects of veratridine and TTX are not restricted to GABA only. Transport of L-glutamic acid is also inhibited by veratridine (Figure 2). However, under standard conditions the effect is not noticeable on the maximum level of uptake, but after ~2 min the onset of an enhanced efflux in the presence of veratridine is noted (data not shown). In the L-glutamic acid system (Kanner & Sharon, 1978) the maximum extent of its uptake is reached much faster than in the GABA system (within 30 s). It seems likely that at these short times, not much dissipation of the sodium ion gradient occurs. This contention is supported by the experiments depicted in Figure 2. The membrane vesicles are diluted into the assay medium containing veratridine but without the solute. After the dissipation of the sodium ion gradient was allowed for 4 min, the transport reaction was started with radioactivity labeled L-glutamate. Under these conditions, the inhibition

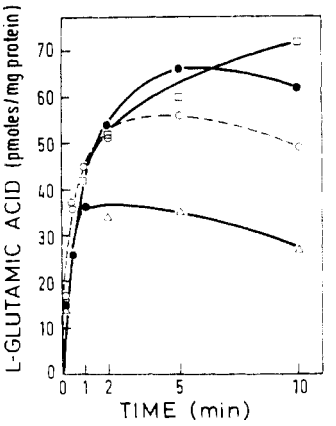

FIGURE 2: Effects of veratridine and TTX on L-glutamic acid transport. Membrane vesicles were loaded with 0.1 M potassium phosphate + 1 mM $MgSO_4$, and 20 μL of the suspension, 42.2 μg of protein, was diluted into 0.1 M NaCl containing no additions (○), veratridine, 25 μM (△), TTX, 1 μM (□), or both veratridine, 25 μM, and TTX, 1 μM (●). After 4 min, t = 0, transport reactions were initiated by addition of L-[^{3}H]glutamic acid (0.02 μM, 0.4 μCi). The rest of the transport assay was conducted as described under Methods.

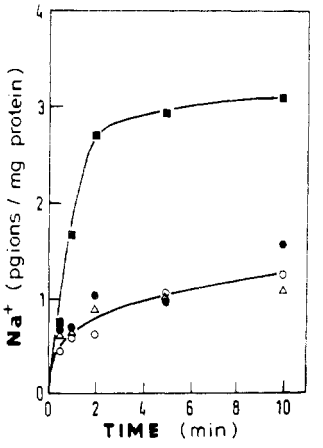

FIGURE 3: Effects of veratridine and TTX on sodium ion transport. Transport of $^{22}Na^+$ was performed as described under Methods. Membrane vesicles (59 μg of protein) were diluted into reaction media containing no additions (○), veratridine, 25 μM (■), TTX, 1 μM (△), or both veratridine, 25 μM, and TTX, 1 μM (●). The same concentrations of toxins were also added to the vesicles prior to the dilution into the reaction media.

was already detected at the early time points and also affected the maximum extent of transport. In contrast to L-glutamate transport, the extent of inhibition of GABA transport was affected much less by preincubation of the vesicles with veratridine in the reaction medium.

The TTX-sensitive inhibition of neurotransmitter transport is dependent on the nature of the internal cation. Although GABA transport is efficiently inhibited by veratridine when the vesicles are loaded with potassium ions, the inhibition by the neurotoxin is rather poor when the loading medium contains Tris, lithium, or sodium ions (Table I). One possible explanation for this observation is that whereas the outward movement of potassium is not rate limiting for the dissipation of the sodium gradient, this may be the case with the other ions. However, it seems unlikely that these effects are due to a decreased permeability of the latter ions as compared to potassium, since similar effects are also observed in the presence of the proton conductor CCCP (data not shown). When the internal anion phosphate was replaced by Mes or Tricine, similar inhibitions by veratridine on GABA transport were noted. When the external anion chloride was isoos-

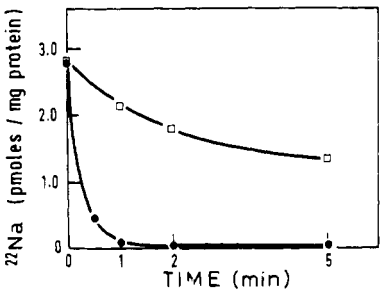

FIGURE 4: Influx and subsequent efflux of $^{22}Na^+$. Membrane vesicles (91 μg of protein per time point) were allowed to accumulate $^{22}Na^+$ for 5 min in the presence of 25 μM veratridine. Subsequently, TTX (1 μM) was added and after 1 min of additional incubation the vesicles were diluted into 2 mL of 0.1 M NaCl with (●) or without (□) nigericin (5 μM final concentration). At the times indicated (time of dilution $t = 0$) the incubation mixtures were filtered and washed with 2 mL of ice-cold 0.1 M choline chloride. Zero-time values (vesicles were added to ice-cold NaCl containing all the additions, filtered, and washed with choline chloride) were subtracted from the experimental values.

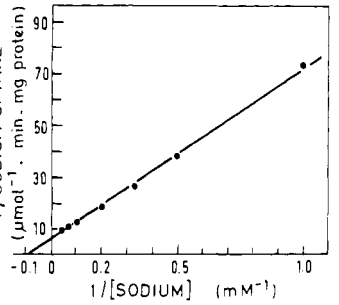

FIGURE 5: Kinetics of veratridine-dependent sodium ion transport. Transport assays were performed as described under Methods. Increasing sodium ion concentrations were obtained by replacing part of the choline chloride by sodium chloride (keeping the sum at 100 mM). 126 μg of membrane protein was used per assay, and 0.4 μCi of carrier-free $^{22}Na^+$ was also present. In order to determine the initial velocity, transport was measured at time zero and at 30 s without and with 100 μM veratridine. The difference between transport in the presence and absence of veratridine is taken to be veratridine-dependent sodium transport. At all veratridine concentrations tested (cf. Figure 6) $^{22}Na^+$ transport proceeded linearly upto 30 s and without any noticeable lag, even without prior preincubation of the vesicles with the veratridine.

motically replaced by sulfate, phosphate, or glucuronate, inhibition of L-glutamic acid transport by veratridine was hardly altered (GABA transport requires external chloride).

Direct uptake measurements with $^{22}Na^+$ provide additional evidence for the presence of functional action potential sodium ion channels in the membrane vesicles (Figure 3). The uptake is about threefold enhanced by veratridine, and this stimulation is abolished by TTX (1 μM). The latter toxin by itself does not have much effect on the influx of $^{22}Na^+$. Therefore, it seems that only a small fraction of the sodium channels are open in the untreated vesicles. The $^{22}Na^+$ taken up exchanges slowly with unlabeled sodium ions upon dilution (Figure 4). On the other hand, when nigericin, an ionophore capable of exchanging sodium ions across the membrane, is present in the dilution medium, an extremely rapid loss of the radioactivity is observed (Figure 4). This suggests that the sodium taken up in fact is transported across the membrane.

The absolute rates of sodium ion transport in these experiments are rather low, since low sodium concentrations are employed. The veratridine-dependent influx of $^{22}Na^+$ exhibits saturation kinetics. Reasonably similar to results obtained with neuroblastoma cells (Catterall, 1977), uptake was linear up to 2 to 3 mM. From Lineweaver–Burk plots a K_m of 11 mM and a V_{max} of 150 nmol/(min mg) have been determined

Table II: Effect of the Composition of the Loading Medium on Veratridine-Stimulated Sodium Fluxes[a]

loading medium	veratridine-stimulated $^{22}Na^+$ flux [pmol/(min mg)]	
	−CCCP	+CCCP
potassium phosphate	1.26	1.23
Tris–phosphate	0.36	0.30
lithium phosphate	0.26	0.19
sodium phosphate	1.03	0.80

[a] Membrane vesicles were loaded with the indicated media at 0.1 M. All media also contained 1 mM $MgSO_4$. Veratridine (25 μM) dependent $^{22}Na^+$ transport was measured after 1 min in the absence or presence of 10 μM CCCP. In order to allow for a direct comparison between vesicles loaded with the various media, the concentration of unlabeled sodium carried over with the sodium-loaded vesicles has not been taken into account for the calculation of the specific activity of the $^{22}Na^+$ flux.

Table III: Effect of Internal and External Anions on Veratridine-Stimulated Sodium Ion Fluxes[a]

external medium	loading medium	veratridine-stimulated $^{22}Na^+$ flux (% of control)
choline chloride	potassium phosphate	100
Tris-chloride	potassium phosphate	74
Tris-sulfate	potassium phosphate	74
Tris-phosphate	potassium phosphate	81
choline chloride	K-Mes	84
choline chloride	K-Tricine	51

[a] Membrane vesicles were loaded with the indicated media at 0.1 M loading media that also contained 1 mM $MgSO_4$. They were subsequently diluted into 0.1 M (with respect to the cation) of the indicated external media containing 0.2 μCi of carrier-free $^{22}Na^+$. The osmolarity of the Tris–sulfate and Tris–phosphate media was adjusted with sucrose (50 and 33 mM, respectively). Transport was assayed as described under Methods.

(Figure 5). The V_{max} is in the same order of magnitude as that obtained with lobster channels incorporated in liposomes (Villegas et al., 1977).

Sodium influx mediated by veratridine also is a process which is dependent on the nature of the cations present in the vesicles' interior. A marked decrease in the rate of veratridine-dependent $^{22}Na^+$ influx is noted when internal sodium or potassium ions are replaced by either Tris or lithium (Table II). With addition of the membrane vesicles to the reaction mixtures, ions of the loading solution are carried over. This results in low concentrations of these ions in the external medium. Control experiments, using as an internal medium either 95 mM choline chloride and 5 mM Tris–chloride or 95 mM choline chloride and 5 mM lithium chloride, result in 0 and 25% inhibition, respectively, as compared with 100 mM choline chloride. Replacement of the choline chloride by 100 mM Tris–chloride yields an inhibition of only 26% of the influx rate of sodium ions (Table III). This indicates that Tris or lithium ions at the inside of the vesicles result in a decreased rate of sodium influx through the sodium channels. These decreased rates persist in the presence of the proton conductor CCCP (Table II). Since the amount of sodium ions transported is very small, it is likely that in the presence of CCCP there is no limitation of the sodium flux by the outward movement of the internal cations of the loading medium. The inhibition of 25% observed with 5 mM external lithium or sodium (see also legend to Table II) can be accounted for by the saturability of the sodium channel (Figure 5) and the fact that lithium traverses the channel almost as efficiently as

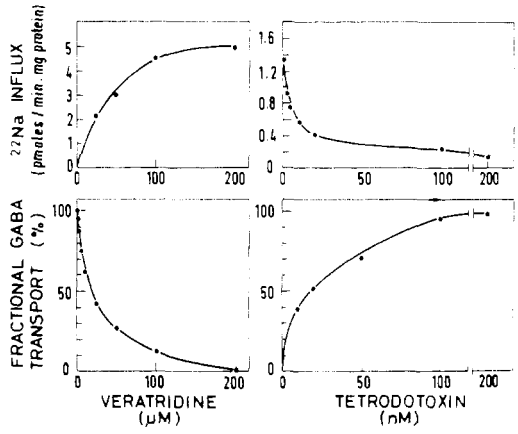

FIGURE 6: Concentration dependence of inhibition of GABA transport and stimulation of sodium transport by veratridine and that of the reversal of both processes by TTX. Sodium ion and GABA transport were measured as described under Methods. The indicated amounts of veratridine or TTX were present during the assays only. Fractional GABA transport measured after 6 min of transport (veratridine curve) represents the transport at the indicated veratridine concentrations minus that at 200 μM inhibitor divided by the difference in GABA transport at zero and 200 μM, respectively. Fractional GABA transport in the case of TTX titration was determined with 25 μM veratridine present in all assays. It is defined as transport in the presence of the indicated concentration of TTX minus that in its absence divided by the difference in transport at 1 μM TTX and no TTX. The values of sodium fluxes measured at 30 s for the veratridine titration reflect veratridine-sensitive transport. In the case of the TTX titration, veratridine-sensitive $^{22}Na^+$ transport, using 25 μM veratridine in the presence of the indicated TTX concentration, is recorded.

sodium (Hille, 1970). No marked changes in veratridine-dependent $^{22}Na^+$ transport are seen when either the external or the internal anions are varied (Table III).

The concentration dependence of the inhibition of the extent of GABA transport by veratridine (half-maximal effect 18 μM) is similar to that of the initial rate of veratridine-dependent $^{22}Na^+$ transport (half-maximal effect 30 μM) (Figure 6). Similarly, a parallel is observed for the reversal of the effect of veratridine on both processes by TTX (Figure 6). Half-maximal effects were reached at 17 nM for transport of GABA and at 10 nM for transport of $^{22}Na^+$. These concentration dependencies for both toxins are very similar to those on $^{22}Na^+$ transport observed using excitable neuroblastoma cells (Catterall, 1977). They are in the same order of magnitude as data on sodium fluxes in the reconstituted system (Villegas et al., 1977). It is of interest to note that grayanotoxin I is much less potent in our system than veratridine. The grayanotoxin-dependent rate of $^{22}Na^+$ transport was between 5 and 10% of that of the veratridine-dependent rate (both toxins were tested at 200 μM). The striking parallel of the concentration dependencies of either toxin on both processes (Figure 6) provides additional evidence for (a) the notion that action potential sodium ion channels are present in those vesicles catalyzing sodium-coupled neurotransmitter transport and (b) that opening of the channels provokes enhanced dissipation of the electrochemical gradient for sodium ions, resulting in inhibition of neurotransmitter transport.

Discussion

Functional action potential Na^+ channels are present in synaptosomes (Blaustein & Goldring, 1975; Li & White, 1977; White, 1977; Blaustein & King, 1976). In this communication we have shown that this functionality is preserved in membrane vesicles isolated from synaptosomal fractions by osmotic lysis (Figures 3 and 4). The isolated membrane vesicles, which are devoid of endogenous substrates or subcellular organelles, thus may represent a very useful tool for future investigations of the properties of this important channel.

In this study we have investigated the link between the activity of the Na^+ channel and sodium-coupled neurotransmitter transport. Veratridine, which opens the channel, inhibits transport both of GABA (Figure 1) and of L-glutamic acid (Figure 2). On the other hand, tetrodotoxin, which closes the channel, reverses the inhibition caused by veratridine (Figures 1 and 2). GABA transport is an electrogenic process which can be driven by two independent driving forces, the gradients of sodium and chloride (both out > in; Kanner, 1978). When transport of GABA was investigated, it appeared that the inhibition by veratridine is much more pronounced under conditions when the predominant driving force is the sodium gradient as compared to that of chloride (Figure 1). Thus, this inhibition by veratridine is caused predominantly because of its effect on the Na^+ channels. There is a similar concentration dependence of inhibition of GABA transport and stimulation of sodium fluxes by veratridine. The same is true for the concentration dependence of TTX on the reversal of the effect of veratridine on both processes (Figure 6). All of these observations, taken as a whole, strongly suggest that opening of the channels by veratridine results in enhanced dissipation of the electrochemical sodium ion gradient which in turn results in inhibition of neurotransmitter transport.

Although with GABA transport a small inhibition by veratridine was often noted at early times, it is clear that the inhibition increases progressively with time (Figure 1). In the case of L-glutamic acid transport, which is much faster than GABA transport (Kanner & Sharon, 1978; Kanner, 1978), inhibition by veratridine of the maximal extent was only noted when the vesicles were diluted in the sodium-containing assay medium several minutes prior to initiation of transport. These observations strongly suggest that under our experimental conditions bulk dissipation of the sodium gradient is required rather than an effect on the membrane potential. The latter would be instantaneous. When studying $^{22}Na^+$ fluxes, no evidence was found for the alternative possibility to explain the above data—a lag in the action of veratridine on the sodium channels (Figure 3). The contention that veratridine inhibits neurotransmitter transport by dissipation of the sodium gradient is further supported by the observation that similar inhibition by veratridine was also seen in the presence of the proton conductor CCCP. Under these conditions, with equal proton concentrations present on both sides of the membrane, it is likely that any preexisting membrane potential is collapsed by the ionophore. Moreover, by use of the cyanine dye diS-C_3-(5) (Sims et al., 1974), membrane potentials (interior negative) were detected but no effects by veratridine or TTX were observed (data not shown). The interpretation of the latter negative result, however, is not straightforward because of the possible heterogeneity of the vesicle preparation. The dye is expected to interact with all vesicles while only part of them may contain functional sodium channels.

On the other hand, with intact synaptosomes maximal inhibition of GABA transport by veratridine was already detected at the earliest time points measured (Blaustein & King, 1976). It was therefore concluded that the inhibition was a result of collapse of the membrane potential (Blaustein & King, 1976). One of the possible reasons for the difference between the mode of inhibition of veratridine on GABA transport in isolated membrane vesicles as compared to intact synaptosomes is that in the latter case the sodium ion gradient is not artificially imposed but generated and maintained by the $(Na^+ + K^+)$-ATPase. A dissipation of the sodium gradient

will result in enhanced activity of this pump, while under the experimental conditions used with the vesicles this ATPase is not operative. An alternative possibility is that in the isolated vesicles the contribution of the membrane potential to the overall driving force for GABA transport may be smaller than in intact synaptosomes.

The above experiments indicate that a large majority of both GABA and glutamate transport systems are located in membrane vesicles also containing the sodium channels. Thus, it seems that in rat brain these transport systems are by and large located in the presynaptic plasma membrane rather than in glial cell membranes. For the case of GABA this is in harmony with similar experiments performed on synaptosomal preparations (Blaustein & King, 1976) as well as with autoradiographic studies (Iversen & Bloom, 1972).

Another interesting finding of these studies is the apparent dependence, even in the presence of CCCP, of the activity of the sodium channel on internal ions (Tables I and II). This activity is seriously hampered in the presence of internal Tris or lithium ions (Table II). This phenomenon probably also explains the relatively poor inhibitions of GABA transport by veratridine with either internal Tris or lithium ions present (Table I). On the other hand, the poor inhibition of GABA transport in the presence of internal sodium ions (Table I) apparently results from the fact that in this case the major driving force is the chloride ion gradient (out > in) rather than the sodium ion gradient (out > in); with internal sodium ions the activity of the channel is not impaired (Table II). It is not clear at present whether the effect of inhibition by internal ions such as Tris and lithium is due to their inhibitory action or if sodium or potassium promotes stimulation of activity of the channel by acting on a site exposed from the inside of the membrane.

It appears that the effects of veratridine and TTX on neurotransmitter transport provide additional support for the notion that these systems are sodium-coupled (Kanner, 1978; Kanner & Sharon, 1978). The presence of functional sodium ion channels in this preparation will hopefully give a stimulus to experiments directed toward the goal of providing a better insight in the mechanism of action of the action potential sodium ion channel.

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