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THE SODIUM CHANNEL IN NON-IMPULSIVE CELLS

INTERACTION WITH SPECIFIC NEUROTOXINS

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Summary

The cell line C_9 used in this paper has a resting potential of −50 mV (±10 mV) but is unable to generate an action potential upon electrical stimulation. The cell membrane has receptors for the selectivity filter toxin tetrodotoxin as well as for the gating system toxins, veratridine, scorpion toxin and sea anemone toxin. The Na^+ channel which remains silent to electrical stimulation in the absence of toxins can be chemically activated by the gating system toxins. This has been demonstrated by electrophysiological techniques and by $^{22}Na^+$ flux studies. The electrophysiological approach has shown that the sea anemone toxin is able to induce a spontaneous slow-wave activity inhibited by tetrodotoxin. $^{22}Na^+$ influx analyses have shown that veratridine and the sea anemone toxin produce an important increase of the initial rate of $^{22}Na^+$ influx into the C_9 cell. The stimulation of $^{22}Na^+$ entry by these gating system toxins is similar to that found using spiking neuroblastoma cells. Veratridine and the sea anemone toxin on one hand as well as veratridine and the scorpion toxin on the other hand are synergistic in their action to stabilize an open and highly permeable form of the sodium channel. Stimulation of $^{22}Na^+$ entry into the cell through the sodium channel maintained open by the gating system neurotoxins is completely suppressed by tetrodotoxin.

Introduction

Recent papers have demonstrated that non-neuronal cells, the electrical response of which does not involve fast sodium channels, like the pancreatic β-cell [1,2] or the chick embryo cardiac cell in culture [3,4] for example,

Abbreviation: Hepes, *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid.

respond to veratridine, one of the toxic compounds considered to be specific of fast Na^+ channels. Work from this laboratory has also suggested that membranes of differentiated neuroblastoma cells in culture comprise side by side both functional and silent 'fast' Na^+ channels [5].

The approach taken in the paper is to use a cell line called C_9 which was isolated from an *N*-ethyl-*N*-nitrosourea-induced rat tumor [6,7] and a series of neurotoxins known to be specific of fast Na^+ channels. These toxins include tetrodotoxin, the most classical toxin of fast Na^+ channel which specifically blocks the selectivity filter of the channel without affecting its gating system [8,9] and veratridine and polypeptide toxins such as a sea anemone toxin and a scorpion toxin which selectively alter the gating system of the Na^+ channel. Veratridine depolarizes excitable membranes by a selective increase in the resting sodium permeability [10,11]; it forces the opening (activation) of the Na^+ channel and prevents its closing (inactivation). Scorpion and sea anemone toxins considerably slow down the closing of the Na^+ channel [12—17].

Materials and Methods

Cell cultures. The C_9 cell line was derived from a bladder metastasis of a *N*-ethyl-*N*-nitrosourea-induced rat brain tumor [6,7]. The cells were propagated in 100-mm Petri dishes (Corning) and grown in a Dulbecco's Modified Eagle Medium containing 3.7 g/l of $HNaCO_3$ and supplemented with 7% fetal calf serum, 50 μg/ml streptomycin and 100 units/ml penicillin. Cultures were maintained at 37°C in a water-saturated atmosphere of 5% CO_2/95% air. For experiments, cells from stocked cultures were dissociated and seeded in 35-mm Petri dishes (Corning) at a density of $2 \cdot 10^5$ cells/dish. The growth medium was replaced on day 3 or 4 with a fresh medium and cells used two days later.

Electrophysiological measurements. Culture dishes containing C_9 cells were directly used in electrophysiology after replacing the culture medium by an Earle medium (NaCl: 140 mM; KCl: 5.4 mM; $MgSO_4$: 0.8 mM; $CaCl_2$: 1.8 mM; Hepes/Tris: 25 mM; glucose: 5 mM) buffered at pH 7.4. The culture dish was placed on the warm stage of an inverted microscope (Leitz-Diavert) and the temperature was maintained between 35°C and 37°C.

Glass capillary microelectrodes were used for intracellular recording and stimulation. They were filled with 3 M KCl and had resistances ranging between 30 and 50 MΩ. The reference electrode was a Ag|AgCl half-cell placed in the external medium. The microelectrode was connected to a negative capacitance electrometer amplifier (WPI, M707) with an active bridge network which made possible simultaneous injections of current and recording through a single microelectrode. Electrical activity was continuously digitized and stored by a digital computer (Intertechnique-Plurimat S).

Measurements of $^{22}Na^+$ uptake. Initial rates of $^{22}Na^+$ uptake by C_9 cells were determined at 37°C. The culture medium was first removed by suction and immediately replaced by 1.5 ml of a standard medium which consisted of 25 mM Hepes buffer adjusted to pH 7.4 with Tris and containing 140 mM NaCl, 5.4 mM KCl, 1.8 mM $CaCl_2$, 0.8 mM $MgSO_4$, 5 mM glucose and 0.1 mg/ml bovine serum albumin. Cells were then equilibrated for 5 min with the assay medium and effectors usually added 4 min before starting the $^{22}Na^+$ uptake

experiment. This time of association was sufficient to get a maximum response. Ouabain was added 30 s before addition of $^{22}Na^+$. The uptake experiment was started by addition of 2 μCi/ml of $^{22}Na^+$. Uptakes were determined by removing the radioactive assay medium and washing three times at 20°C with 1.5 ml of a wash medium consisting of 140 mM NaCl, 5.4 mM KCl, 1.8 mM $CaCl_2$, 0.8 mM $MgSO_4$ in a 25 mM Tris buffer at pH 7.4. The washing procedure lasted less than 15 s, a time sufficient to remove the extracellular radioactive ion without significant loss of the intracellular radioactivity. Washed cells were then suspended in 0.1 N NaOH and radioactivity was measured using Picofluor and a Packard 2450 scintillation spectrometer. In each series of experiments, a 'zero-time' assay was carried out by adding $^{22}NaCl$ to the assay medium and then washing the cells within 10 s by the standard procedure. The zero-time value was subtracted from every uptake measurement. Uptake rates in nmole $\cdot$ min^{-1} $\cdot$ mg^{-1} of cell protein were calculated from the measurements of $^{22}Na^+$ taken up and from determinations of cell protein by the method of Hartree [18]. Each experimental point in a series of experiments to obtain a given curve has been done in duplicate.

Purification of sea anemone toxin II. The purification of toxin II from the sea anemone *Anemonia sulcata* was carried out according to Béress et al. [19, 20] with minor modifications [21]. Fresh stock solutions of this toxin were prepared every 3—4 days at a concentration of 1 mM and kept at 4°C.

Other chemicals and toxins. They were obtained from the following sources: veratridine from Aldrich; ouabain, choline chloride and bovine serum albumin (fraction V) from Sigma; tetrodotoxin (free of citrate) from Sankyo; Dulbecco's Modified Eagle Medium HG (cat. No. H21) and fetal calf serum from Gibco; $^{22}NaCl$ from the Commissariat à l'Energie Atomique (Saclay). The scorpion neurotoxin from *Androctonus australis Hector* used in this work (scorpion toxin II) was kindly given to us by Dr. H. Rochat and his colleagues.

Results

Electrophysiological properties of the C_9 cells in culture

The mean resting potential of C_9 cells is dependent upon the stage of culture. After 3 days in culture, cells are in logarithmic growth; their mean resting potential is —25 mV (±5 mV). After 5 days in culture, cells are at confluence; their mean resting potential is —50 mV (±10 mV). Only this later stage of development was used in the present study. As shown in Fig. 1, C_9 cells exhibited passive responses to depolarizing current pulses without evidence of graded spikes. Prepulses to —100 mV lasting 1 s do not change this behavior, i.e. do not permit to observe action potentials. Some other electrical characteristics of this cell are also presented in Fig. 1. The relationship between the resting potential and the log of the external K^+ concentration above 2 mM is linear with a slope of —35 mV/decade (Fig. 2A). Under normal conditions, i.e. in the absence of toxins, the resting potential is nearly independent of the external Na^+ concentration. It also does not seem to be affected by variations of the external calcium concentration between 0.18 mM and 18 mM (not shown).

Addition of ouabain known to block the (Na^+ + K^+)-ATPase at a concentra-

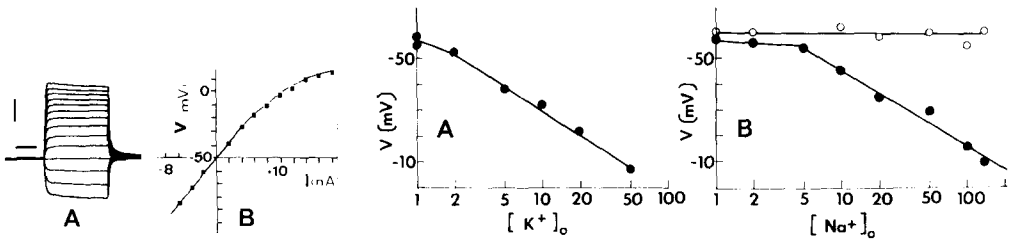

Fig. 1. (A) Superimposed traces of changes in membrane potential occurring in response to eleven successive current pulses ranging from —6 nA to 16 nA (outward current is positive). Time scale: 20 ms; voltage scale: 20 mV. (B) The steady *I-V* relationship of the same cells as in (A). The experiments have been carried out in the Earle medium described in Materials and Methods.

Fig. 2. (A) Effect of external K^+ concentration on the resting membrane potential, $[Na^+]_0$ = 5 mM. (B) Effect of external Na^+ concentration in the absence (○) and in the presence (●) of veratridine (10^{-4} M). $[K^+]_0$ = 5.4 mM.

tion of 0.13 mM did not affect the resting potential.

The alkaloid veratridine is known to increase the resting membrane permeability to Na^+ in a variety of excitable preparations [10,11]. The resting potential of C_9 cells becomes very dependent upon external sodium concentration after treatment with veratridine at a concentration of 0.1 mM (Fig. 2B). The relationship between the resting potential and the log of the external Na^+ concentration is linear above 5 mM with a slope of —30 mV/decade. Under normal conditions, i.e. at $[Na^+]_0$ = 140 mM, veratridine at a concentration of 1 μM has no effect on the resting potential. At 10 μM, veratridine depolarizes C_9 cells to —15 mV; at 0.1 mM veratridine, the equilibrium value of the resting potential is only a few millivolts.

The sea anemone toxin prolongs the action potential of a variety of excitable cells by selectively slowing down the rate of inactivation of Na^+ channels [12—14]. A treatment of the C_9 cell with a 1 μM concentration of the sea anemone toxin II gives rise to a spontaneous slow-wave activity (Fig. 3A). The amplitude of these slow waves can reach 50 mV. The slow wave does not present an overshoot. The induction of a slow-wave signal by the sea anemone toxin II is readily suppressed by tetrodotoxin as shown in Fig. 3A. High concentrations of the order of 10 μM tetrodotoxin have to be used to suppress the sea anemone toxin II effect; tetrodotoxin concentrations between 10 nM and 100 nM are without effect.

Veratridine and sea anemone toxin II act in synergy on the membrane of the C_9 cell. Fig. 3B shows the effects of a 1 μM concentration of veratridine, which by itself has no effect on the resting potential of the C_9 cells, on C_9 cells which has been previously treated with 1 μM sea anemone toxin II to induce the slow-wave behavior. Under these conditions, 1 μM veratridine provoked a decrease of the depolarization rate of the slow waves, a decrease of their amplitude, and finally a complete stop of the spontaneous activity when the cell reaches a depolarized state near —20 mV. Similarly, if C_9 cells are first preincubated in 1 μM veratridine and if the sea anemone toxin II is then added at a concentration of 10 nM which is below the threshold concentration for the induction of slow waves in the absence of other types of toxines, then, slow oscillations of

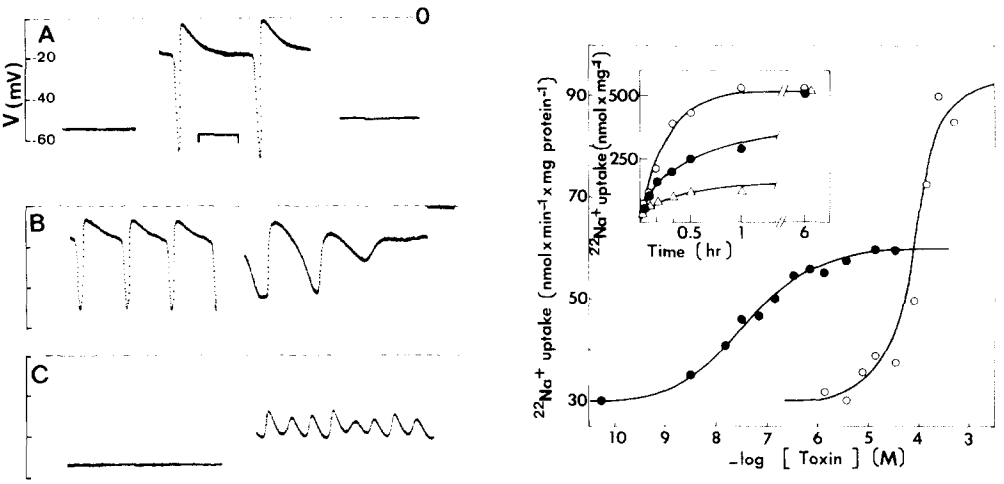

Fig. 3. (A) From left to right: control resting potential of a C_9 cell; spontaneous slow-wave activity after application of sea anemone toxin II (1 μM); after addition of tetrodotoxin (10 μM), the slow-wave activity is blocked. (B) Left: slow-wave activity induced by sea anemone toxin II (1 μM); right: the transformation of this slow wave activity after addition of veratridine (1 μM), the membrane becomes depolarized. (C) Left: absence of effect of veratridine (1 μM); right: after addition of sea anemone toxin II (20 nM), the membrane depolarizes and slow waves of small amplitude occur. In A—C the zero voltage line is indicated. Time calibration: 10 s.

Fig. 4. Sea anemone toxin II and veratridine-activated $^{22}Na^+$ influx into C_9 cells. Inset. The time courses of $^{22}Na^+$ influxes into C_9 cells are measured as described under Materials and Methods, in the presence of 0.5 mM ouabain and without Na^+ channel toxin (△———△) or with 10 μM sea anemone toxin II (●———●) or 133 μM veratridine (○———○). Main panel. Sea anemone toxin II and veratridine dose-response curves: the initial rates of $^{22}Na^+$ influx into C_9 cells are determined as described under Materials and Methods in the presence of 0.5 mM ouabain and the indicated concentrations of sea anemone toxin II (●———●) or veratridine (○———○).

small amplitude (10—20 mV) are induced together with a small depolarization to a resting potential near —35 mV (Fig. 3C).

Activation of $^{22}Na^+$ influx by toxins which alter the gating system of the sodium channel

Veratridine and sea anemone toxin II which are well-known to alter the gating system of the Na^+ channel in a variety of excitable cell systems are also well-known to stimulate the rate of $^{22}Na^+$ entry through the sodium channel of nerve [5,22], skeletal muscle cells [23] and cardiac cells in culture [4,21]. Fig. 4 shows that these toxins stimulate $^{22}Na^+$ influx into non-spiking C_9 cells. All experiments were carried out as usual [5], in the presence of 0.5 mM ouabain to completely inhibit Na^+ efflux via the $(Na^+ + K^+)$-ATPase system. Under these conditions, the inset of Fig. 4 shows that sea anemone toxin II and veratridine stimulate the initial rate of $^{22}Na^+$ influx into C_9 cells. Dose-response curves for the actions of veratridine and sea anemone toxin II on the Na^+ channel of the C_9 cell are presented in Fig. 4. The development of sea anemone toxin II action is observed at concentrations ranging from 5 to 500 nM. The half-maximum effect of the toxin is observed at 38 nM. The development of veratridine action is observed between 10 and 300 μM; its half-maximum effect is observed at 86 μM.

A characteristic of spiking neurones in culture such as neuroblastoma cells which is also shared by spiking cardiac or skeletal muscle cells is that sea anemone toxin II and veratridine act in synergy on the Na^+ channel (Ref. 5 and unpublished results) The same property is found for C_9 cells. The initial rate of $^{22}Na^+$ influx measured for C_9 cells treated by a mixture of 10 μM sea anemone toxin II and 133 μM veratridine reaches a value of 425 $\cdot$ min^{-1} $\cdot$ mg^{-1} protein of cell protein. This stimulation is considerably higher than that observed with either 10 μM anemone toxin II (60 nmol $\cdot$ min^{-1} $\cdot$ mg^{-1} protein), or 133 μM veratridine (75 nmol min^{-1} $\cdot$ mg^{-1} protein).

Dose-response curves for sea anemone toxin II at different concentrations of veratridine and dose-response curves for veratridine at different concentrations of sea anemone toxin II clearly demonstrate this synergy in Fig. 5.

Scorpion neurotoxins are polypeptide toxins with no homology with the sea anemone toxins [25,26]. The main effect of the scorpion toxin used in this work is to slow down the inactivation of the Na^+ channel.

A synergy has also been found between a scorpion neurotoxin and veratridine in their action on the Na^+ channels of spiking neurones [5,24]. The same type of synergy is shown in Fig. 6 for the non-spiking C_9 cell.

Scorpion toxin II from *A. australis Hector* does not stimulate significantly by itself the rate of $^{22}Na^+$ influx into the C_9 cells as previously observed for neuroblastoma cells [5] but a strong stimulation is observed in the presence of veratridine which gives a dose-response curve for the scorpion toxin with a half-maximum effect at 5 nM.

Tetrodotoxin is well-known for its ability to specifically block action potential Na^+ channels of the fast type in various excitable systems [8,9]. As shown in Fig. 7, $^{22}Na^+$ influxes activated by sea anemone toxin II, by veratridine, or by a mixture of sea anemone toxin II and veratridine are always fully inhibit-

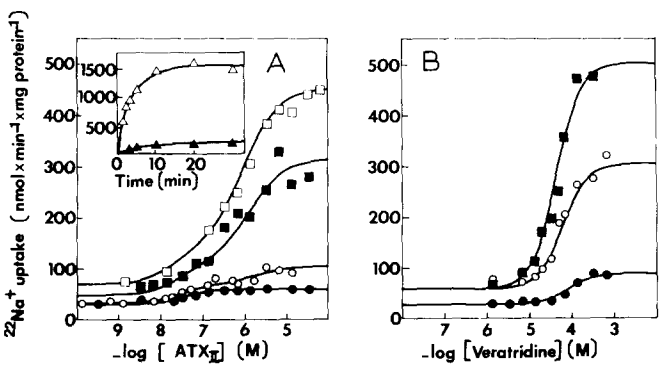

Fig. 5. Synergistic activation of the $^{22}Na^+$ uptake in C_9 cells induced by sea anemone toxin II and veratridine. The initial rates of $^{22}Na^+$ uptake were measured in the presence of 0.5 mM ouabain. (A) The dose-response curves describing the activating effects of sea anemone toxin II were determined without (●———●) or with 10 μM (○———○), 50 μM (■———■) or 133 μM veratridine (□———□). (B) Veratridine dose-response curves were determined in the absence (●———●) or in the presence of 0.5 μM (○———○) or 10 μM sea anemone toxin II (■———■). Inset. $^{22}Na^+$ uptakes were carried out for the indicated times in the presence of 0.5 mM ouabain and without (▲———▲) or with 10 μM sea anemone toxin II plus 133 μM veratridine (△———△). $^{22}Na^+$ uptakes were expressed in nmol Na^+/mg of cell protein.

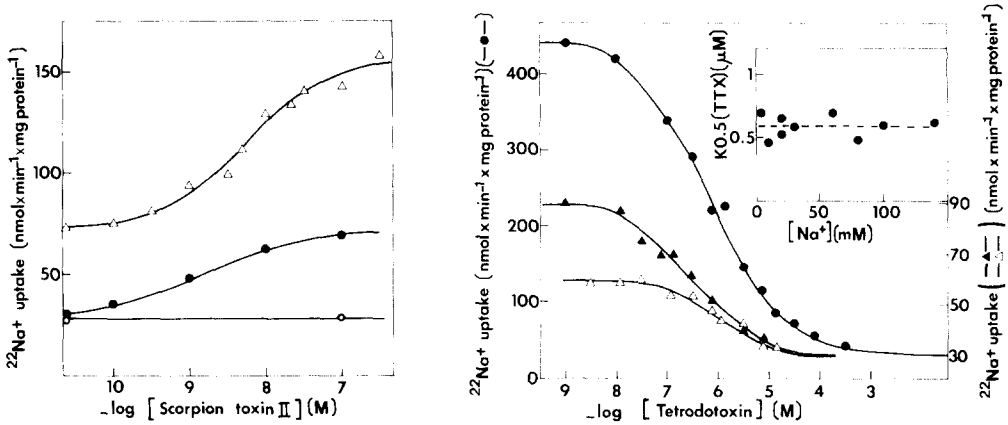

Fig. 6. Synergism between scorpion toxin II from *A. australis Hector* and veratridine in activating $^{22}Na^+$ influx into C_9 cells. The initial rates of $^{22}Na^+$ uptake were measured as described under Materials and Methods in the presence of 0.5 mM ouabain. The scorpion toxin II has no effect in the absence of veratridine (○———○). Its dose-response curves were determined in the presence of 13 μM (●———●) or 133 μM veratridine (△———△).

Fig. 7. Tetrodotoxin inhibition of sea anemone toxin II and veratridine-dependent $^{22}Na^+$ uptake in C_9 cells. $^{22}Na^+$ uptakes were measured at 37° C in the presence of 0.5 mM ouabain. The initial rates of $^{22}Na^+$ uptakes were measured during 1 min. Tetrodotoxin was introduced 30 s before initiating the uptake. Longer times of incubation of the cells up to 10 min give the same results. Experiments were carried out in the presence of 10 μM sea anemone toxin II (△), 133 μM veratridine (▲) or 10 μM sea anemone toxin II plus 133 μM veratridine (●). Inset. Variations of $K_{0.5(\text{tetrodotoxin})}$ as a function of extracellular Na^+ concentration. The $K_{0.5(\text{tetrodotoxin})}$ was determined from inhibition experiments similar to that shown in the main panel, in the presence of 10 μM sea anemone toxin II and 133 μM veratridine and at different external Na^+ concentrations.

able by tetrodotoxin. As expected, the basal $^{22}Na^+$ uptake observed in the presence of ouabain and in the absence of the Na^+ channel toxins is insensitive to tetrodotoxin.

Under standard external Na^+ concentration conditions of 140 mM, the tetrodotoxin concentration required for half-maximal inhibition ($K_{0.5(\text{tetrodotoxin})}$) of $^{22}Na^+$ influxes stimulated by sea anemone toxin II (10 μM) or veratridine (133 μM) are 1.1 and 0.27 μM, respectively. $K_{0.5(\text{tetrodotoxin})}$ is 0.65 μM when measured in the presence of a mixture of sea anemone toxin II (10 μM) and veratridine (133 μM). Tetrodotoxin inhibition experiments have been performed at different sodium concentrations between 1 and 140 mM with C_9 cells previously treated by 10 μM sea anemone toxin II and 133 μM veratridine to stimulate $^{22}Na^+$ influx through the sodium channel. The results are shown in the inset of Fig. 7 where $K_{0.5(\text{tetrodotoxin})}$ is plotted as a function of the external Na^+ concentration. $K_{0.5(\text{tetrodotoxin})}$ does not vary greatly with the Na^+ concentration, all values being between 0.45 and 0.7 μM.

Discussion

The main conclusions of $^{22}Na^+$ flux studies through the fast Na^+ channel in spiking neuroblastoma cells [5] are the following: (i) veratridine and the sea anemone toxin II stimulate $^{22}Na^+$ entry through the Na^+ channel of neuroblastoma cells; (ii) tetrodotoxin suppresses this stimulation, and (iii) scorpion

toxins by themselves are unable to stimulate $^{22}Na^+$ influx but they do it in synergy with veratridine. Veratridine and sea anemone toxin II are also synergistic in their action.

The cultured cells used in this paper have a good resting potential of about —50 mV but, in contrast to neuroblastoma cells, they are unable to generate an action potential. However, $^{22}Na^+$ flux studies carried out in the presence of veratridine, sea anemone toxin II and the scorpion toxin give essentially the same results as those described for neuroblastoma cells [5] or for cardiac cells [4,21] in culture. Comparative properties of association of these toxins with spiking neuroblastoma cells on one hand and non-impulsive C_9 cells on the other hand are presented in Table I. It is perfectly clear from this table that $^{22}Na^+$ flux studies would not permit to detect in the absence of the electrophysiological tool which of the C_9 cell or the neuroblastoma cell is the electrogenic cell. The only marked difference between the two types of cells is the apparent affinity for tetrodotoxin. This apparent affinity differs by two orders of magnitude and the non-spiking cell has the lowest affinity for tetrodotoxin. However, one should not draw any relationship between this low affinity for tetrodotoxin and the unability to generate an electrical signal. Skeletal myotubes in culture generate action potentials, they have a functional fast Na^+ channel although they also have a low affinity for tetrodotoxin (G. Romey et al., unpublished results).

The electrophysiological study of C_9 cells has essentially confirmed the results obtained with $^{22}Na^+$ flux studies. It has shown that although the cell has much of the machinery of a fast Na^+ channel, i.e. a receptor for each one of the toxins tested in this work, the channel is not in a functional state in the membrane which therefore remains non-excitable.

The results of this paper clearly show that immature (or silent) sodium channels might well exist in non-impulsive cells. These channels can be activated chemically in using the gating system toxins but they cannot be activated by an electrical depolarization. The absence of any graded response during depolarizing current steps could be explained in two different ways: (i) the transient opening of the Na^+ channels is unable to give an active response because either the density of Na^+ channels is not sufficient or the value of the leakage conductance is too high compared to that of the activated Na^+ conductance, and (ii) the kinetics of the Na^+ channel are inadequate. The first interpretation seems unlikely. Table I shows that the maximum Na^+ permeability for tetrodotoxin-sensitive Na^+ channels opened by a mixture of veratridine and sea anemone toxin II is nearly three times higher in the C_9 cell as compared to the neuroblastoma cell. Therefore it would seem that the molecular machinery of the fast Na^+ channel is in a concentration three times greater in the C_9 cells. The leakage conductance measured by polarizing pulses is independent of the presence of sea anemone toxin II, the values being between 5 and 10 MΩ. The most probable interpretation is the second one. A fast Na^+ channel necessitates adequate kinetics for the activation and inactivation processes. Experiments in Fig. 3 suggest that some of the non-impulsive Na^+ channels may have inactivation kinetics faster than activation kinetics. In such a case electrical depolarization will not permit the transient accumulation of an open form of the Na^+ channel. The channel will remain closed at all

TABLE I

A COMPARATIVE STUDY BETWEEN C_9 AND NEUROBLASTOMA CELLS: PROPERTIES OF ASSOCIATION OF SEA ANEMONE TOXIN II AND VERATRIDINE WITH THE Na^+ IONOPHORE AND THEIR EFFECTS ON THE $^{22}Na^+$ INFLUXES

The maximum Na^+ permeability ($P_{Na^+}(max)$) is related to the maximum $^{22}Na^+$ influx ($J_{Na^+}(max)$), the external Na^+ concentration $[Na^+]_0$ and the membrane potential (V) by the Goldman equation [27]:

$$J_{Na^+}(max) = P_{Na^+}(max) \times [Na^+]_0 \frac{F}{RT} \frac{V}{e^{VF/RT} - 1};$$

$[Na^+]_0$ = 140 mM and the mean value of V is −5 mV for both C_9 cells and neuroblastoma cells in the presence of saturating concentrations of veratridine and sea anemone toxin II (unpublished data).

	C_9 cells	Neuroblastoma cells [5]
Unstimulated $^{22}Na^+$ influx (insensitive to tetrodotoxin)	30 nmol · min^{-1} · mg^{-1} of protein	15 nmol · min^{-1} · mg^{-1} of protein
$^{22}Na^+$ influx stimulated by a saturating concentration of sea anemone toxin II (tetrodotoxin inhibitable)	30 nmol · min^{-1} · mg^{-1} of protein	23 nmol · min^{-1} · mg^{-1} of protein
$^{22}Na^+$ influx stimulated by a saturating concentration of veratridine (tetrodotoxin inhibitable)	60 nmol · min^{-1} · mg^{-1} of protein	80 nmol · min^{-1} · mg^{-1} of protein
$^{22}Na^+$ influx stimulated by a mixture of veratridine and sea anemone toxin II at saturating concentrations (tetrodotoxin inhibitable), $J_{Na^+}(max)$	500 nmol/min per mg protein	147 nmol/min per mg of protein
Membrane area S	13.2 cm^2 · mg^{-1} of protein	11.2 cm^2 · mg^{-1} of protein
$P_{Na^+}(max)$	$4.9 \cdot 10^{-6}$ cm · S^{-1}	$1.7 \cdot 10^{-6}$ cm · S^{-1}
$K_{0.5}$ (sea anemone toxin II)	0.038 μM	0.15 μM
$K_{0.5}$ (veratridine)	86 μM	44 μM
$K_{0.5}$ (scorpion toxin)	scorpion toxin II : 5 nM	scorpion toxin I : 0.25 nM
$K_{0.5}$ (tetrodotoxin)	0.5 μM	5 nM

potentials. A possible way to correct this situation is to treat the cell with the sea anemone toxin which specifically and considerably slows down the inactivation of the Na^+ channel. In such a situation, if after treatment with the sea anemone toxin II the inactivation step becomes slower than the activation step one should then observe the generation of an electrical signal. This is indeed the observation which is made in Fig. 3. Sea anemone toxin II turns out to be a very interesting toxin to reveal electrophysiologically fast Na^+ channel.

Acknowledgments

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