Vol. 107, No. 1, 1982 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
July 16, 1982 Pages 202-208

PROPERTIES OF Na^+ CHANNELS IN FIBROBLASTS

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Received May 28, 1982

SUMMARY : The Na^+ channels of Chinese Hamster lung fibroblasts have receptor sites for tetrodotoxin, batrachotoxin, veratridine, dihydrograyanotoxin, scorpion and sea anemone toxins. The binding properties of these toxic compounds were determined and shown to be very similar to those found in a variety of excitable cells. Electrophysiological experiments indicate that these Na^+ channels cannot be electrically activated unless previously treated by veratridine.

INTRODUCTION : The voltage dependent Na^+ channel that can be inhibited by tetrodotoxin is an essential component of the membrane of excitable cells, such as neuronal cells (1, 2), cardiac (3) and skeletal muscle cells (4), in which it is essential for the generation of action potentials. This Na^+ channel has also been found in a variety of non-excitable cells, including C9 cells (5), Schwann cells (6), glial cells (7) and fibroblasts from different cell lines (8). In this paper we describe some of the properties of the Na^+ channel present in a cell line from Chinese Hamster lung fibroblasts.

MATERIALS AND METHODS : Veratridine was obtained from the Sigma Chemical Company and TTX from Sankyo. BTX was kindly given to us by Drs J. Daly and T. Tokuyama. DHG was a generous gift from Drs. M. Nakajima and Ryozo Irize. Sea anemone toxins II and V were prepared from Anemonia sulcata according to Schweitz et al. (9). The scorpion Androctonus australis toxin II was purified as previously described (10). $^{22}NaCl$ was purchased from the CEA. [^3H] en-TTX (26 Ci/mmol) was prepared according to Chicheportiche et al. (11).

Cells from the Chinese Hamster lung fibroblast line (ATCC CCl39) were grown as previously described (8). For electrophysiological experiments confluent cultures of fibroblasts were incubated in the following medium : 115 mM NaCl, 5.4 mM KCl, 0.4 mM $MgSO_4$, 1.8 mM $CaCl_2$, 5 mM glucose, 25 mM Hepes-NaOH at pH 7.4. The temperature was maintained at 34°C ± 1°C. Intracellular recordings were made using glass microelectrodes filled with 3 M KCl, which had resistances between 30 and 50 MΩ. The microelectrode was connected to a ne-

ABBREVIATIONS : TTX : tetrodotoxin, [^3H] en-TTX : tritiated ethylenediamine tetrodotoxin, BTX : batrachotoxin, DHG : dihydrograyanotoxin$_{II}$, ScTX : scorpion neurotoxin, ATX : sea anemone toxin.

0006-291X/82/130202-07$01.00/0

gative capacitance electrometer amplifier (WPI, M 707) with an active bridge network which made possible simultaneous injections of current and recordings through the same microelectrode. Only recordings that were stable for at least 20 minutes were considered.

$^{22}Na^+$-uptake experiments and binding assays of [^{3}H] en-TTX to fibroblast homogenates were carried out using experimental conditions defined previously (4).

RESULTS AND DISCUSSION : Density inhibited CCL39 fibroblasts had a mean resting potential of 51 ± 4 mV. The input resistance around the resting potential varied between 5 and 15 MΩ, depending on the cell size. The membrane of CCL39 fibroblasts exhibited passive responses to depolarizing pulses of current, indicating that the cell is of the non-impulsive type. The current-voltage relationship was linear over the range -100 to -20 mV. Treatment of CCL39 fibroblasts with 100 µM veratridine for a period of 10 min had no effect on either the resting potential or the input resistance around the resting potential. However, slow action potentials could be ellicited in veratridine-treated cells by depolarizing pulses of current after adjustment of the membrane potential to a steady hyperpolarized level of -80 to -90 mV (Fig 1 A). The threshold potential was about -50 mV. The application of 10 µM ATX_{II} in the absence of veratridine had no effect on the electrical properties of these cells but the addition of 1 µM ATX_{II} to cells that had been previously treated with 100 µM veratridine increased the amplitude of the slow action potentials observed with veratridine alone (Fig 1 B).

The properties of Na^+ channels in fibroblasts were further characterized using $^{22}Na^+$ flux studies. Na^+ channels in excitable cells possess several classes of receptor sites for neurotoxins (1-4). One class of site is specifically recognized by molecules such as tetrodotoxin and saxitoxin, which block the Na^+ channel. A second class of site recognizes the lipid-soluble neurotoxins (veratridine, BTX, DHG). A third type of receptor site is specific for polypeptide neurotoxins from sea anemone and scorpion venom. Lipid soluble toxins alter the gating system of the Na^+ channel and stabilize an open conformation of the channel by removing inactivation (12, 13). In doing so they increase the rate of $^{22}Na^+$ uptake in cultured excitable cells (1-5). Polypepti-

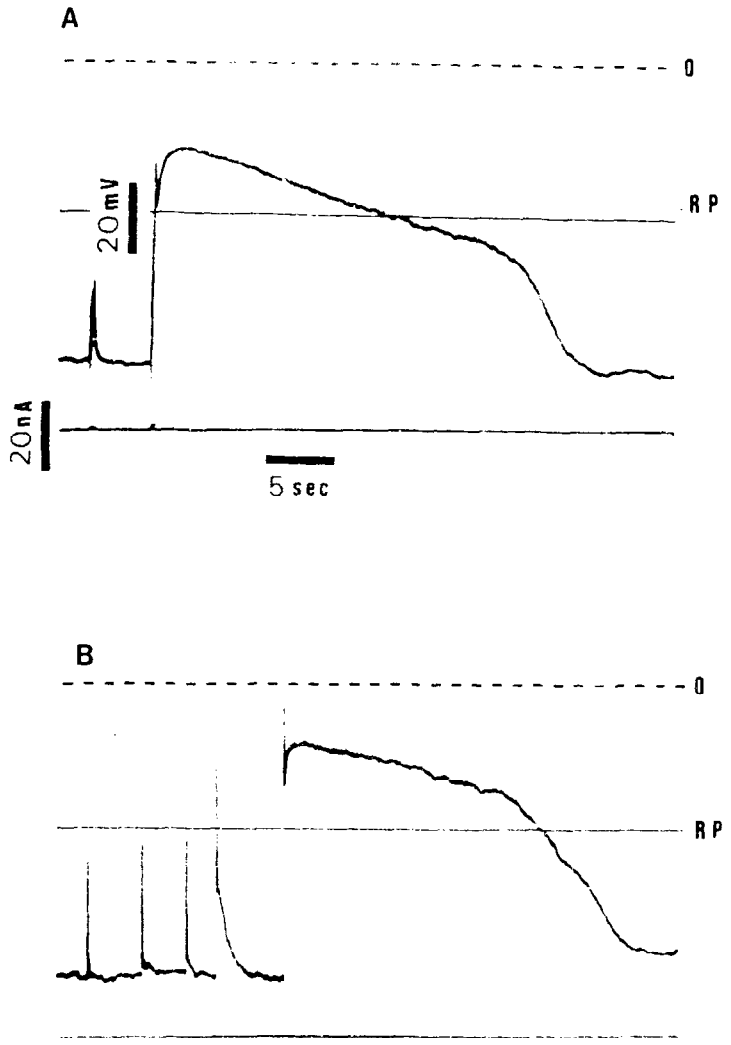

Fig 1. Electrophysiological properties of CC139 fibroblasts.
A. Induction of excitability in CC139 fibroblasts by 100 μM veratridine. Subthreshold response and action potential ellicited by brief depolarizing pulses of current.
B. Enhancement by 1 μM ATX_{II} of the amplitude of the action potential produced by 100 μM veratridine. Membrane potential was adjusted to a steady level of -90 mV (A) and -80 mV (B) by passing a constant current. Upper trace : the zero voltage line. Lower trace : current trace. The resting potential (RP) is indicated.

de toxins slow down the inactivation step of the Na^+ channel (14) and act in synergy with lipid soluble toxins to increase the rate of $^{22}Na^+$ uptake (1-5).

The initial rate of $^{22}Na^+$ uptake by fibroblasts was increased from 5 to 10 nmol/min/mg of protein in the presence of 10 μM BTX and to 22 nmol/min/mg of protein in the presence of 10 μM BTX plus 10 μM ATX_{II}. Mixtures of ATX_{II} and veratridine or DHG also increased the initial rate of $^{22}Na^+$ uptake by

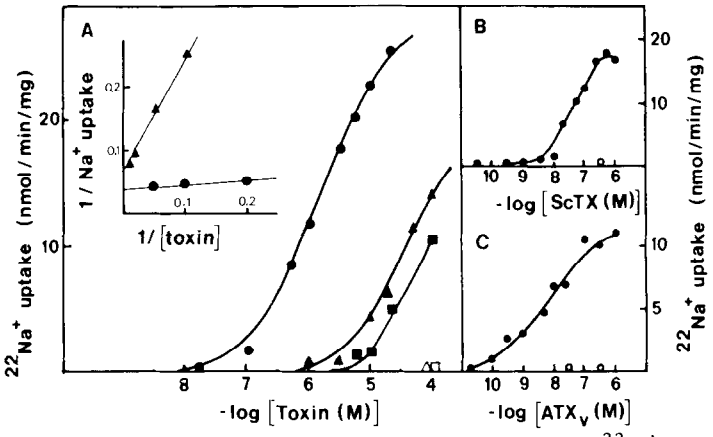

Fig 2. The stimulating effect of neurotoxins on the rate of $^{22}Na^+$ uptake by CCl39 fibroblasts.

A. Effects of veratridine (▲), BTX (●) and DHG (■) on the rate of $^{22}Na^+$ uptake measured in the presence of 10 μM ATX_{II} (closed symbols) or in its absence (open symbols). The basal, TTX insensitive rate of $^{22}Na^+$ uptake (5 nmol/min/mg of protein) was substracted from all experimental data. Inset; Double reciprocal plot of veratridine (▲) and BTX (●) effects on the initial rate of $^{22}Na^+$ uptake.

B. Effect of scorpion neurotoxin on $^{22}Na^+$ uptake measured in the presence of 100 μM veratridine (●) or in its absence (○).

C. Effect of ATX_V on $^{22}Na^+$ uptake measured in the presence of 1 μM BTX (●) or in its absence (○). Flux experiments were performed on Na^+-depleted fibroblasts as previously described (4). The uptake period was 1 to 3 minutes. The external Na^+ concentration was 10 mM. Ouabain (0.5 mM) was used to prevent $^{22}Na^+$ efflux catalyzed by the (Na^+,K^+)ATPase.

fibroblasts. Dose-response curves for the effect of BTX, veratridine and DHG on $^{22}Na^+$ uptake are presented in Fig 2 A. Double reciprocal plots of the data indicate that BTX was the most potent toxin with a half maximum effect ($K_{0.5}$) at 1 μM. $K_{0.5}$ values for veratridine and DHG were 40 and 60 μM, respectively. This order of efficiency is similar to that observed for neuroblastoma cells and for skeletal muscle cells in culture (1, 4). The flux of $^{22}Na^+$ through the Na^+ channel in fibroblasts was higher at saturating concentrations of BTX (28 nmol/min/mg of protein) than at saturating concentrations of veratridine or DHG (18 nmol/min/mg of protein). Fig 2 B and C show the activating effect of polypeptide neurotoxins on the rate of $^{22}Na^+$ uptake by fibroblasts, measured in the presence of lipid-soluble toxins. The half maximum effect of ATX_V in the presence of 1 μM BTX was observed at 0.1 μM (Fig 2 C). The half maximum effect for ScTX in the presence of 100 μM veratridine was observed at 50 nM (Fig 2 B).

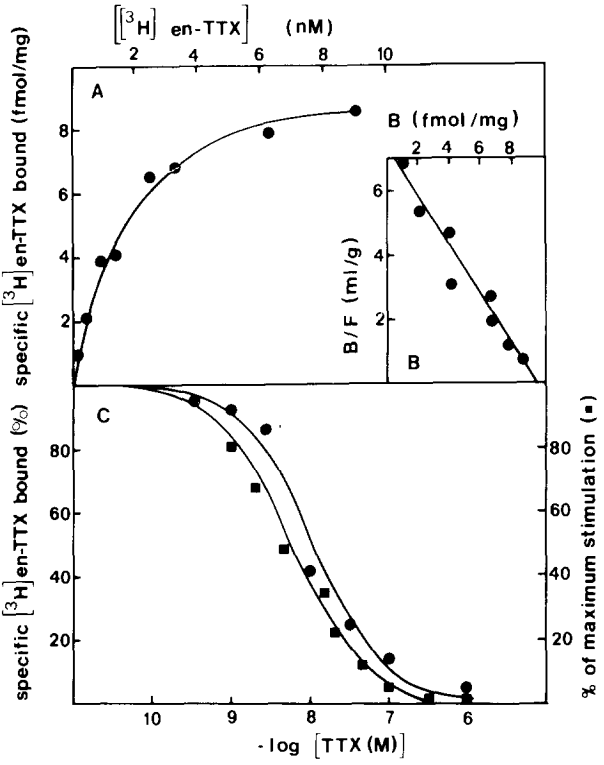

Fig 3. Interaction of TTX with the Na^+ channel of fibroblasts

A. Specific binding of [^{3}H] en-TTX to fibroblast homogenates as a function of free [^{3}H] en-TTX concentration.

B. Scatchard plot of the data.

C. Inhibition by TTX of the specific [^{3}H] en-TTX binding to the Na^+ channel of fibroblasts (●). The EC_{50} for TTX inhibition of the specific [^{3}H] en-TTX binding is 10 nM. The equilibrium dissociation constant of the TTX-receptor (K_D (TTX)) complex is derived from

$$K_D\ (TTX) = \frac{EC_{50}}{1 + \frac{[[^3H]\ en\text{-}TTX]}{K_D\ ([^3H]\ en\text{-}TTX)}}$$

were [[^{3}H]en-TTX] is the free radioligand concentration at half dissociation (5 nM) and K_D ([^{3}H] en-TTX) is the dissociation constant of the [^{3}H] en-TTX receptor complex derived from Fig 2 B (1.2 nM). Inhibition by TTX of the rate of $^{22}Na^+$ uptake stimulated by a mixture of 100 µM veratridine and 10 µM ATX_{II} (■).

The stimulation of $^{22}Na^+$ uptake produced by toxin mixtures that open the Na^+ channel was suppressed by TTX, as shown in Fig 3C. Half maximum inhibition occurred at 5 nM TTX.

The biochemical characterization of Na^+ channels in fibroblasts was carried out with the use of a highly radiolabelled derivative of TTX : [^{3}H] en-TTX. Specific [^{3}H] en-TTX binding to fibroblasts was a saturable process (Fig 3 A) and a Scatchard plot of the specific binding is linear (Fig 3 B),

indicating the presence of a single class of sites, a dissociation constant for the [^{3}H]en-TTX receptor complex of 1.2 nM and a maximum binding capacity of 9 fmol/mg of cell protein. Specific [^{3}H]en-TTX binding was competitively inhibited by TTX (Fig 3 C). The EC_{50} value was 10 nM and the true dissociation constant of the TTX receptor complex was 2 nM (see legend of Fig 3), a value very similar to that found for TTX inhibition of toxin-activated rates of ^{22}Na uptake (5 nM, Fig 3 C). The dissociation constant obtained from [^{3}H]en-TTX binding data is similar to those found for other excitable cells (15). However there is a difference in the number of Na^+ channels expressed per mg of cell protein. Na^+ channels are 5 times less abundant in fibroblasts than they are in neuroblastoma cells, chick skeletal or cardiac muscle cells (15).

In conclusion, Na^+ channels in fibroblasts have receptor sites for TTX, BTX, veratridine, DHG, scorpion and sea anemone toxins and the binding properties of these toxic compounds are similar to those found in excitable cells. Na^+ channels in fibroblasts are chemically activated by BTX or by mixtures of lipid soluble toxins and polypeptide toxins. However, there is no electrophysiological evidence that they can be electrically activated to produce normal action potentials. This apparent lack of electrical excitability of fibroblasts is probably due to one of the following reasons.
(i) The number of Na^+ channels per cell is too low to produce a high enough Na^+ current to generate an action potential. (ii) The Na^+ channel is normally inactivated at the resting potential and remains inactivated even after the long hyperpolarization to -80 to -90 mV used in the electrophysiological experiments presented in Fig 1. (iii) The kinetics of the gating system are inadequate ; the activation of the m gate being slower than the closing of the h gate, as previously suggested (5). Recording of single channel activities will probably be necessary to distinguish among these three possibilities.

AKNOWLEDGEMENTS : We are grateful to M.T. Ravier, and M. Valetti for expert assistance and to Dr. K.P. Wheeler for a careful reading of the manuscript. This study was supported by the Centre National de la Recherche Scientifique, l'Aide au Développement pour la recherche sur le Cancer, the Institut National de la Santé et de la Recherche Médicale grant no 80.30.10 and the Délégation Générale de la Recherche Scientifique et Technique grant no 81.E.05.75.

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