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Sodium Uptake Associated with Activation of Action Potential Ionophores of Cultured Neuroblastoma and Muscle Cells

(excitable membranes/hybrid cells/veratridine/tetrodotoxin)

WILLIAM A. CATTERALL AND MARSHALL NIRENBERG

Laboratory of Biochemical Genetics, National Heart and Lung Institute, National Institutes of Health, Bethesda, Md. 20014

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ABSTRACT **Veratridine, an activator of action potential Na^+ ionophores, stimulated passive Na^+ uptake by electrically excitable neuroblastoma and muscle cells but had no effect on clonal cell lines defective in Na^+-ionophore activity. Veratridine-dependent Na^+ uptake was completely inhibited by tetrodotoxin, a specific inhibitor of the action potential Na^+ ionophore. Half-maximal inhibition was obtained with 11 nM tetrodotoxin. Thus, veratridine-dependent Na^+ uptake provides a specific and convenient means of assaying populations of cultured cells for action potential Na^+-ionophore activity.**

Clonal lines of mouse neuroblastoma C1300 provide a model system in which the development and regulation of neuronal communication can be explored by biochemical and genetic techniques in cell culture. Measurement of synaptic receptor function and action potential generation in cell culture is a central problem in the study of development and regulation of neuronal communication with these clonal cell lines. Microelectrode techniques involving recordings from single cells have yielded much useful information (1–5). However, biochemical methods for determining the activity of action potential and of synaptic receptor reactions that can be applied to entire populations of cultured cells would be useful.

Kasai and Changeux (6) have reported extensive studies of $^{22}Na^+$ efflux catalyzed by acetylcholine receptor ionophores in membrane vesicle preparations from eel electroplax and demonstrated that isotope flux measurements correlate well with electrophysiological current measurements. In this report, a method is described for determining the activity of the action potential Na^+ ionophore based on measurements of $^{22}Na^+$ uptake by populations of cultured cells.

MATERIALS AND METHODS

Chemicals were obtained from the following sources: $^{22}NaCl$ and [^{14}C]inulin from Amersham-Searle; [^3H]H_2O, [*methyl*-^3H] thymidine, and [^{14}C]sucrose from New England Nuclear Corp; ouabain from Sigma; veratridine from K and K Chemicals; tetrodotoxin (purified by Sankyo Chemical Co.) from Calbiochem; fetal-bovine serum from Colorado Serum Co.; 1X crystalline trypsin from Worthington; and DMEM (the Dulbecco–Vogt modification of Eagle's minimal essential medium, GIBCO H-21) from GIBCO. Other chemicals used in this study were of reagent grade purity.

Cell Lines. The origin of the clonal cell lines used has been described. In brief, N18 and N103 are single-cell clones of mouse neuroblastoma C1300 (7). N18TG2 is a 6-thioguanine-resistant subclone of N18 (8). C6BU1 is a bromodeoxyuridine-resistant clone of glioma C6*. B82 is a bromodeoxyuridine-resistant L-cell clone lacking the enzyme thymidine kinase (9). Neuroblastoma x L cell hybrid clones NL304, NL305, NL308, and NL309(3) were obtained by Sendai virus-induced fusion of N18TG2 and B82 and selection in HAT medium (10). Neuroblastoma x glioma hybrid lines NG108-5 and NG108-15 were obtained by Sendai virus-induced fusion of N18TG2 and C6BU1 and selected in HAT medium*. L8 is a clonal line of rat-skeletal muscle and was the gift of Dr. D. Yaffee.

Cell Culture Conditions. Cell lines N18, N103, N18TG2, C6BU1, and B82 were grown in DMEM supplemented with either 5% or 10% fetal-bovine serum in a humidified atmosphere of 10% CO_2–90% air. Hybrid cell lines were grown in the same medium supplemented with 0.1 mM hypoxanthine, 1 μM aminopterin, and 16 μM thymidine (HAT medium). L8 was grown in DMEM supplemented with 3% fetal-bovine serum and 3% horse serum.

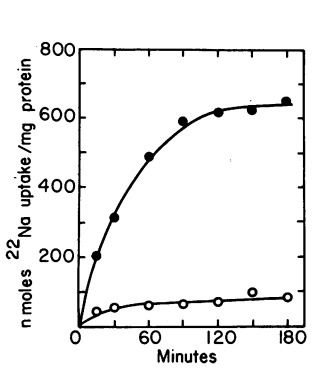
Most cell types were subcultured with the aid of 0.05% crystalline trypsin, but, in some experiments, clones N18, NG108-5, and NG108-15 were subcultured without trypsin.

For experiments on cell lines N18, N18TG2, N103, B82, C6BU1, and the somatic cell hybrids, cultures were seeded in multi-well plates (16 mm diameter, 2 cm^2, Linbro Chemical Co., TC-16-24) at about 10% of final density and were labeled by growth in medium supplemented with 16 μM[^3H]thymidine (0.1 μCi/ml) for at least four generations before use to allow determination of recovery of cells during assays as described below. Experimental cultures of cell lines C6BU1 and B82 were grown similarly without added [^3H]thymidine. Cells in stationary phase were used, usually from 6 to 8 days after subculture. Multiple layers of cells were present in many areas of the cultures of most clones under these conditions. Results similar to those reported below were also obtained when cultures of clones N18, NG108-5, NG108-15, and B82 in petri dishes (60-mm diameter, Falcon Plastics) were studied at about 25–50% confluence.

Primary cell cultures of chick skeletal muscle from 11-day embryos were grown in petri dishes (60-mm, Falcon Plastics) by a modification of the method of Konigsberg (11) essentially as described (12). Cultures of the muscle cell clone L8 were seeded at 3×10^4 cells per petri dish (60-mm, Falcon Plastics). Muscle cultures were used after well-developed myotubes appeared, usually after 8–9 days for primary chick-embryo muscle cultures and 11 days for cultures of L8.

Measurement of $^{22}Na^+$ Uptake. Cultures were removed from the incubator, and the culture medium was immediately removed and replaced with assay medium (0.5 ml per well or 2.0 ml per petri dish) consisting of 50 mM *N*-2-hydroxyethylpiperazine *N'*-2-ethanesulfonic acid (adjusted to pH 7.4

* Hamprecht, B., Amano, T. & Nirenberg, M., manuscript in preparation.



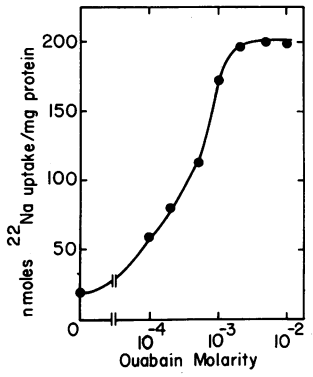


FIG. 1 (*left*). Effect of ouabain on $^{22}Na^+$ uptake. Cultures of neuroblastoma clone N18 were seeded at 20,000 cells per cm^2 and grown to a density of about 1.3 × 10^5 cells per cm^2 (130 μg of cell protein per cm^2) in the presence of 10% fetal-bovine serum. Uptake of $^{22}Na^+$ was measured for the indicated times at 36° in the presence (●) or absence (○) of 5.0 mM ouabain. Each point represents the average of duplicate cultures.

FIG. 2 (*right*). Effect of ouabain concentration on $^{22}Na^+$ uptake. Cultures of neuroblastoma × glioma line NG108-15 were seeded at 5000 cells per cm^2 and grown to a density of about 5 × 10^4 cells per cm^2 (95 μg of cell protein per cm^2) in the presence of 5% fetal-bovine serum. Uptake of $^{22}Na^+$ was measured for 30 min at 36° in the presence of the indicated concentrations of ouabain. Each point represents the average of duplicate cultures.

with NaOH), 130 mM NaCl, 5.4 mM KCl, 1.8 mM $CaCl_2$ 0.8 mM $MgSO_4$, 5.5 mM glucose, and 1.0 mM NaH_2PO_4 (adjusted to pH 7.4 with NaOH). The measurement of $^{22}Na^+$ uptake was begun by aspirating off this medium and replacing it with assay medium (0.5 ml per well or 2.0 ml per petri dish) containing, in addition to the components described above, from 2.0 to 5.0 μCi of ^{22}NaCl per ml and appropriate effectors as described in the figure legends. These operations were performed with cultures immersed in a water bath maintained at 36° and with media equilibrated at 36°.

Uptake measurements were terminated at timed intervals by aspirating off the radioactive assay medium and washing the culture three times, as rapidly as possible, with wash medium at room temperature (2.5 ml per well or 10 ml per petri dish) consisting of 164 mM NaCl, 5.4 mM KCl, 1.8 mM $CaCl_2$, 0.8 mM $MgSO_4$, and 5.0 mM NaH_2PO_4 (adjusted to pH 7.4 with NaOH). The washed cells were removed by incubation at room temperature with 0.4 N NaOH (1.0 ml per well or 2.0 ml per petri dish). To an aliquot (0.5 ml) of each of the resulting suspensions was added 1.0 ml of 1.0 M Tris·HCl(pH 7.4) and the radioactivity in ^{3}H- and ^{22}Na-labeled compounds was determined by liquid scintillation counting in 10 ml of a scintillation mixture consisting of 5.4 g of 2,5-diphenyloxazole and 67.5 mg of 1,4-bis-(2-phenyloxazolyl) benzene per liter dissolved in a 2:1 (v/v) mixture of toluene and Triton X-100.

Cultures were incubated for more than four generations with [^{3}H]thymidine as described above. Within a set of identical cultures, the [^{3}H]thymidine incorporated was proportional to cell protein. Thus, the [^{3}H]thymidine recovered after the cultures were washed was proportional to the cell protein recovered and could be used to correct for variable recoveries of cell protein in different cultures within an experiment. In each experiment, a set of "zero time" assays was conducted by adding radioactive assay medium to the cultures and immediately (within 10 sec) washing the cultures as usual. Uptake of $^{22}Na^+$ in nmol/mg of cell protein was calculated from the ratio of corrected $^{22}Na^+$ cpm (total $^{22}Na^+$ cpm − "zero time" value) to [^{3}H]thymidine cpm for each individual culture and the [^{3}H]thymidine cpm per mg of cell protein determined for three cultures chosen at random from each set of identical cultures. Protein was determined by a modification of the method of Lowry *et al.* (13). The reproducibility of values of nmol of $^{22}Na^+$ uptake per mg of protein determined in this manner was better than ±10%. For cell lines not labeled with [^{3}H]thymidine, protein was determined directly by a modification of the Lowry method (13).

RESULTS

The action potential consists of at least two separate processes distinguishable by pharmacologic (14) or genetic (8, 10) means: an initial increase in Na^+ permeability which disappears after a few milliseconds, followed by a more prolonged increase in K^+ permeability (15, 16). The increase in Na^+ permeability is primarily responsible for the rising, depolarizing, phase of the action potential spike. The increase in K^+ permeability contributes to the falling, repolarizing, phase of the action potential spike and the hyperpolarizing potential following the spike. Although both action potential reactions are usually assayed by microelectrode techniques, they can also be assayed by measurement of fluxes of radioactive ions induced by electrical stimuli (17). In this report we describe biochemical methods for assaying clonal cell populations for Na^+-permeability reactions of the action potential by initiating a steady-state reaction with the alkaloid, veratridine.

Initiation of Na^+ Uptake. The relatively low intracellular Na^+ concentration is maintained by the action of Na^+, K^+-activated ATPase which exchanges internal Na^+ for external K^+ against the concentration gradients of these ions (18). Thus, Na^+ uptake can be initiated by inhibiting the synthesis of intracellular ATP or by inhibiting Na^+, K^+-activated ATPase with ouabain.

In the absence of ouabain, $^{22}Na^+$ uptake by neuroblastoma cells was relatively low, consisting of an exchange of external $^{22}Na^+$ with internal Na^+ (Fig. 1). However, in the presence of ouabain, uptake of $^{22}Na^+$ into the cells increased markedly. The time course of ^{22}Na uptake by different cell lines was approximated by an exponential curve with a half-time of 30–60 min. Measurement of the intracellular volume of line NG108-15, with [^{3}H]H_2O and [^{14}C] sucrose or [^{14}C]inulin as internal and external volume markers, respectively, suggested that $^{22}Na^+$ equilibrated completely with the intracellular space. The measured intracellular volume was about 4.3 μl per mg of cell protein. Since $^{22}Na^+$ uptake approached 600 nmol per mg of cell protein for NG108-15, the final intracellular concentration of $^{22}Na^+$ was 140 ± 20 mM compared to the extracellular concentration of 152 mM. The difference between the extents of uptake of $^{22}Na^+$ in the presence and absence of ouabain (Fig. 1) suggests that these cells maintain an internal Na^+ concentration of 15–20 mM against an external concentration of 152 mM. Other experiments (not shown) indicated that a more rapid uptake of Na^+ was initiated by inhibition of Na^+, K^+-activated ATPase with ouabain than by inhibition of ATP synthesis. Thus, ouabain inhibition was used in the experiments described below

Treatment of cell cultures with various concentrations of ouabain indicated that the stimulation of uptake was half-

TABLE 1. *Effect of veratridine on different cell lines*

Cell type	Cell line	Electrical characteristics*	Veratridine-dependent $^{22}Na^+$ uptake† (nmol/mg of protein per 10 min)	Veratridine stimulation† (ratio)
Mouse neuroblastoma	N103	A^-B^+ (5)	10	1.1
"	N18	A^+B^+ (5)	210	4.7
"	N18TG2	A^+B^+ (8)	165	4.0
Mouse L cell	B82	A^-B^- (8)	6	1.0
Rat glioma	C6BU1	A^-B^- (‡)	4	1.0
Neuroblastoma x L-cell hybrids				
N18TG2 x B82	NL308	A^+B^+ (10)	95	2.4
"	NL309 (3)	A^+B^+ (10)	80	2.2
"	NL304	A^-B^+ (10)	3	1.0
"	NL305	A^-B^- (10)	0	1.0
Neuroblastoma x glioma hybrids				
N18TG2 x C6BU1	NG108-5	A^+B^+ (‡)	205	4.9
"	NG108-15	A^+B^+ (‡)	170	4.3
Rat skeletal muscle	L8	A^+B^+ (27)	345	5.7
Primary chick-embryo skeletal muscle		A^+B^+ (28)	310	5.9

Experiments were done essentially as described in the legend to Fig. 3 and in *Methods*. Muscle cells were seeded at 1500 cells per cm^2 and grown in petri dishes (60 mm diameter). Other cell lines were seeded at about 10% of final cell density (5000–20,000 cells per cm^2) and grown to confluence.

* Electrical characteristics are listed according to the nomenclature described (8). A^+-cell lines exhibit a depolarizing response to a pulse of depolarizing current presumably due to an increase in Na^+ permeability during depolarization. A^--cell lines lack this characteristic action potential response. B^+-cell lines exhibit a delayed hyperpolarizing response to a pulse of depolarizing current, presumably due to a delayed increase in K^+ permeability during depolarization. B^--cell lines lack this characteristic action potential response. *Numbers in parentheses* are references to the electrophysiological data presented.

† Veratridine stimulation is reported as the ratio of the rate of $^{22}Na^+$ uptake in the presence of veratridine to that in its absence calculated according to Eq. 2. Veratridine-dependent $^{22}Na^+$ uptake is reported as the difference in $^{22}Na^+$ uptake during incubations (10 min) between cultures treated with 0.1 mM veratridine and untreated control cultures. Control rates of uptake were similar for all cell clones corresponding to 80–120 nmoles of $^{22}Na^+$ per mg of cell protein in 10 min.

maximal at a concentration of 0.5 mM and complete at 2.0 mM (Fig. 2). The requirement for relatively high ouabain concentrations is expected, since rat and mouse Na^+, K^+-activated ATPases have an unusually low affinity for ouabain (19). Ouabain concentrations of 5.0 mM were used in the subsequent experiments.

Stimulation of Passive Na^+ Uptake by Veratridine. At least six neurotoxins can activate action potential Na^+ ionophores: the alkaloids veratridine (20), batrachotoxin (21), aconitine (22), and grayanotoxin (29), and the polypeptide toxins of scorpion and coelenterate venoms(23, 30). The effect of veratridine on the passive Na^+ permeability of cultured neuroblastoma cells was examined to determine whether this compound can induce a steady-state permeability increase that can be measured as an increase in the rate of $^{22}Na^+$ uptake. Treatment of cultures of neuroblastoma with veratridine markedly increased the rate of $^{22}Na^+$ uptake (Fig. 3*A*). Similar results were obtained with cells that were subcultured with trypsin 6 days before use.

If one assumes that the decay of the $^{22}Na^+$ concentration gradient across the cell membrane is described by a single exponential, then the instantaneous rate of uptake is given by

$$d(^{22}Na^+)_{in}/dt = k[(^{22}Na^+)_{in}^{\infty} - (^{22}Na^+)_{in}^{t}] \quad [1]$$

where $(^{22}Na^+)_{in}^{\infty}$ is the concentration of $^{22}Na^+$ inside the cell at $t = \infty$, i.e., at equilibrium. The solution of this equation when $(^{22}Na^+)_{in} = 0$ at $t = 0$ is

$$\ln \frac{(^{22}Na^+)_{in}^{\infty}}{(^{22}Na^+)_{in}^{\infty} - (^{22}Na^+)_{in}} = k' = \frac{\ln 2}{T_{1/2}}(t) \quad [2]$$

where $T_{1/2}$ is the half-time for equilibration in minutes. Plots of the data of Fig. 3*A* according to Eq. 2 are linear (Fig. 3*B*), indicating that the time course of $^{22}Na^+$ uptake of both control and veratridine-treated cultures is described adequately by a single exponential. Stimulations of the rate of $^{22}Na^+$ uptake calculated from the half-times for equilibration in experiments like that illustrated in Fig. 3 ranged from 4- to 6-fold for cell lines N18, NG108-5, and NG108-15.

The effect of veratridine concentration upon $^{22}Na^+$ uptake is shown in Fig. 4. The stimulation of ^{22}Na uptake was half-maximal at about 4×10^{-5} M veratridine and approached completion at concentrations in the range of 1 to 2×10^{-4} M. Investigation of higher veratridine concentrations was limited by solubility. A veratridine concentration of 1×10^{-4} M was used in the experiments described below. The arithmetic concentration curve shown as an inset in Fig. 4 suggests that about 80% of the maximum response was attained at this concentration.

Effect of Tetrodotoxin. Tetrodotoxin is a specific inhibitor of the action potential Na^+ ionophores (14, 24). Thus, if the increase in $^{22}Na^+$ uptake caused by veratridine is a specific measure of steady-state activity of the action potential Na^+ ionophores, the increase in $^{22}Na^+$ uptake should be inhibited by tetrodotoxin at a concentration that inhibits action potentials in nerve preparations.

The stimulation of $^{22}Na^+$ uptake by veratridine is completely inhibited by 1 μM tetrodotoxin (Fig. 5). Treatment of cultures with tetrodotoxin at concentrations between 1×10^{-10} and 1×10^{-6} M gave the results illustrated in Fig. 6. Half-maximal inhibition of the veratridine-dependent $^{22}Na^+$ uptake occurred at 1×10^{-8} M. Inhibition was greater than 95% complete at 3×10^{-7} M tetrodotoxin. Half-maximal inhibitions were achieved at concentrations between $1.0 \times \times 10^{-8}$ and 1.5×10^{-8} M, and complete inhibitions at about 3×10^{-7} M tetrodotoxin with cell lines N18, NG108-5, and NG108-15. Action potential generation by nerve preparations from various species is inhibited half-maximally in the range of 0.3 to 8.0×10^{-8} M tetrodotoxin (24). A single class of binding sites for [^{3}H]tetrodotoxin having a dissociation constant of 0.3 to 1.0×10^{-8} M has been detected by equilibrium dialysis studies of garfish olfactory nerve, lobster walking-leg nerve, and rabbit vagus nerve (25, 26). Thus, the inhibition of veratridine-dependent $^{22}Na^+$ uptake by tetrodotoxin occurs at concentrations identical to those that inhibit action potentials in many nerve preparations. These data, considered together with the specificity of tetrodotoxin binding, provide evidence that the veratridine-dependent $^{22}Na^+$ uptake reflects activation of the Na^+ ionophores associated with the action potential.

Effect of Veratridine on Different Cell Lines. In order to provide further evidence for the specificity of veratridine-dependent $^{22}Na^+$ uptake as a measure of action potential Na^+ ionophores, we studied several types of cultured cells with defined electrophysiological properties (Table 1). Three neuroblastoma clones were examined. N103, which is defec-

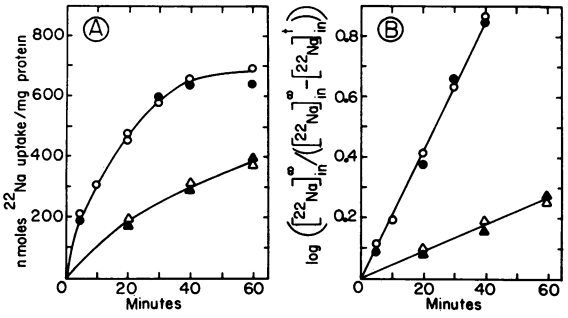


FIG. 3. Stimulation of $^{22}Na^+$ uptake by veratridine. (*A*) Cultures of neuroblastoma clone N18 were seeded at 20,000 cells per cm^2 and grown to a density of about 2.1 × 10^5 cells per cm^2 (210 μg of cell protein per cm^2) in the presence of 10% fetal-bovine serum. Uptake of $^{22}Na^+$ was measured for the indicated times at 36° in the presence of 5.0 mM ouabain (▲, △) or 5.0 mM ouabain and 0.1 mM veratridine (●, ○). Each point represents the average of duplicate cultures. *Open symbols*, data from cells subcultured with the use of trypsin (0.05%); *closed symbols*, data from cells subcultured by repetitive pipetting without trypsin. (*B*) The data in *A* are plotted on semilogarithmic axes as in Eq. 2 using $(^{22}Na^+)_{in}^{\infty}$ = 700 nmol/mg of cell protein.

tive in the depolarizing step (A) but not in the repolarizing step (B) of the action potential (A^-B^+) (5), was not affected appreciably by veratridine. In contrast, veratridine markedly stimulated the $^{22}Na^+$ uptake of the electrically excitable cells (A^+B^+) (5, 8) of clones N18 and N18TG2, a mutant subclone used for cell hybridization studies (8, 10). Veratridine had no effect on a mouse L-cell clone or a rat-glioma clone also used for cell hybridization studies and known to be unable to generate action potentials (A^-B^-)* (8). Four neuroblastoma x L-cell hybrid clones were examined. Veratridine stimulated $^{22}Na^+$ uptake by two hybrid clones, NL308 and

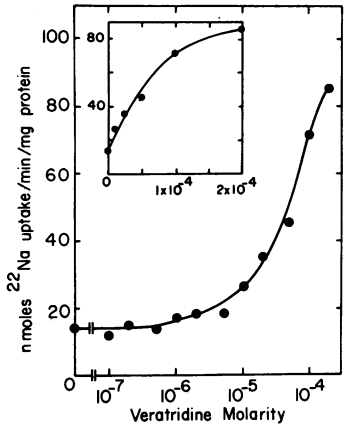


FIG. 4. Effect of veratridine concentration on stimulation of $^{22}Na^+$ uptake. Cultures of neuroblastoma × glioma line NG108-5 were seeded at 5000 cells per cm^2 and grown to a density of about 8 × 10^4 cells per cm^2 (210 μg of cell protein per cm^2) in the presence of 5% fetal-bovine serum. Uptake of $^{22}Na^+$ was measured for 10 min at 36° in the presence of 5.0 mM ouabain and the indicated concentrations of veratridine. Each point represents the average of four replicate cultures. Values for the initial rates of $^{22}Na^+$ uptake were calculated from Eq. 1 using $(^{22}Na^+)_{in}^{\infty}$ = 550 nmol/mg of cell protein. An arithmetic plot of the data at higher concentrations is presented in the *inset* to illustrate more clearly the saturation of the veratridine stimulation at concentrations in the range of 1 to 2 × 10^{-4} M.

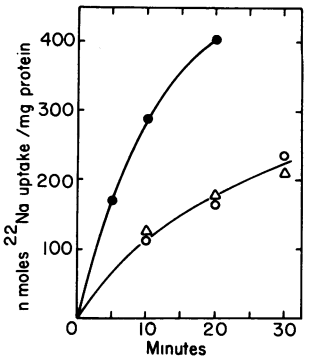


FIG. 5. Inhibition of veratridine-dependent $^{22}Na^+$ uptake by tetrodotoxin. Cultures of neuroblastoma × glioma line NG108-5 were seeded at 5000 cells per cm^2 and grown to a density of about 6 × 10^4 cells per cm^2 (160 μg of cell protein per cm^2) in the presence of 10% fetal-bovine serum. Uptake of $^{22}Na^+$ was measured for the indicated times at 36° in the presence of 5.0 mM ouabain (△), 5.0 mM ouabain and 0.1 mM veratridine (●), or 5.0 mM ouabain, 0.1 mM veratridine, and 1 μM tetrodotoxin (○). Each point represents the average of duplicate cultures.

NL309(3), which are electrically excitable (A^+B^+) (10), but had little or no effect on $^{22}Na^+$ uptake by either NL304, specifically defective in the depolarizing step of the action potential (A^-B^+) (10), or NL305, defective in both action potential steps (A^-B^-) (10). Two electrically active neuroblastoma x glioma hybrid cell lines* were also examined and were found to respond well to veratridine. Similarly, veratridine markedly stimulated $^{22}Na^+$ uptake by primary chick-embryo skeletal muscle and a clonal line of rat-skeletal muscle.

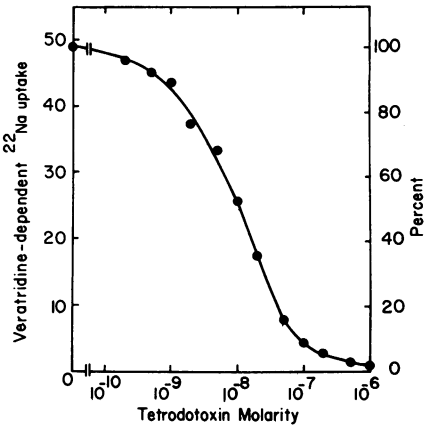


FIG. 6. Effect of tetrodotoxin concentration on inhibition of veratridine-dependent $^{22}Na^+$ uptake. Cultures of neuroblastoma clone N18 were seeded at 20,000 cells per cm^2 and grown to a density of about 1.2 × 10^5 cells per cm^2 (120 μg of cell protein per cm^2) in the presence of 10% fetal-bovine serum. Uptake of $^{22}Na^+$ (nmol of $^{22}Na^+$ per min per mg of protein) was measured for 10 min at 36° in the presence of 5.0 mM ouabain, 0.1 mM veratridine, and the indicated concentrations of tetrodotoxin, Each point represents the average of triplicate cultures. Values for the initial rates of $^{22}Na^+$ uptake were calculated from Eq. 1 using $(^{22}Na^+)_{in}^{\infty}$ = 700 nmol/mg of cell protein. Veratridine-dependent $^{22}Na^+$ uptake is the difference in initial rate of $^{22}Na^+$ uptake between cultures treated with 0.1 mM veratridine plus the indicated concentrations of tetrodotoxin and control cultures treated with neither veratridine nor tetrodotoxin. The initial rate of $^{22}Na^+$ uptake for control cultures was 17 nmol/min per mg of protein.

Thus, veratridine stimulated $^{22}Na^+$ uptake by seven cell lines that are capable of generating depolarizing action potentials but had no effect on five cell lines lacking a depolarizing action potential step. These results provide further evidence that the veratridine-dependent $^{22}Na^+$ uptake reflects steady-state activity of the Na^+ ionophores that are activated transiently during the action potential.

DISCUSSION

Three characteristics of the stimulation of $^{22}Na^+$ uptake measured in these experiments indicate that it represents steady-state activation of the Na^+ ionophores associated with action potential generation. (*i*) The stimulation of $^{22}Na^+$ uptake is dependent on veratridine, which has been shown in electrophysiological studies to activate action potential Na^+ ionophores (20). (*ii*) The $^{22}Na^+$ uptake is inhibited by tetrodotoxin at concentrations that specifically inhibit the generation of action potentials in a wide variety of systems. (*iii*) Analysis of 12 cell lines differing in their electrical properties showed that veratridine-dependent $^{22}Na^+$ uptake was associated only with those cell lines capable of generating depolarizing action potentials. Thus, veratridine-dependent $^{22}Na^+$ uptake provides a specific measure of the Na^+-permeability reaction of the action potential. This assay of the action potential Na^+ ionophore is rapid, sensitive, and reproducible and can be used to determine the excitability of populations of cells and the modulation of excitability by growth conditions or other effectors.

The estimated membrane current expected from the veratridine-dependent $^{22}Na^+$ uptake is about 20 $\mu A/cm^2$ of membrane-surface area (7.5×10^7 ions per min per μm^2) for clone N18. A value of 64 $\mu A/cm^2$ (24×10^7 ions/min per μm^2) for the action potential current was obtained in electrophysiological studies of a selected population of morphologically well-differentiated N18 cells that were grown under similar conditions but were tested after adjustment of the membrane potential (5). Because of the differences in cell-population sampling and membrane potential, a rigorous comparison of these data requires further work. However, the similarity of these estimated membrane currents suggests that veratridine induces an activity of the Na^+ ionophores that is similar in magnitude to that during an action potential.

Previous studies have shown that cultures of most clonal lines of neuroblastoma cells contain small, electrically passive, neuroblast-like cells as well as large neuron-like cells with well-developed processes and electrically excitable membranes. Populations of passive cells with action potential currents of about 1 $\mu A/cm^2$ or populations enriched with excitable cells having action potential currents of about 250 $\mu A/cm^2$ can be obtained depending on growth conditions (5). Thus, the acquisition of electrically excitable membranes is regulated *in vitro*. The procedures described should be useful in unravelling the action potential reactions and the regulation of some molecules required for transmission of information in the nervous system.

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