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Membrane excitability expressed in human neuroblastoma cell hybrids

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The voltage-sensitive Na^+ channel is responsible for the action potential of membrane electrical excitability in neuronal tissue. Three methods were used to demonstrate the presence of neurotoxin-responsive Na^+ channels in two hybrid cell lines resulting from the fusion of excitable human neuroblastoma cells with mouse fibroblasts. Only one of the two electrically active hybrid cell lines maintained the sensitivity of the neuroblastoma parent to tetrodotoxin (TTX). The other hybrid, although electrically active, was not responsive to TTX or scorpion venom. Comparisons of the patterns of expression of membrane excitability and of chromosome complements in these human neuroblastoma cell hybrids suggest that the phenotype of membrane excitability is composed of genetically distinct elements.

Voltage-sensitive Na^+ channel; Karyotype; Neurotoxin; Cell hybrid; (Human, Mouse)

1. INTRODUCTION

The voltage-sensitive Na^+ channel is responsible for the rising phase of the action potential during electrical excitability in neuronal, muscle and endocrine tissue. The molecular basis for the functional properties has been studied by molecular biological [1] and biochemical methods [2]. Several facts have emerged from these studies which suggest that more than one polypeptide may be involved in the formation and maintenance of Na^+ channels with normal kinetics. First, three distinct Na^+ channels have been described in rat brain as a result of cloning and sequencing of complementary DNAs specific for the large polypeptide termed the α subunit [1]. Second, although mRNA from the α subunit can direct the functional expression of a voltage-sensitive channel full inactivation was observed only by the addition of low molecular mass mRNAs [3]. Third, a low molecular mass subunit, β_1 was preferentially labeled with β-ScTX in cultured brain neurons [4]. Fourth, Na^+ channel activity in a rat brain system appears to require both α and β_1 subunits [5].

The presence of a smaller subunit is not universal, however, since a single polypeptide, an α subunit, from the electric eel [6] and mouse neuroblastoma [7] forms active Na^+ channels when reconstituted into artificial phospholipid vesicles. To complicate the issue is the fact that small peptides synthesized from the information obtained from the cDNA sequence [1] formed partially functional Na^+ channels in an artificial bilayer [8].

Somatic cell hybrids formed from fusion of human neuroblastoma cells CHP 134, which ex-

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Abbreviations: Hybrid cell lines: N-5, N4BTP; N-13, N4HTP-13; N-8, N4DTP-8; and N-10, N2DTP-10; TPMP, tritiated triphenylmethyl phosphonium bromide; HAT, hypoxanthine, aminopterin and thymidine; VTD, veratridine; TTX, tetrodotoxin; ScTX, *Leiurus quinquestriatus* scorpion venom

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press membrane excitability [9], with mouse fibroblasts, IT-22 [10], which do not, are useful for studying the expression of this differentiated phenotype [11]. CHP 134 contains a stable near diploid chromosomal complement [10]. Using hybrids which resulted from such a fusion we present data which support the concept that multiple components are necessary for the full expression of the excitable Na^+ channel and that there is discordancy in the chromosomal origin of these components.

2. MATERIALS AND METHODS

The parent cell lines, human neuroblastoma, CHP 134 and mouse fibroblasts, IT-22, were grown as described [9]. The fusion was accomplished using inactivated Sendai virus; hybrid cells were selected in HAT medium and ouabain and cloned from single cells [10]. The human chromosome complements of each clone were determined by the analysis of G-banded and G-11 stained metaphases [12]. Isoenzyme analysis was used to distinguish mouse and human forms of individual enzymes mapped to particular chromosomes [13].

The cells were exposed to 2% DMSO for 4 days prior to analysis [9]. Ion flux through the Na^+ channel was measured by the efflux of ^{86}Rb (6 Ci per mg; New England Nuclear) before and after stimulation with 100 μM VTD (Sigma) and ScTX (5 μg/ml; Sigma) [14]. Membrane potentials were calculated from the equilibrium distribution of TPMP [15]. Recordings of resting membrane potentials and spontaneous electrical activity were made by standard electrophysiological techniques [15]. The membrane potentials were measured both before and after the addition of the depolarizing neurotoxin, 100 μM VTD. All studies were repeated in the presence of 1 μM TTX (Calbiochem-Behring), a neurotoxin that specifically inhibits ion flux through the Na^+ channel [2,14].

3. RESULTS AND DISCUSSION

3.1. *Hybrids with excitable membranes*

^{86}Rb efflux in response to VTD and ScTX was examined in all the hybrids. Two hybrids, N-5 and N-13, responded to VTD but only one, N-5, also responded to ScTX and TTX. The rate constants of VTD/ScTX-stimulated ^{86}Rb efflux [9,14] for CHP 134 and the VTD responsive hybrids N-5 and N-13, were 0.688 min^{-1}, 0.409 min^{-1}, and 0.44 min^{-1}, respectively. N-13 responded to VTD alone since concomitant addition of ScTX did not produce the synergistic increase in ^{86}Rb efflux previously reported for CHP 134 [9]. Moreover, the effect of VTD was not inhibited by TTX, as it was in N-5 and CHP 134.

It was necessary to verify by other methods that N-13 did not respond to TTX even though it was stimulated by VTD. Therefore, the membrane potentials were measured directly by microelectrode puncture or by the equilibrium distribution of TPMP (table 1). The introduction of VTD to the medium resulted in depolarization of N-13 and CHP 134 as determined by both intracellular recording and the equilibrium distribution of TPMP (table 1). Concurrent exposure of the cells to VTD and TTX resulted in depolarization of N-13 as with VTD alone, whereas depolarization of CHP 134 cells was inhibited by TTX (table 1). Therefore, N-13 did not express the TTX sensitivity associated with membrane electrical activity.

The action potentials recorded from N-13 were similar in appearance to those recorded for CHP 134, although the latter were larger in amplitude and had a shorter rise time. In both, the action potential was characterized by a relatively slow rising spike followed by a prolonged repolization phase. A few cells showed patterns resembling those recorded from mouse neuroblastoma × mouse L-cell hybrids [16], while others exhibited prolonged slow depolarization of the type that has been associated with Ca^{2+} channels in cultured mouse neuroblastomas [17]. The resting membrane potentials (table 1) were higher than those reported for mouse neuroblastoma [18], although consistent with those for mammalian neurons [19].

3.2. *Hybrids without excitable membranes*

Many hybrids obtained were inactive. One hybrid, N-10 was examined with respect to ^{86}Rb efflux and membrane potentials. No action potentials could be detected in N-10; the introduction of VTD gave no change in the resting potential of these cells. In addition, the equilibrium distribution of TPMP was unchanged (table 1). Examples of the rate constants for the efflux of ^{86}Rb after addition of VTD/ScTX for two inactive hybrids. N-10 and N-8 were 0.133 min^{-1} and 0.21 min^{-1}, respectively. The rate constants for ^{86}Rb flux prior to the addition of neurotoxins were less than 0.24 min^{-1} in all of the hybrid clones and the parent lines.

3.3. *Karyotypes of hybrid clones*

In the limited study of the 4 hybrid cell lines with

Table 1

Membrane electrical activity in human neuroblastoma CHP 134 and neuroblastoma hybrid clones, N-10 and N-13

Cell line	Membrane potential (mV)						Spontaneous action potentials
	Microelectrode[a]			TPMP[b]			
	No toxin	VTD	VTD/TTX	No toxin	VTD	VTD/TTX	
CHP 134	−75 ± 3(82)	−48 ± 2(51)	−68 ± 8(27)	−81	−57	−75	yes
	−73 ± 3(83)			−88	−60		
N-10	−70 ± 6(68)	−67 ± 8(43)	−65 ± 7(30)	−86	−90		no
				−86	ND	−67	
				−91	−91		
N-13	−63 ± 4(73)	−50 ± 5(67)	−52 ± 6(33)	−75	−65	−55	yes
				−97	−84		

[a] Measured by intracellular recording at rest and in the presence of 100 μM VTD and 1 μM TTX. Numbers in parentheses represent the number of cells from which microelectrode measurements were made

[b] Calculated from the equilibrium distribution of TPMP

differing responses to neurotoxins, the retention or loss of no single human chromosome could be correlated with the continued expression of membrane electrical activity. From the data presented (table 2), chromosomes 1, 6, 9 and 15 were ruled out as being involved in membrane electrical activity, as none of these chromosomes were present in either of the excitable hybrids, N-5 and N-13. The genes responsible for VTD sensitivity may be on chromosomes 3, 8, 10, 16 and/or 22, as these chromosomes were present in both excitable hybrids N-5 and N-13 and absent in either one or the other of the nonexcitable hybrids, N-3 or N-10. Genetic factors that determine sensitivity to TTX may reside on chromosomes 2, 4, 12, 18, 19, or 20, because these were the only chromosomes that differed between TTX sensitive, N-5 and TTX insensitive, N-13. Roses [20] has suggested on the basis of studies utilizing a cDNA probe to the Na^+ channel that a gene may reside on chromosome 4 and/or 16. A precise assignment of the genes responsible for the expression of neurotoxin sensitivity to particular human chromosomes will require a detailed analysis of many additional toxin sensitive and insensitive hybrids and additional cDNA probes.

The fusion of human neuroblastoma cells that express membrane electrical activity with mouse

Table 2

Chromosomal assignment of excitable membrane properties

Clone	Human chromosomes[a]																					
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22
Excitable membrane[b]																						
N-5 TTX sensitive	−	+	*	*	+	−	no	*	−	+	+	+	*+	+	−	*	*	+	+	+	+	*
N-13 TTX insensitive	−	−	*	no	+	−	no	*	−	+	+	−	*+	+	−	+	*	−	−	−	+	*
Nonexcitable membrane[c]																						
N-8	*+	+	*	*	+	+	no	*	−	−	+	−	*+	+	−	*	*	+	+	+	+	*
N-10	+	+	no	no	+	+	no	no	+	+	+	+	+	+	−	no	*	+	+	+	+	no

[a] + and −: presence or absence based on human isoenzyme assigned to that chromosome
* and no: presence or absence of particular human chromosomes in metaphase spreads of cells from each hybrid clone

[b] Spontaneous action potentials and/or neurotoxin-stimulated ^{86}RB efflux

[c] No response to neurotoxins of membrane potential and/or ^{86}Rb efflux

fibroblasts that lack this capacity resulted in two hybrid clones which retained functional properties of voltage-dependent Na^+ channels. The fact that the VTD, ScTX and TTX sensitivities segregated discordantly in these hybrids, suggests that the expression of a fully functional Na^+ channel with particular characteristics may involve the interaction of genes specifying one or more protein subunits, as well as trans-acting factors and genes whose products (e.g. glycosyltransferases) are responsible for post-translational modification of the proteins [21]. All of these are consistent with our finding that the interaction of genes on more than one chromosome is required for full expression of membrane electrical excitability in human cells.

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