REVIEW

Voltage-dependent $Na_v1.7$ Sodium Channels: Multiple Roles in Adrenal Chromaffin Cells and Peripheral Nervous System

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ABBREVIATIONS

ACh, acetylcholine; Atr, atropine; DRG, dorsal root ganglion; HERG, human ether-a-go-go-related gene; IEM, inherited erythermalgia; oxo, oxotremorine; PbTx-3, *Ptychodiscus brevis* toxin-3; PEPD, paroxysmal extreme pain disorder; PKC, protein kinase C; SCG, superior cervical ganglion; STX, saxitoxin; TTX, tetrodotoxin.

1. Overview of Na^+ channel family

Besides generating and propagating action potentials in the established neuronal circuits, multiple lines of evidence have documented that sodium channels engrave neuronal network per se from early in embryonic development through adulthood via the following actions (e.g. axon myelination; differentiation of neurites into single axon and multiple dendrites, forming neuronal polarity; attractive and repulsive navigation of axon growth cone; experience/learning-driven cognition) (Wada 2006). Particularly, deprivation of visual experience impaired visual system development. Even in adult human brains, piano playing increased axon myelination. Abnormal remodeling of wild-type or otherwise silent sodium channel isoforms is responsible for various neuronal diseases (e.g. pain; multiple sclerosis) (Wallace *et al.* 1998; Diss *et al.* 2001; Meisler & Kearney 2005; Wada 2006).

Sodium channels consist of the principal α-subunit (~ 260 kDa), without or with a noncovalently-attached β_1- or β_3-subunit, and a disulfide-linked β_2- or β_4-subunit (Isom 2002; Catterall *et al.* 2005; Wada 2006). The α-subunit is composed of four homologous domains (I-IV), each containing six transmembrane segments (S1-S6), and forms the ion-pore and the five distinct toxin binding sites, including site 1 for tetrodotoxin (TTX)/saxitoxin (STX), site 2 for veratridine, site 3 for α-scorpion toxin, site 4 for β-scorpion toxin and site 5 for *Ptychodiscus brevis* toxin (PbTx) (Wada *et al.* 1992; Yuhi *et al.* 1994; Cestèle & Catterall 2000; Shiraishi *et al.* 2001a; Oliveira et al., 2004; Catterall *et al.* 2002, 2005; Schiavon *et al.* 2006). The nine α-subunit isoforms ($Na_v1.1$-$Na_v1.9$) arise from nine different genes (SCN1A-SCN5A and SCN8A-SCN11A) (Catterall *et al.* 2005). β-Subunit is type 1 transmembrane protein harboring a single membrane-spanning region (Isom 2002; Kim *et al.* 2005; Wong *et al.* 2005). Surprisingly, the extracellular domain of β-subunit contains a single immunoglobulin V-set fold structure, that enables the β-subunit to interact with cell adhesion molecules (e.g. neurofascin), extracellular matrix proteins (e.g. tenascin), and intracellular scaffold proteins (e.g. ankyrin) (Isom 2002). In this context, it should be noted that β-subunits normally accelerate gating kinetics in the oocyte expression system (Ji *et al.* 1994; Wallace *et al.* 1998). On the contrary, more recent data obtained in mammalian expression systems suggest also other forms of modulating the biophysical properties such as the mid point of the steady-state inactivation (Meadows *et al.* 2002). Moreover, they modulate cell surface targeting of sodium channels, as evidenced by expression studies (Isom 2002).

Interestingly, Kim *et al.* (2005) and Wong *et al.* (2005) documented that either β-subunit was sequentially cleaved by α-, β-, or γ-secretase, enzymes involved in Alzheimer's disease; the β-subunit cleavage event culminated in the regulation of cell adhesion and migration of Chinese hamster ovary cells and neuroblastoma.

Klugbauer *et al.* (1995) documented that bovine adrenal gland expresses new member of TTX/STX-sensitive human neuroendocrine sodium channel (hNE-Na), also called PN1 (Toledo-Aral *et al.* 1997) and $Na_v1.7$ (Catterall *et al.* 2002, 2005); $Na_v1.7$ was distributed to peripheral sympathetic and sensory ganglia (e.g. superior cervical ganglion [SCG] and dorsal root ganglion [DRG]) (Toledo-Aral *et al.* 1997). In DRG neurons and PC12 cells, $Na_v1.7$ was localized predominantly in the axon growth cone (Toledo-Aral *et al.* 1997), a neuronal compartment thought to be pivotal to correct synapse formation (Wada 2006).

2. $Na_v1.7$ in adrenal chromaffin cells

2.1. Regulation of Na^+ influx via $Na_v1.7$

In 1976, the pioneering studies documented that cultured adrenal chromaffin cells from adult human, gerbil, and rat generated action potentials spontaneously or in response to depolarized current, the latter action potentials being blocked by TTX (Biales *et al.* 1976; Brandt *et al.* 1976). Acetylcholine (ACh) released from the splanchnic nerve terminals normally stimulates adrenaline and noradrenaline release from chromaffin cells. These neurotransmitters are preferentially released from two sets of cells that can be recognized by specific antibodies, by the histamine response or the presence of human ERG (ether-a-go-go-related gene) potassium channels (Gullo *et al.* 2003). It has been originally shown that stimulation of nicotinic (but not muscarinic) receptors by ACh increased the spike frequency in a concentration-dependent manner between 0.1 and 100 μM (Brandt *et al.* 1976; Kidokoro *et al.* 1982).

By using either the specific muscarinic or nicotinic agonists (oxotremorine or nicotine, respectively) or ACh without or with atropine (Atr, muscarinic antagonist), we here show that the rat chromaffin cells respond with dramatically different hyper- or depolarizing responses. This is shown in Fig. 1, upper panel (for different oxotremorine [oxo] concentrations) and nicotine; and in Fig. 1, lower panel (for two ACh concentrations [1 and 10 μM]). These data clearly suggest that chromaffin cells spontaneously fire from resting potentials of about -55 mV and, if stimulated by nicotine or ACh + Atr, respond with a firing increase that can reach complete depolarization and firing blockade. On the contrary, low concentrations of oxotremorine are sufficient to decrease the spontaneous firing (Fig. 1, upper, 1st panel). Higher concentrations of oxotremorine produce a transient and strong hyperpolarization of about 20 mV caused by the muscarinic ACh receptor-induced $[Ca^{2+}]_i$ increase (Fig. 1, upper, 2nd and 3rd panels). Physiological stimulations by ACh produce sequentially the muscarinic and the nicotinic responses (Fig. 1, lower, 1st and 4th panels), and atropine is able to cancel the former one.

FIG. 1 near here

In cultured bovine adrenal chromaffin cells, veratridine increased $^{22}Na^{+}$ influx, stimulating $^{45}Ca^{2+}$ influx via voltage-dependent calcium channels and catecholamine secretion (Wada *et al.* 1985a, b); these cellular events induced by veratridine were enhanced by α- or β-scorpion venom, or PbTx-3 (Wada *et al.* 1992; Yuhi *et al.* 1994; Yokoo *et al.* 2000; Shiraishi *et al.* 2001a, b, 2003; Kajiwara *et al.* 2002), or inhibited by various drugs [e.g. TTX, phenytoin (Wada *et al*. 1985a, b, 1987), ketamine (Takara *et al.* 1986), antidepressants (Arita *et al.* 1987), neuroprotective riluzole (Yokoo *et al.* 1998, 2000), antihypertensive carvedilol (Kajiwara *et al.* 2002), and local anesthetics (Shiraishi *et al.* 2003)].

2.2. Biophysical properties of $Na_v1.7$

Of the 9 genes encoding distinct isoforms identified in the human genome, some are expressed specifically only in one tissue, but others are distributed in many different tissues. The proteins are assembled with a 4-fold symmetry consisting of structurally homologous domains (D1–D4), each containing six membrane-spanning segments (S1–S6) and a region (S5–S6 pore loop) controlling ion selectivity and permeation. In particular, the four S4 segments function as voltage sensors and, according to their domain position, control both the probability of opening (activation) and partially the mechanism of inactivation (located on the cytoplasmic face).

Originally, Hodgkin and Huxley (1952) described, by a series of kinetic relationships dependent on membrane potential, their voltage-clamp data. They introduce the concept of three functional states, namely closed, open and inactivated. These definitions are still valid and help investigators to compare the biophysical properties of wild-type channels by using peptide toxins such as α- or β-scorpion toxins to study the voltage-dependence of inactivation or activation, respectively.

It is now well established that chromaffin cells express a particular isoforms of sodium channels, originally named PN1 (for peripheral nervous system and now called $Na_v1.7$; Toledo-Aral *et al.* 1997; Catterall *et al.* 2002, 2005), and typical of neuroendocrine cells (Klugbauer *et al.* 1995). Incidentally, one of the first characterizations of the role of this channel in rat chromaffins was illustrated in a famous paper where the exceptional properties of the current-clamp version of the patch technique were described by the Nobel Prize Erwin Neher and coworkers (Fenwick *et al.* 1982).

The $Na_v1.7$ voltage-dependent activation and steady-state inactivation relationships do not differ much from those of the other TTX-sensitive channels present in the same human chromosome 2, such as $Na_v1.1$, $Na_v1.2$, $Na_v1.3$. These properties also depend: 1) on methodological procedures used by the different investigators, 2) on the different isoforms used (rat, mouse or human) and 3) on the presence of ancillary subunits β1-β4. The $Na_v1.7$ activation properties is characterized by the half voltage activation, $V_{½}$, which is in the range -20/-8 mV. The $V_{½}$ of the inactivation curve is in the range -80/-70 mV if tested with short preconditionings (fast steady-state inactivation) and around -40 mV if tested with long preconditionings (slow inactivation) (Cummins *et al.* 2004). The native, TTX-sensitive, sodium currents present in rat chromaffin cells are shown in Fig. 2. The normalized conductance can be fitted to a Boltzmann relationship where the $V_{½}$ value was -7.6 mV (n=7).

FIG. 2 near here

It was recently reported that a scorpion toxin (OD1) is able to produce, at nanomolar concentrations, dramatic effects on the inactivation properties and indirectly to the peak currents of the $Na_v1.7$ isoform (Maertens *et al.* 2006). The sodium currents recorded from cells expressing these channels can be modulated by protein kinase C (PKC) activators (Vijayaragavan *et al.* 2003). This same isoform was found to be highly expressed in prostate cancer cell lines (Diss *et al.* 2001). It is presently unknown if this specificity is involved in the cellular metastatic and invasive ability of this tumor type.

2.3. Up- and down-regulation of $Na_v1.7$

Importantly, up-regulation or hyperactivity of wild-type $Na_v1.7$ is involved in eliciting intolerable pain in various acquired diseased states (e.g. inflammation; diabetic neuropathy) (Hong *et al.* 2004; Nassar *et al.* 2004), thus $Na_v1.7$ being the first convincing molecular target of therapeutic drugs against pain (Wada 2006).

TABLE 1 near here

Table 1 summarizes up- and down-regulation mechanisms of $Na_v1.7$ caused by various extra- and intra-cellular signals in cultured bovine adrenal chromaffin cells. Particularly, insulin-like growth factor-I up-regulated $Na_v1.7$ gene expression via mechanisms distinct from those of insulin (Yamamoto *et al.* 1996; Wada *et al.* 2005). PKC-α and PKC-ε down-regulated $Na_v1.7$ via PKC isoform-specific mechanisms; PKC-α promoted

endocytosis of cell surface $Na_v1.7$, whereas PKC-ε destabilized $Na_v1.7$ mRNA (Yanagita *et al.* 1999, 2000). High and low sustained (but not low transient) rise of $[Ca^{2+}]_i$ down-regulated $Na_v1.7$ via promoting PKC-α/calpain-induced endocytic internalization of cell surface $Na_v1.7$; in addition, only high sustained $[Ca^{2+}]_i$ rise lowered $Na_v1.7$ and β_1-subunit mRNA levels (Shiraishi *et al.* 2001b). In nonstimulated adrenal chromaffin cells, constitutive phosphorylation/activity of extracellular signal-regulated kinase-1/-2 (but not p38 mitogen-activated protein kinase and c-Jun N-terminal kinase) destabilized $Na_v1.7$ mRNA via translational event, negatively regulating steady-state levels of $Na_v1.7$ mRNA and cell surface functional sodium channels (Yanagita *et al.* 2003). Inhibition of calcineurin by therapeutic immunosuppressive drugs cyclosporin A or FK506 up-regulated functional $Na_v1.7$ via retarding internalization of cell surface $Na_v1.7$ (Shiraishi *et al.* 2001a). For more information, the readers are advised to refer to our review articles (Wada *et al.* 1998, 2004; Wada 2006) and references in Table 1.

2.4. Functional roles of $Na_v1.7$

Ito *et al.* (1980) provided first evidence that veratridine caused exocytic secretion of catecholamines from perfused guinea-pig adrenal glands. In cultured bovine adrenal chromaffin cells, veratridine-induced $^{22}Na^+$ influx was the prerequisite to $^{45}Ca^{2+}$ influx via voltage-dependent calcium channels and exocytosis of catecholamines (Wada *et al.* 1985b). In addition, veratridine caused Ca^{2+}-dependent phosphorylation/activation of tyrosine hydroxylase, stimulating catecholamine synthesis (Uezono *et al.* 1989); veratridine-induced Ca^{2+} influx increased diacylglycerol formation, leading to membrane translocation/activation of PKC-α (Uezono *et al.* 1992; Yanagita *et al.* 1999, 2000).

In *in situ* rat adrenal chromaffin cells of acute slices (experimental preparations preserving the fine structures), Martin *et al.* (2001) documented that spontaneous $[Ca^{2+}]_i$ oscillations occurred simultaneously in neighboring cells, Ca^{2+} spreading between cells via gap junction-mediated cell-to-cell communication. The $[Ca^{2+}]_i$ oscillations were reversibly abolished by 0.5 μM TTX or 0.5 mM Cd^{2+} (an inhibitor of voltage-dependent calcium channels). In mouse adrenal chromaffin cells in slices, Moser (1998) showed that spike-like currents spread into neighboring cells via gap junction, which were inhibited by 10 μM TTX.

$Na_v1.7$ channel is crucial for generating excitability through all-or-none action potentials. The fact that this type of channel is present mainly in endocrine cells and in cells crucial for nociception has attracted the interest of many investigators. These cells receive primarily slow stimulatory signals and their response consists of a firing regime where a fast

spiking is not necessary and not critically important as it is for fast spiking CNS neurons or for myelinated and unmyelinated axons.

The biophysical properties that govern the neuroendocrine cell spiking are linked to the time constants of the inactivation gates, namely the development of, and the recovery from the inactivated state. In fact, the time constant of development of inactivation (τ_{dev}) at potentials around resting is important because, depending on the properties of the depolarizing potentials (slow or fast), the amount of channels available (to be opened for generating the action potential) at the threshold, would critically depend on how fast this process develops. When membrane potential increases from resting, conformational states can proceed either from the route CLOSED > OPEN > INACTIVATED or from the route CLOSED > INACTIVATED.

Data indicate that τ_{dev} is very different between different isoforms. In particular, channel types $Na_v1.6$ and $Na_v1.7$ have been compared (Herzog *et al.* 2003). $Na_v1.6$ and $Na_v1.7$ channels show, at -70 mV, values of τ_{dev} around 20 and 155 ms respectively. This suggests that in cells expressing the latter channel, the $Na_v1.7$ proteins are less likely to undergo conformational changes from closed- to inactivated-state during slow depolarizations which normally are present in spontaneously firing chromaffin cells. On the contrary, $Na_v1.6$ channels, present in the Ranvier nodes (Caldwell *et al.* 2000), acting as repeaters (because they are subject to fast depolarizing currents originating in the preceding node), can work perfectly. If the $Na_v1.6$ and $Na_v1.7$ channel were swapped in their localization, nodes would respond also to slow and random changes of membrane potentials and chromaffin cells would not respond to slow depolarizations, because sodium channels would be rapidly and totally inactivated before having the possibility to open.

From the inactivated state, the protein can reach the closed state only from negative potentials and after an exponential time course called “recovery from inactivation” and characterized by a time constant (τ_{rec}), but there is an exception to this rule. It has been shown that there is only one isoform, namely $Na_v1.6$, in which the protein can reach the open state from the inactivated state: this current is called “resurgent current” and can be recorded either in Purkinje cerebellar neurons or under the action of a particular β-scorpion toxin (Raman & Bean 1997; Schiavon *et al.* 2006).

The ability of producing high frequency firing is strongly linked to τ_{rec}, the time constant of recovery from the inactivation state in which all the channels are confined after an action potential. Indeed, it has been shown that a channel like $Na_v1.6$, present in the Ranvier nodes and in CNS (subthalamic nucleus; Tri *et al.* 2004), where very fast spiking rate were recorded, has τ_{rec} of the order of 6.5 ms (at -70 mV). On the contrary, at the same membrane

potential, $Na_v1.7$ channels have τ_{rec} around 72 ms (Herzog *et al.* 2003). This suggests that $Na_v1.7$ channels are structured for a relatively slow firing rate, typical of chromaffin cells where, indeed, other channels, mostly calcium channels, are responsible of the $[Ca^{2+}]_i$ increase and the neurotransmitter release machinery.

3. $Na_v1.7$ and pain

Two gain-of-function mutations of $Na_v1.7$ (van Genderen *et al.* 1993; Yang *et al.* 2004; Drenth *et al.* 2005; Dib-Hajj *et al.* 2005; Waxman & Dib-Hajj 2005; Fertleman *et al.* 2006; Catterall & Yu 2006; Han *et al.* 2006, 2007; Wada 2006) and one loss-of-function mutation of $Na_v1.7$ (Cox *et al.* 2006; Wada 2006) convincingly demonstrated the involvement of $Na_v1.7$ in pain perception.

A gain-of-function mutation of $Na_v1.7$ proved to be responsible for human disease erythermalgia, a burning pain syndrome in hands and feet first described in 1878 (Waxman & Dib-Hajj 2005; Wada 2006). Interestingly, expression studies by Rush *et al.* (2006) documented that $Na_v1.7$ point mutation (L858H) rendered DRG neurons hyperexcitable, while causing SCG neurons hypoexcitable; these opposite effects of L858H were thought to be attributed to the selective existence of $Na_v1.8$ in DRG neurons, but not in SCG neurons. In erythermalgia, however, $Na_v1.7$ mutation in sympathetic ganglion caused sympathetic dysfunctions (Mørk *et al.* 2002; Davis *et al.* 2003). Abnormal hyperexcitability of sympathetic nervous system is known to be involved in the pathogenesis of chronic intolerable pain (e.g. fibromyalgia), accompanied by peripheral sympathetic dysfunction (e.g. orthostatic hypotension) (Martinez-Lavin 2004). Thus, it may be noted that pharmacological blockade of lumbar sympathetic ganglia or surgical thoracic sympathectomy dramatically relieved intolerable pain and microcirculation disturbances in human patients suffered from non-inherited form of secondary erythermalgia (Seishima *et al.* 2000; Wada 2006).

Woods and colleagues (Cox *et al.* 2006) found Pakistan families with $Na_v1.7$ channelopathy-associated insensitivity to pain. In mice, total deletion of $Na_v1.7$ caused perinatal death (Nassar *et al.* 2004); however, the patients lacking functional $Na_v1.7$ were alive, showing no signs of dysfunction in their sympathetic ganglion neurons (Cox *et al.* 2006). Compensatory expression of otherwise silent sodium channel isoform might occur in sympathetic ganglion cells, as was the case in the retinal ganglion cells of mouse lacking $Na_v1.6$ (Van Wart & Matthews 2006).

4. Gain-of-function mutations of $Na_v1.7$ in humans

4.1. Inherited erythermalgia (IEM)

Inherited erythermalgia (*erythro*s = red; *melos* = extremities; *algos* = pain) is a familial painful neuropathy characterized by burning pain and redness of the skin of the extremities in response to warm stimuli or moderate exercise (van Genderen *et al.* 1993). The pattern of inheritance is autosomal dominant (Drenth *et al.* 2005).

Six mutations of $Na_v1.7$ have been found directly linked with IEM (Drenth *et al.* 2005; Waxman & Dib-Hajj 2005). F216S and N395K mutations localize in DI/S4 and DI/S6, respectively. The mutation F1449V is located in the intracellular loop connecting domains III and IV. In HEK293 transfected cells (α, β1 and β2 subunits), this substitution cause an 8 mV hyperpolarizing shift in voltage-dependent activation and a 4 mV depolarizing shift in fast activation of $hNa_v1.7$ channels. These effect is expected to increase the percentage of channels available for activation close to resting potentials and thus increase cell excitability. In fact, current-clamp recordings demonstrate a lower threshold for single action potential and high frequency firing in response to graded stimuli in DRG neurons transfected with the mutant channels (Dib-Hajj *et al.* 2005). Three mutations (L858F, L858H, I848T) occur within DII/S4-S5 linker (van Genderen *et al.* 1993; Yang *et al.* 2004; Waxman & Dib-Hajj 2005; Han *et al.* 2006). These mutations enhance the activation of sodium channels (Yang *et al.* 2004; Catterall & Yu 2006). Biophysical analysis of I848T and L858H, in HEK293 cells (α-subunit), revealed a significantly shift in the voltage dependence activation in the hyperpolarizing direction. Deactivation (transition from open to closed state) of $Na_v1.7$ was also slowed in both mutations, although the effect was much larger for the L858H. Both mutations increase also the size of ramp currents produced by $hNa_v1.7$ channels in response to slow depolarizations. These changes in the functional properties of $Na_v1.7$ are likely to contribute to increase excitability of spinal sensory neurons that express this sodium channel isoform and may underline the abnormal pain sensations in patients with IEM (Cummins *et al.* 2004). Han et al. (2006) provided an electrophysiological characterization of L858F mutation in HEK293 cells. It revealed a 9 mV hyperpolarized left shift of activation for L858F channels. The time constant of deactivation (at -100 mV) for L858F channels was up to 18 times larger than for wild-type $hNa_v1.7$ channels. The mutation caused a significant depolarizing shift of steady-state inactivation curve and a faster recovery from inactivation. Onset of inactivation at -80 mV was slowed, resulting in an increased amplitude of the ramp currents evoked by slow depolarization. In conclusion, L858F might facilitate a more rapid response to successive generator potentials, thus producing high frequency firing (Han *et al.* 2006).

IEM is characterized by attacks of burning pain in the extremities induces by warmth and exercise. Pharmacological treatment is often ineffective, but the pain could be relieved by

cooling the extremities in ice cold water. Han et al. (2007) tested the effect of temperature on gating properties of wild-type $hNa_v1.7$ and L858F channels. Reduction in temperature to 16°C caused a significant shift in a depolarizing direction of the activation curve. This brings the threshold of activation of the mutant channels closer to that of wild-type, and it is likely to contribute to the alleviation of painful symptoms by cooling extremities in patients with L858F erythermalgia mutation (Han *et al.* 2007).

4.2. Paroxysmal extreme pain disorder

Paroxysmal extreme pain disorder (PEPD) is an inherited condition characterized by paroxysms of rectal, ocular, or submandibular pain with flushing (Fertleman *et al.* 2006). PEPD is the second inherited pain disorder to be attributable to mutation in SCN9A.

Paroxysmal extreme pain syndrome differs from erythermalgia in the following points: (1) paroxysmal extreme pain syndrome is essentially a visceral pain condition, often unprovoked, whereas IEM is a pain syndrome in extremities, triggered by exercise and temperature change; (2) carbamazepine effectively ameliorates paroxysmal extreme pain syndrome, but not erythermalgia; (3) in contrast to IEM in which mutations enhance the activation of sodium channels, the mutations that cause PEPD impair $hNa_v1.7$ inactivation. Thus there is a defined genotype/phenotype correlation between these two distinct syndromes (Catterall & Yu 2006). Of the eight mutations that cause PEPD, three (I1461T, F1462V, T1464I) are located in the inactivation gate itself, two of which occur within the highly conserved IFM amino acid motif in the linker region between domain III and IV. Three (V1298F, V1298D, V1299F) occur in the completely conserved amino acids V1298 V1299 in DIIIS4-S5 loop, believed to interact with the domain III-IV inactivating motif. One (M1627K), in DIV/S4-S5 loop alters a methionine conserved residues that may form part of the inactivation gate. A mutation (R996C) in the not conserved residue 996 in the intracellular linker between II and III domains, may have a slight effect on channel function, and this is consistent with the less severe nature of phenotype in patients presenting this mutation (Fertleman *et al.* 2006). These disease mutations maps almost exactly onto the well-known structure-function relationships responsible of the sodium channel inactivation.

Fertleman *et al.* (2006) investigated the electrophysiological properties of three of these mutations (I1461T, T1464I, M1627K) in HEK293 cells (no β-subunit added). All these mutant channels present altered inactivation associated with persistent currents, which were maintained for several hundred millisecond in the first two mutations. There is a depolarizing right shift in the steady-state inactivation curves for all three mutants, too. In conclusion, these mutations cause non-inactivating sodium currents in $hNa_v1.7$ channels, which likely

cause PEPD syndrome by inducing repetitive firing in peripheral nerves that conduct pain information (Catterall & Yu 2006; Fertleman *et al.* 2006), in which $hNa_v1.7$ expression pattern and function are known (Nassar *et al.* 2004; Meisler & Kearney 2004).

5. Loss-of-function mutation of $Na_v1.7$ in humans

5.1. Channelopathy-associated insensitivity to pain

Pain is an essential sense evolved as a protective mechanism to minimize tissue and cellular damage, and hence prolong survival. Channelopathy-associated insensitivity to pain is a new syndrome which affected individuals that had never felt any pain, any time, in any part of their body. Neurological examination revealed that each could correctly perceive the sensation of touch, warm, cold temperature, proprioception, tickle and pressure, but not pain stimuli (Cox *et al.* 2006). This syndrome is caused by loss-of-function of the voltage gated sodium channel gene SCN9A.

A study of affected individuals from three families revealed distinct single homozygous nonsense mutations: a base substitution resulting in the amino acid change W897X in the linker between DII/S5-S6; a base deletion that led to the amino acid change I767X in DII/S2; a substitution resulting in the amino acid change S459X in the intracellular loop between domains I and II. These $hNa_v1.7$ non-sense mutations are expected to cause prematurely truncated proteins or non-sense-mediated messenger RNA decay and hence loss-of-function of $hNa_v1.7$ in nociceptive neurons. Patch-clamp experiments in HEK293 cells transfected with mutated channels (and the β1- and β2-subunits) confirmed the complete loss-of-function of $Na_v1.7$ channels. The authors suggest that the firing of action potential may be substantially compromised in nociceptor neurons lacking $Na_v1.7$ and this cause an insensitive to pain in patients which thus suffer of permanent injury because they fail to notice illness and fail to learn pain-avoiding behaviors (Cox *et al.* 2006).

Acknowledgments

We thank Ms. Keiko Kawabata for secretarial assistance in preparing this manuscript. This study was supported by The Grant-in-Aid for The 21st Century COE (Centers of Excellence) Program (Life Science) from the Ministry of Education, Culture, Sports, Science, and Technology, Japan to AW and by grants from Ministero dell'Università e della Ricerca Scientifica e Tecnologica (MURST-COFIN 2005-2007; FISR-Neurobiotecnologie: Fisiopatologia del sistema nervoso; FIRB-2001) to E.W. F.G. and E.S. are post-doc students in Physiology at Milano-Bicocca University, Department of Biotechnology and Biosciences.

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Figure legends

Fig. 1 A comparison of the responses obtained, in the same cell, during the application of nicotinic and muscarinic agonists and antagonists. *Upper panels*: responses obtained under the application of various concentrations of oxotremorine (oxo), compared to the effect of 10 μM nicotine (nico). *Lower panels*: responses obtained under the application of various concentrations of ACh and ACh + atropine (Atr); atropine concentration was 1μM. Notice that atropine removes the muscarinic response of ACh (hyperpolarizing action). In this cell, the response to 10 μM ACh + Atr was much more effective than the action of 10 μM nicotine. From 12th International Symposium on Chromaffin Cell Biology, Canary Islands (2003), poster session, page 69.

Fig. 2 $Na_v1.7$ sodium channel in rat chromaffin cells. Normalized peak conductance curve obtained from Na_v 1.7 sodium currents recorded in rat chromaffin cells (n = 7) using the patch-clamp technique. The continuous line is the Boltzmann equation which best fitted to the experimental data with the following parameters: $V_{1/2}$ = -7.63 ± 0.35, slope = 7.4 ± 0.31. Inset: superimposed sodium currents, in an exemplary cell (cap. 4.18 pF, Rs 3.8 MOhm), evoked by protocol shown above. Scale bars: 200 pA, 1 ms. In brief: from an holding potential of -120 mV, cell membrane is clamped to depolarizing step ranging from -80mV to +20 mV to evoke $Na_v1.7$ sodium currents. Traces are TTX subtracted.

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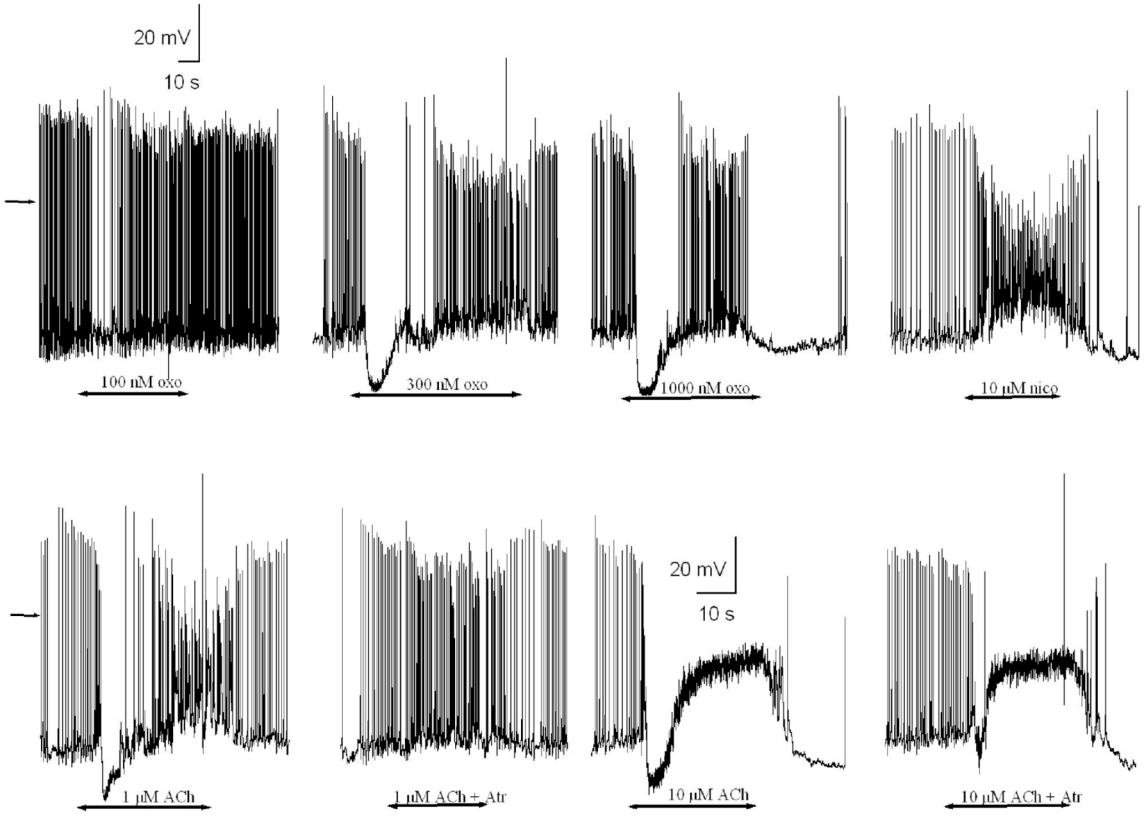

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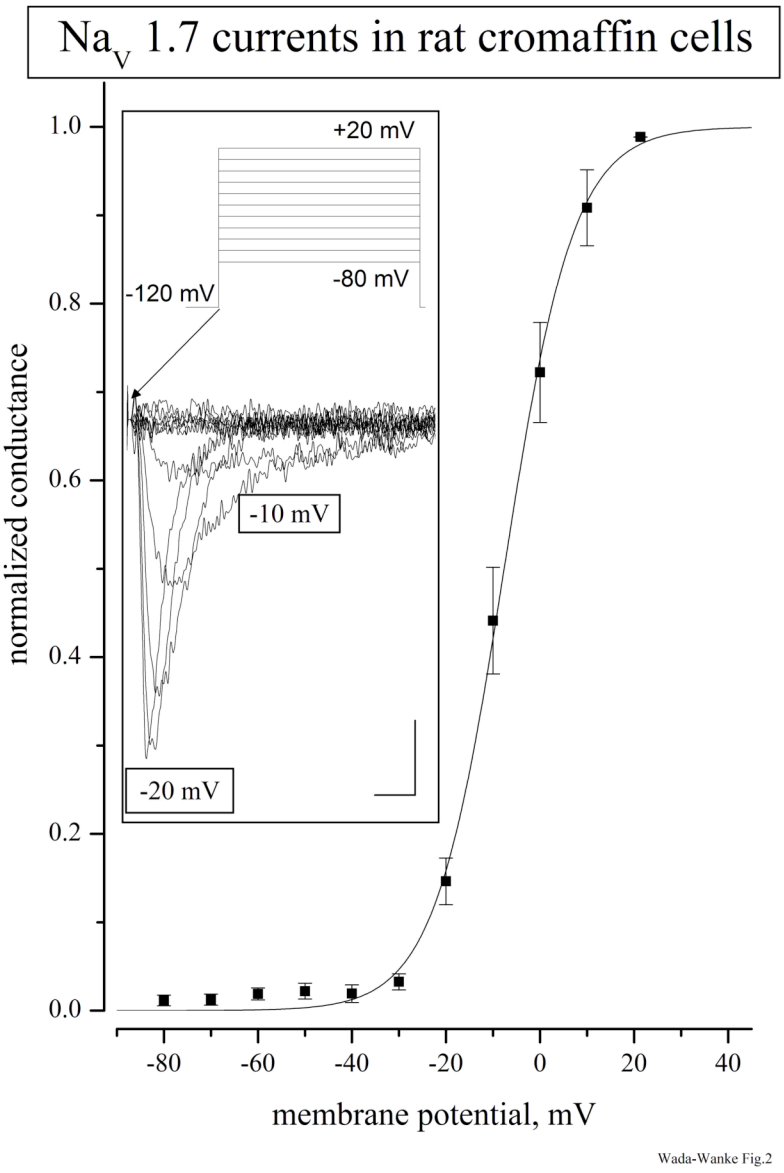

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Table 1. Cell surface $Na_v1.7$ sodium channels: up- and down-regulation mechanisms in adrenal chromaffin cells

	Cell surface [^{3}H]STX binding	$^{22}Na^+$ influx	Na_v 1.7 mRNA			β_1-subunit mRNA	Internalization	References
			Level	Stability	Transcription	Level		
Intracellular signals								
PKC-α	⇓	⇓	⇒			⇒	⇑	Yanagita *et al*. 1999, 2000
PKC-ε	⇓	⇓	⇓	⇓	⇒	⇑	⇒	
$[Ca^{2+}]_i$ rise								Shiraishi *et al*. 2001b
high sustained	⇓	⇓	⇓			⇓	⇑	
low sustained	⇓	⇓	⇒			⇒	⇑	
low transient	⇒	⇒						
ERK	⇓	⇓	⇓	⇓	⇒	⇒	⇒	Yanagita *et al*. 2003
Calcineurin	⇓	⇓	⇒			⇒	⇑	Shiraishi *et al*. 2001a
mTOR	⇓						⇒	
PKA	⇑	⇑						Yuhi *et al*. 1992
Extracellular signals								
Insulin	⇑	⇑	⇒			⇒		Yamamoto *et al*. 1996
IGF-I	⇑	⇑	⇑	⇒	⇑			Wada *et al*. 2005
Valproic acid	⇑	⇑	⇑			⇑		Yamamoto *et al*. 1997
Riluzole	⇒	⇓						Yokoo *et al*. 1998
Neuroprotective NS-7	⇑	⇓	⇒			⇒		Yokoo *et al*. 2000
Carvedilol	⇑	⇓	⇒			⇒		Kajiwara *et al*. 2002
Bupivacine	⇑	⇑	⇒			⇒		Shiraishi *et al*. 2003
Ropivacaine	⇑	⇓						
Lidocaine	⇒	⇓						

PKA, cyclic AMP-dependent protein kinase; ERK, extracellular signal-regulated kinase; IGF-I, insulin-like growth factor-I: mTOR, mammalian target of rapamycin; PKC, protein kinase C; STX, saxitoxin. See refs. Wada *et al*. 1998, 2004; Wada 2006.