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L-GLUTAMATE EFFECTS ON ELECTRICAL POTENTIALS OF SYNAPTIC PLASMA MEMBRANE VESICLES

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The electrogenic nature of the L-glutamate-stimulated Na^+ flux was examined by measuring the distribution of the lipophilic anion [^{35}S]thiocyanate (SCN^-) into synaptic membrane vesicles that were incubated in a NaCl medium. Concentrations of L-glutamate from 10^{-7} to 10^{-4} M added to the incubation medium caused an enhanced intravesicular accumulation of SCN^-. Based on the SCN^- distribution in synaptic membrane vesicles it was calculated that 10 μM L-glutamate induced an average change in the membrane potential of +13 mV. L-Glutamate enhanced both the Na^+ and K^+ conductance of these membranes as determined by increases in SCN^- influx. Other neuroexcitatory amino acids and amino acid analogs (D-glutamate, L-aspartate, L-cysteine sulfinate, kainate, ibotenate, quisqualate, *N*-methyl-D-aspartate, and DL-homocysteate) also increased SCN^- accumulation in synaptic membrane vesicles. These observations are indicative of the activation by L-glutamate and some of its analogs of excitatory amino acid receptor ion channel complexes in synaptic membranes.

Resealed synaptic plasma membrane vesicle preparations from brain tissue are thought to consist largely of presynaptic membrane sacs and of a few postsynaptic membrane vesicles. These plasma membrane fractions apparently retain a high degree of functional and structural integrity. This conclusion is based on the demonstration that the transport systems for the putative neurotransmitters L-glutamic acid and γ-aminobutyric acid are as active in these membrane preparations as they are in the intact nerve ending particles (synaptosomes) [1–3]. The resealed synaptic membranes also retain a Na^+-Ca^{2+} antiporter activity which is almost equal to that seen in brain synaptosomes [4]. In addition, we have shown that these neuronal plasma membrane subfractions are enriched in L-[^{3}H]glutamic acid binding sites [5,6] and that exposure of these membranes to the neuroexcitatory amino acid L-glutamate brings about a rapid stimulation of Na^+ flux across the vesicular membrane [7,8]. It is for this reason that we have used these membranes as a model system for the study of the organization and functional characteristics of the putative neuronal receptor ion channel complex for L-glutamic acid.

L-Glutamic and L-aspartic acid have been shown to cause transient membrane conductance for Na^+, K^+ and Ca^{2+} in vertebrate neurons and in invertebrate muscle cells which lead to electrical depolarization of these cells [9–15]. In order for L-glutamic acid to produce a membrane depolarization in these cells, one would have to assume that the flux of cations, such as Na^+, across the plasma membrane is an electrogenic process. If the same conditions also prevail in the isolated synaptic plasma membrane vesicle preparations which

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we have been using, then the stimulation of Na^+ influx by L-glutamate should bring about a change in the vesicle membrane electrical potential ($\Delta\Psi$) such that the intravesicular compartment becomes more positive.

Changes in the membrane $\Delta\Psi$ of synaptosomal preparations and of other biological systems have been measured through the use of voltage-sensitive fluorescent probes (see, for example, Ref. 16) or through changes in the distribution of radioactively labeled lipophilic ions (see, for example, Refs. 17, 18). The distribution of the radioactively labeled lipophilic anion thiocyanate (SCN^-) depends on the electrochemical gradients across the membrane and has been used in some studies as a measure of the presence of an interior positive $\Delta\Psi$ in chromaffin granule ghosts [19] and in the plasma membrane vesicles of neurospora [20]. The $\Delta\Psi$ in such membrane preparations can be calculated from the distribution of this lipophilic anion according to the Nernst equation

$$\Delta\Psi = (RT/nF)\ \ln([SCN^-]_{in}/[SCN^-]_{out}).$$

This lipophilic anion was also employed to demonstrate the development of positive $\Delta\Psi$ in intact neurons in culture following either exposure of the neurons to elevated $[K^+]$ or activation of Na^+ membrane conductance by veratridine [17]. Since the purpose of the present study was to determine the possible electrogenic nature of the enhanced Na^+ flux caused by exposure of the synaptic plasma membranes to L-glutamic acid and to various glutamate analogs, the accumulation of [^{35}S]thiocyanate by synaptic membrane vesicles was used as a measure of the development of a transmembrane $\Delta\Psi$ (interior positive) in these membrane preparations. In addition, the possible contribution to the membrane $\Delta\Psi$ of changes in ion fluxes for K^+ and Cl^-, as well as for Na^+, were evaluated by this technique.

Materials and Methods

Materials

[^{35}S]Thiocyanate (30 Ci/mol) was purchased from New England Nuclear, Boston, MA. Valinomycin, L-glutamine, L-aspartic acid, kainic acid, glycine, γ-aminobutyric acid, *N*-methyl-D-aspartate, ibotenic acid, DL-homocysteic acid and L-glutamic acid were obtained from Sigma Chemical Co., St. Louis, MO. All other chemicals were reagent grade.

Preparation of synaptic membrane vesicles

Membrane vesicles from adult, male Sprague-Dawley rats were prepared and stored as previously described [7]. Under these conditions the L-[^{3}H]glutamate binding activity and the glutamate-induced Na^+ uptake process of the vesicles was stable for 3–4 weeks. The amount of protein employed in all tissue preparations and assays was measured by the Lowry procedure [21].

[^{35}S]*Thiocyanate uptake assays*

The synaptic plasma membranes used for the SCN^- uptake assays were rapidly thawed at 37°C and internally loaded with a solution of 0.32 M sucrose/10 mM Tris-H_2SO_4/0.5 mM EDTA (pH 7.4) (sucrose buffer), according to the procedures described previously [7]. The final protein concentration of the membrane suspension was 2 mg/ml. Aliquots (20 μl) of this membrane vesicle suspension were diluted at room temperature into 100 μl of the incubation medium which contained 100 mM NaCl/5 mM Tris-H_2SO_4/1 mM $MgSO_4$/1 mM NaSCN/120 mM sucrose (pH 7.4)/1 μCi K^{35}SCN, the latter being added as a tracer. A lower SCN^- concentration (100 μM) was also used in many experiments. The results obtained were similar to those determined in the presence of 1 mM SCN^-, but SCN^- uptake was smaller and less stable under the conditions of reduced SCN^- concentration as compared to the values obtained with 1 mM SCN^-. Various concentrations of L-glutamate or of other amino acid analogs or ion carriers were present in the incubation medium in some experiments, as is indicated in the legends to the figures. At various times following the initiation of the incubation, 100 μl of the incubated sample were filtered directly through 0.45 μm Millipore filters (HAWP). The filters were washed with 5 ml of ice-cold stop solution consisting of 100 mM choline chloride/10 mM Tris-H_2SO_4/120 mM sucrose (pH 7.4). The radioactivity associated with the filters was measured by scintillation spectrometry in a 2-ethoxyethanol/toluene (0.2:0.8, v/v) scintillation fluid.

Results

Characteristics of the L-glutamate-induced SCN^- influx into synaptic membrane vesicles

The electrogenic nature of the glutamate-induced Na^+ influx into synaptic membrane vesicles was determined by measuring the accumulation of the lipophilic anion $^{35}SCN^-$ in the intravesicular space under control incubation conditions and in the presence of L-glutamic acid. In order to eliminate the contribution of K^+ diffusion (from the intravesicular to the extravesicular space) to the development of an electrical potential, the synaptic membrane vesicles were loaded internally with the sucrose buffer solution described under Materials and Methods instead of the potassium phosphate buffer used in the Na^+ uptake assays reported previously [7]. When these sucrose-loaded vesicles were incubated in a medium containing 100 mM NaCl, there was a gradual influx of SCN^- into the intravesicular space (Fig. 1). Maximum levels of SCN^- influx into the membrane vesicles were achieved within 45–60 s from the initiation of the incubation period. The apparent equilibrium level of $^{35}SCN^-$ accumulation varied between 1 and 2 nmol/mg protein for different membrane preparations. This variation in the maximum amount of SCN^- retained within the intravesicular space was probably due to differences in the intravesicular volume of the isolated synaptic plasma membranes, as well as, due to variable degrees of membrane fragility. The average plateau level of intravesicular SCN^- approximated 1.3 nmol/mg protein (1.33 ± 0.29 nmol/mg protein, $n = 6$; Fig. 1). In the absence of any ionic gradient except that for the NaSCN, i.e., in sucrose-loaded membranes incubated in the same sucrose buffer medium that contained 1 mM $^{35}SCN^-$, an equal value for equilibrium SCN^- accumulation was obtained (1.33 nmol/mg at 2 min; Fig. 1). An almost identical value for SCN^- accumulation was obtained following incubation of the membranes for 2 h at room temperature under the conditions described above (data not shown). This observation would indicate that the synaptic membranes are quite permeable to SCN^- and that a stable equilibrium distribution is achieved within 1—2 min of incubation at room temperature. Based on the amount of SCN^- accumulated at equilibrium in the absence of other ion gradients, the volume of the intravesicular space in which SCN^- distributed was determined to be equal to 1.33 μl/mg protein. This estimate of the intravesicular volume for SCN^- accumulation was not very different from the intravesicular volume of synaptic membranes (1.96 ± 0.33 μl/mg, $n = 11$) that was previously determined on the basis of the equilibrium concentration level of ^{22}Na trapped within that space [8]. The equilibrium concentration of SCN^- within the membrane vesicles that were incubated in the NaCl-containing medium (Fig. 1) would be 1.0 mM if the intravesicular volume were assumed equal to 1.33 μl/mg protein. Thus, the concentration of intravesicular SCN^- was identical to the extravesicular concentration of this ion (1.0 mM) and was indicative of the lack of an electrical potential difference ($\Delta\Psi$) across the

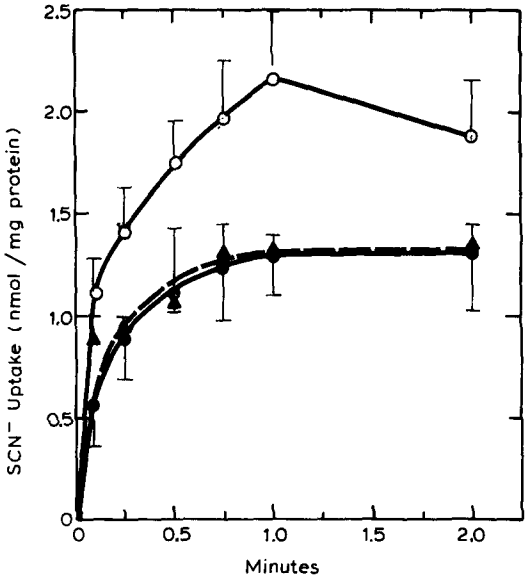

Fig. 1. L-Glutamate effects on SCN^- uptake. Synaptic membrane vesicles (40 μg) preloaded with sucrose buffer were diluted into 100 μl of external solution containing 100 mM NaCl/5 mM Tris-H_2SO_4/1 mM $MgSO_4$/1 mM $Na^{35}SCN$/120 mM sucrose. Assays were conducted in duplicate in the presence (○) or in the absence (●) of 10 μM L-glutamate. The influx of SCN^- was determined by Millipore filtration of the samples as described under Materials and Methods. All measurements were done at room temperature and represent the mean (±S.E.) SCN^- uptake for six membrane preparations. The influx of SCN^- into membrane vesicles that were preloaded with and were incubated in sucrose buffer are also shown (▲).

plasma membranes. It would appear, then, that the influx of Na^+ under these incubation conditions is an electroneutral process. Such electrical neutrality is probably maintained by the diffusion of Cl^- together with Na^+ into the synaptic membrane vesicles, since Cl^- was the major anion in the assay medium.

The same membrane vesicles incubated in a NaCl-containing buffer medium in the presence of 10 μM L-glutamic acid under conditions identical to those described above, exhibited an enhanced accumulation of SCN^- into the intravesicular space at all time periods of incubation (Fig. 1). The maximum SCN^- influx was obtained at 60 s (2.22 ± 0.3 nmol/mg protein, $n = 6$; Fig. 1). Once again, when the intravesicular volume for SCN^- distribution was assumed to be 1.33 μl/mg protein, then the intravesicular concentration of this ion at that time period was calculated to be 1.67 mM. The accumulation of SCN^- in the intravesicular space beyond its chemical equilibrium in the presence of 10 μM L-glutamic acid was indicative of the development of an internal positive membrane $\Delta\Psi$. This $\Delta\Psi$ was calculated according to the Nernst equation and found to be equal to +12.9 mV.

In addition to L-glutamic acid, other neuroexcitatory amino acids were found to stimulate SCN^- accumulation into synaptic membrane vesicles when these vesicles were incubated in a NaCl medium (Fig. 2). For example, the D-enantiomer of glutamic acid frequently produced a stronger stimulation of SCN^- uptake than L-glutamate (e.g., Fig. 2A), while the neuroexcitatory amino acid analog, kainic acid, repeatedly produced an increase in SCN^- influx equivalent to the increases measured following the addition of L-glutamic acid (e.g., Fig. 2B). An example of some of the other neuroexcitatory amino acids or amino acid analogs that were tested is shown in Fig. 2C. As can be seen in that figure, 10 μM *N*-methyl-D-aspartate caused an equal or slightly greater stimulation of SCN^- accumulation into synaptic membrane vesicles as did 10 μM L-glutamic acid. On the other hand, DL-homocysteic acid and ibotenic acid (10 μM) were less active in terms of stimulating SCN^- accumulation in the same membrane vesicle preparation. Another excitatory amino acid

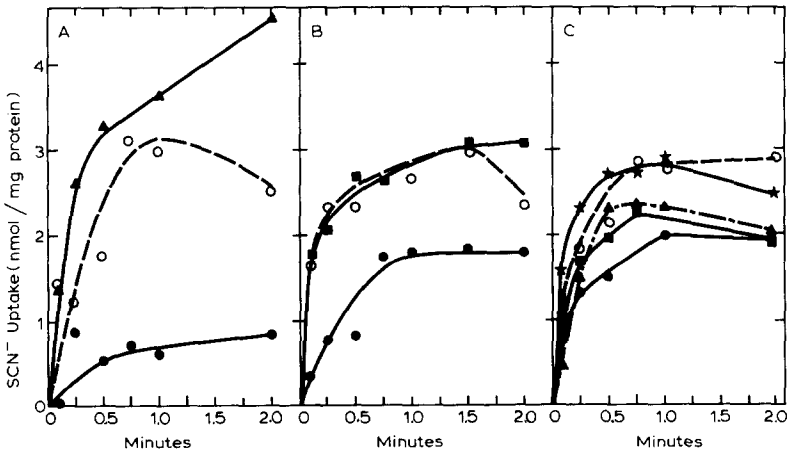

Fig. 2. Effects of various excitatory amino acids and amino acid analogs on SCN^- accumulation by synaptic membrane vesicles. All assays were conducted according to the procedures described for Fig. 1. (A) The uptake of SCN^- (1.1 mM) was determined in the presence of either 10 μM D-glutamate (▲) or 10 μM L-glutamate (○) or in the absence of either agent (●). Each point is the mean of duplicate determinations from a single membrane preparation. Mean deviation of duplicate samples was 6.6%. (B) Uptake of SCN^- (1.0 mM) in the presence of either 10 μM kainic (■) or 10 μM L-glutamic acid (○) was compared to the basal SCN^- uptake (●). Each point is the mean of duplicate determinations from a single membrane preparation, and the mean deviation of duplicate samples was 4.7%. (C) Influx of SCN^- (1.17 mM) was determined in the presence of L-glutamate (○), *N*-methyl-D-aspartate (*), DL-homocysteic acid (▲), ibotenic acid (■), and in the absence of any of these agents (●). All excitatory amino acids and amino acid analogs were present at a concentration of 10 μM. Each point is the mean of duplicate determinations from a single membrane preparation that had a variance of 6%.

anolog, quisqualic acid, once again markedly, and consistently, enhanced SCN^- influx into synaptic membrane vesicles to an even greater degree than L-glutamic acid (data not shown). The maximal increase in SCN^- influx induced by 5 μM quisqualic acid was 32% greater than that obtained in the presence of an equal concentration of L-glutamic acid (mean of four experiments). A comparison of the maximal enhancement of SCN^- accumulation produced by a 10 μM concentration of various dicarboxylic amino acids and amino acid analogs was also obtained in two different synaptic membrane preparations and was expressed in relation to the increases in SCN^- accumulation caused by L-glutamic acid (set arbitrarily at 100). The following relative order of potency was obtained: D-glutamic acid (114) > L-glutamic acid (100) > kainic acid (92) > L-aspartic acid (84) > L-cysteine sulfinic acid (72) > L-glutamine (64) > control (51). On the other hand, γ-aminobutyric acid, a neuroinhibitory transmitter which is thought to increase primarily Cl^- flux through neuronal membranes [22], either had no effect or produced a small decrease in SCN^- accumulation into the synaptic membrane vesicles (Fig. 3).

The L-glutamic acid stimulation of SCN^- accumulation into synaptic membranes was a concentration-dependent process with maximal enhancement of SCN^- influx achieved at L-glutamate concentrations over the range 1–10 μM (Fig. 4). At 100 μM L-glutamate, the response of the membranes to this amino acid was only slightly greater than the basal SCN^- diffusion (Fig. 4). The dose-response characteristics of glutamate-enhanced SCN^- accumulation shown in Fig. 4 were very similar to the dose-dependent L-glutamate stimulation of ^{22}Na influx into synaptic membrane vesicles which we have described previously, including the decrease in the glutamate-induced stimulation of Na^+ fluxes at 100 μM L-glutamic acid [7].

The stimulation of SCN^- influx into synaptic membrane vesicles produced by 5 μM L-glutamic acid was partially antagonized by the introduction into the incubation medium of 100 μM L-glutamate diethyl ester (Fig. 5). Glutamate diethyl ester is a weak but fairly specific antagonist of L-glutamic acid excitation of central nervous system neurons [23] and has been shown to block L-[^{3}H]glutamate

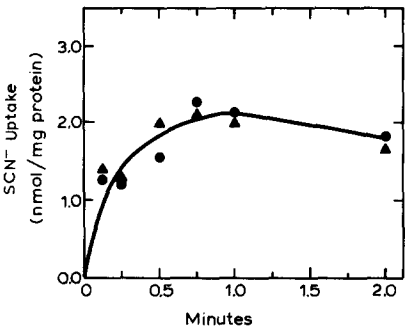

Fig. 3. Accumulation of SCN^- in synaptic membrane vesicles in the presence (▲) or absence (●) of γ-aminobutyric acid (10 μM). The incubations were conducted in the same NaCl medium under the same conditions described in Fig. 1. Each point is the mean of duplicate determinations from a single membrane preparation. Mean deviation of duplicate samples was 7.2%. This same experiment was repeated with another membrane preparation and yielded similar results.

binding to both the synaptic membranes and to the purified glutamate-binding protein [24,25]. The SCN^- influx induced by 10 μM L-glutamic acid

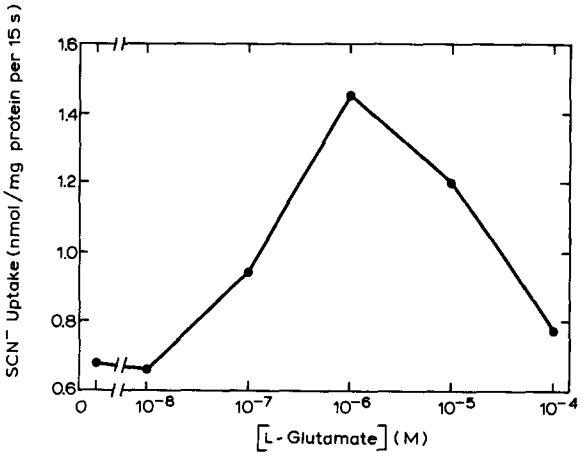

Fig. 4. Dose-response characteristics of L-glutamate-induced increases in SCN^- accumulation in synaptic membrane vesicles. Thiocyanate influx into synaptic membrane vesicles was measured in the presence of various concentrations of L-glutamic acid under conditions identical to those described for Fig. 1. The SCN^- uptake at 15 s obtained from each time kinetic determination with the respective glutamate concentration was used to plot the data shown. Each point is the mean of duplicate determinations from a single membrane preparation, and the mean deviation of duplicate samples was 8.7%. A similar dose-response relationship was obtained when the data from the 30 or 60 s incubation periods were used, although the overall SCN^- uptake was greater than that measured at 15 s.

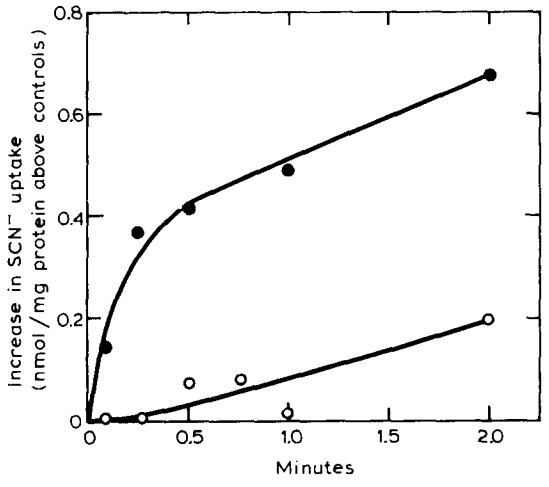

Fig. 5. Inhibition of L-glutamate-induced SCN^- uptake by L-glutamate diethyl ester. The net increase in SCN^- accumulation above control influx brought about by exposure of the membranes to 5 μM L-glutamic acid is shown. Both control and L-glutamate-exposed membrane vesicles were incubated in a NaCl medium in the absence (●) or presence (○) of 100 μM L-glutamate diethyl ester. Each point is the mean of duplicate determinations from two membrane preparations.

was antagonized not only by glutamate diethyl ester, but was also blocked by the metal ligand sodium azide (3 mM) and the metal chelator *o*-phenanthroline (1 mM) (data not shown). Both agents are known to inhibit the binding of L-[^{3}H]glutamic acid to the presumed receptor sites in synaptic membranes and to the glutamate binding protein purified from synaptic membranes [6,26]. A common property of these agents was the stimulation of the basal SCN^- influx by both metal ligands and a relative diminution of the L-glutamate-induced SCN^- influx.

Ionic effects on the glutamate activation of SCN^- accumulation in synaptic membrane vesicles

The stimulation of SCN^- influx into synaptic membrane vesicles produced by D- and L-glutamic acid and by kainic, quisqualic, ibotenic, *N*-methyl-D-aspartic, DL-homocysteic, L-aspartic, and L-cysteine sulfinic acid was indicative of a possible electrogenic enhancement of synaptic membrane Na^+ permeability brought about by exposure of the membranes to each of these neuroexcitatory amino acids and amino acid analogs. When NaCl in the incubation medium of synaptic membranes was replaced by 50 mM Na_2SO_4, the accumulation of SCN^- in the membrane vesicles was generally greater under the Na_2SO_4 incubation condition as compared to that measured in the NaCl medium (2.10 ± 0.4 as compared to 1.52 ± 0.2 nmol/mg protein, averages from four membrane preparations). Since electrical neutrality was not maintained under the conditions of Na^+ and SO_4^{2-} diffusion potentials, it would appear that the basal membrane permeability to SO_4^{2-} was considerably lower than that for Na^+, whereas the permeability for Cl^- was approximately equal to that for Na^+. The stimulation of SCN^- influx into a membrane preparation brought about by the addition of L-glutamate (10 μM) was slightly higher in the presence of Na_2SO_4 (net increase in SCN^- accumulation 0.92 nmol SCN^-/mg protein) as compared to that observed in the NaCl incubation condition (0.72 nmol/mg protein). It would appear that glutamate did not have a direct effect on Cl^- conductance under the NaCl incubation conditions, but that it may have increased the flux of chloride ions indirectly by a process similar to that which caused the increases in SCN^- accumulation. The influx of Cl^- in membranes incubated in NaCl would in turn cause a decrease in SCN^- uptake. This may have accounted for the lower SCN^- accumulation in these vesicles as compared to that observed in membranes incubated in the Na_2SO_4 medium.

When NaCl in the incubation medium was replaced by an equal concentration of KCl, then the basal SCN^- accumulation into synaptic membrane vesicles was enhanced as compared to the SCN^- accumulation in vesicles incubated in a NaCl medium (Fig. 6). This accumulation of SCN^- in the presence of an inward-directed K^+ diffusion potential indicated the development of an internal positive $\Delta\Psi$ under these incubation conditions (= +33.7 mV at its maximum point, uptake = 5.07 nmol/mg protein). The presence of 10 μM L-glutamic acid in the incubation medium caused a further increase in SCN^- influx and the development of an even greater internal positive $\Delta\Psi$ (+42.5 mV at 2 min incubation, uptake = 7.2 nmol/mg protein) (Fig. 6). A similar stimulation of SCN^- accumulation was observed in membrane vesicles that were incubated in the presence of the K^+ ionophore valinomycin at 0.1 μM con-

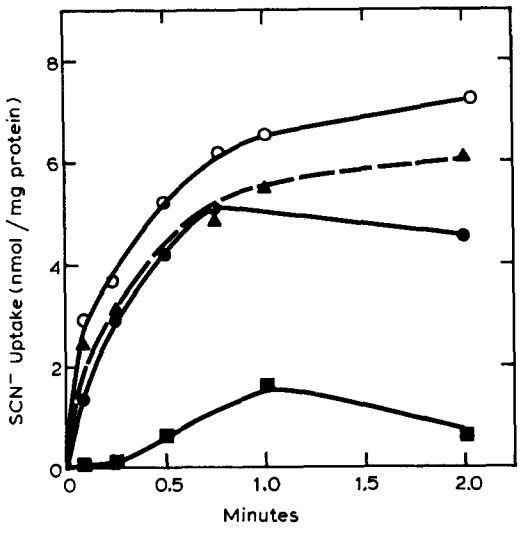

Fig. 6. L-Glutamate and valinomycin effects on SCN^- influx into synaptic membrane vesicles. The membrane vesicles were preloaded with sucrose buffer and 20 μl aliquots were transferred into a medium (100 μl) containing 100 mM KCl, 5 mM Tris-H_2SO_4, 1 mM NaSCN and 120 mM sucrose. The vesicles were incubated in this medium in the presence (○) or absence (●) of 10 μM L-glutamate, or in the presence of methanol alone (■) or of methanol plus 0.1 μM valinomycin (▲). Each data point represents the average of duplicate determinations from a single membrane preparation. Mean deviation of duplicate samples was 8%.

centration (Fig. 6). The solvent in which valinomycin was dissolved (methanol, 1.2 M final concentration in the assay) markedly decreased the basal accumulation of SCN^- as compared to the SCN^- uptake observed with membranes incubated under the control conditions (Fig. 6). Alcohols are known to reduce the passive ion permeability of lipid bilayers [27] and methanol appeared to have such an effect on the synaptic membrane preparations. However, an increase in membrane leakiness brought about by methanol cannot be excluded. As a result of methanol's effects on the basal SCN^- influx, the net stimulation of SCN^- accumulation into the vesicles brought about by 0.1 μM valinomycin was considerably greater than the net glutamate-induced increase in SCN^- accumulation. As would have been expected, the effect of the K^+ carrier valinomycin [28] was to enhance the K^+ conductance across the synaptic membranes and consequently the total SCN^- accumulation into the vesicular space. The observation that L-glutamic acid also increased the levels of intravesicular SCN^- under the KCl incubation conditions, was suggestive of a glutamate -initiated K^+ conductance increase in these membrane preparations. Finally, the increased accumulation of SCN^- in vesicles that were incubated in a KCl medium as compared to that observed in vesicles incubated in NaCl was indicative of a greater membrane permeability to K^+ than to either Na^+ or Cl^-.

Discussion

The idea that the stimulation of Na^+ influx into synaptic membrane vesicles caused by L-glutamic acid and other neuroexcitatory amino acids is an electrogenic process was confirmed by the observation that exposure of the membrane vesicles to these amino acids and to various concentrations of L-glutamic acid (10^{-7}–10^{-4} M) in a NaCl incubation medium brought about an enhancement of [^{35}S]thiocyanate accumulation. The effects of excitatory amino acids on synaptic membrane accumulation of $^{35}SCN^-$ were analogous to the increases in SCN^- influx into intact neurons brought about by veratridine, an activator of voltage-dependent Na^+ channels [17]. However, we have previously shown that L-glutamate-induced stimulation of Na^+ influx is not sensitive to tetrodotoxin, a strong inhibitor of voltage-dependent Na^+ channels [7]. Thus, it is unlikely that the stimulation of SCN^- accumulation in the synaptic membrane preparations caused by L-glutamate was produced through activation of the voltage-dependent membrane channels.

The response of synaptic membranes to increasing concentrations of L-glutamic acid as determined by SCN^- accumulation was biphasic in nature, reaching a maximum over the range 1–10 μM, while concentrations higher than 10 μM brought about smaller increases in SCN^- influx. Possible explanations for the decrease in SCN^- accumulation at high concentrations of L-glutamic acid may include the following: (a) in the presence of these glutamate concentrations, the Na^+ permeability of the membranes was increased very rapidly so that equilibration of all ions, including Cl^- and SCN^-, had occurred by the time the first sample was taken (8 s); (b) alternatively, exposure

to these concentrations of L-glutamic acid may have caused 'desensitization' of physiologic receptors for this amino acid. The fact that intravesicular SCN^- levels measured in the presence of 10 μM glutamate tended to return towards the baseline SCN^- levels (e.g., Fig. 1), might indicate that the membrane potential produced by glutamate-induced increases in Na^+ permeability were transient and would be dissipated under prolonged incubation conditions.

An approximately equal increase in SCN^- accumulation in the presence of L-glutamic acid was detected when either Na^+ or K^+ was the primary cation in the extravesicular medium. The substitution of Cl^- by the less permeable anion SO_4^{2-} increased slightly the L-glutamate-induced SCN^- accumulation. Therefore, it was suggested that L-glutamic acid normally enhances the Na^+ and K^+ permeability of synaptic plasma membranes without affecting directly the Cl^- conductance of these membranes. The effect of L-glutamate on synaptic membrane Na^+ and K^+ permeability could not be ascribed to activation or inhibition of the membrane ($Na^+ + K^+$)-ATPase, since there is no detectable enzymatic activity in these membranes in the absence of ATP [7].

In our previous report [7] we had calculated that the L-glutamate-induced increase in the influx of Na^+ into brain synaptosomes could bring about a maximal change in the membrane $\Delta\Psi$ of approx. +20 mV at equilibrium (calculated by the Nernst equation), if such Na^+ accumulation were not immediately accompanied by an increase in the intrasynaptosomal concentration of a counterbalancing anion. On the basis of the SCN^- distribution in synaptic plasma membrane vesicles determined in the present study, it was possible to detect the development of an internal positive membrane $\Delta\Psi$ in the presence of 10 μM L-glutamic acid +13 mV. These estimates of glutamate-induced changes in membrane $\Delta\Psi$ in synaptosomes and synaptic plasma membrane vesicles are quite similar to the electrophysiologically measured changes in the intraneuronal potential (+10 to +30 mV) that occurred within 30 to 60 s after the extracellular application of L-glutamic acid in cerebral cortical slices [29]. As was mentioned previously [7], the sites with which L-glutamic acid interacts to bring about this increase in cation fluxes across the plasma membranes in our preparations may be located either on presynaptic or postsynaptic membrane components. The membrane preparations used in the present study contain portions of the plasma membranes from both the presynaptic and the postsynaptic neurons [30].

It is even more difficult to determine whether the sites with which L-glutamate interacts to produce its effects on ion fluxes are the physiologic receptor-ion-channel complexes in neuronal membranes or whether they are part of the high affinity dicarboxylic amino acid transport carrier system of synaptic membranes. Since L-glutamic acid is apparently transported into these membrane vesicles by a carrier-mediated process which is activated by the presence of an [Na^+] gradient (out > in) and a [K^+] gradient (in > out) across the vesicular membrane [1], it would be possible to ascribe the development of the internal positive $\Delta\Psi$ in the presence of these ions to an electrogenic Na^+ (or K^+) co-transport with L-glutamate. It would seem unlikely, however, that the changes in the membrane $\Delta\Psi$ described in this study were due solely to the electrogenic properties of L-glutamate transport. First, even though both the L-glutamic and γ-aminobutyric acid transport processes depend on an [Na^+] gradient (out > in) for maximal activity and are similarly affected by changes in the membrane $\Delta\Psi$ [31], γ-aminobutyric acid did not alter the basal SCN^- accumulation by the synaptic membrane vesicles in our study. Second, while 10 μM D-glutamic acid did not produce activation of the L-glutamic acid transport carriers in these membrane preparations [1], it nevertheless caused a stimulation of SCN^- influx which was consistently greater than that of L-glutamic acid (e.g., Fig. 2A). Third, kainic acid, *N*-methyl-D-aspartate and ibotenic acid are transported to a very limited extent, or not at all, by the dicarboxylic amino acid carriers of brain neurons [32–34], while L-aspartate has a relatively high affinity for these membrane carriers [35,36]. Yet, *N*-methyl-D-aspartate and kainate produced an increase in SCN^- accumulation in synaptic membrane vesicles nearly equivalent to that brought about by L-glutamic and L-aspartic acid, and ibotenic acid caused a somewhat smaller stimulation of SCN^- influx. Fourth, the antagonist of L-glutamate-induced physiologic excitation, glutamate diethyl es-

ter, does not affect the activity of the neuronal L-glutamate transport [33], but in the present study, it was found to block the stimulation of SCN^- accumulation caused by L-glutamic acid. Based on these observations it would appear that stimulation of SCN^- accumulation by L-glutamic acid was the result of activation of excitatory amino acid receptor sites on synaptic membranes.

Even if one were to assume that the stimulation by L-glutamic acid of Na^+ or K^+ flux through the synaptic membranes was due to activation of a receptor-associated ion channel process, it would not be correct to infer that all of the amino acids that were examined in this study produced their effects through interaction with the same receptor-ion-channel complex. Both neuropharmacological and biochemical studies have shown that D-glutamate interacts very strongly with a population of amino acid receptors that are most sensitive to *N*-methyl-D-aspartate [37], while kainic acid interacts with receptors which have pharmacologic and biochemical characteristics different from those of either the L-glutamate of the *N*-methyl-D-aspartate receptors [38–40]. It should be noted that the relative efficacy of the various excitatory amino acids, except for kainic acid, in stimulating SCN^- accumulation in synaptic membranes was similar to the relative activity of these agents in enhancing ^{22}Na efflux from brain slices [41]. Kainic acid, on the other hand, was more effective than L-glutamate in terms of stimulating ^{22}Na efflux from both brain slices [41] and synaptic membrane vesicles [8].

The activation of SCN^- accumulation in synaptic membrane vesicles by L-glutamic acid was similar in some respects to the response of physiologic L-glutamate receptors in neuronal or muscle cell preparations. Interaction of L-glutamate with its physiologic receptors has been shown to increase both Na^+ and K^+ membrane conductance [14,15] and the ratio of increases in the permeability of Na^+ and K^+ at the invertebrate neuromuscular junction was estimated to be 0.9 [14]. Thus with respect to changes in ion permeability, the characteristics of the L-glutamate-induced SCN^- accumulation in the synaptic membrane vesicles described in this study are similar to those of physiologic L-glutamate receptors. Despite these similarities, it is not yet possible to conclude with certainty that the ionic fluxes activated by L-glutamic acid in synaptic membrane vesicles represent the activity of the membrane receptor · ion channels studied in physiological systems.

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