Molecular and biophysical properties of smooth muscle-type voltage-gated Na^+ channels

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It is well-known that activation of voltage-dependent Ca^{2+} channels (Ca_V) and voltage-gated Na^+ channels (Na_V) is involved in the generation of action potentials in various types of excitable cells. Although voltage-gated Na^+ currents (I_{Na}) fail to be recorded in the vast majority of smooth muscle tissues, there are several reports regarding the existence of I_{Na} in vascular and visceral smooth muscles, suggesting that I_{Na} are involved in the generation of action potentials. Na_V appear to be selectively expressed in some, but not all smooth muscle, thereby questioning their significance in the physiology of the tissues. Furthermore, little attention has been given to the molecular properties of TTX-sensitive I_{Na} in smooth muscles.

Recent studies have revealed that Na_V consist of three subunits (expressed as a trimer): namely, an α subunit (260 kDa) which forms the core protein of the channel (possessing the TTX-binding sites) and two β subunits (30-40 kDa) which modify the channel function as an auxiliary subunit. To date, eleven isoforms of genes (*Scn1a-11a*) encoding TTX-sensitive and TTX-insensitive Na_V have been identified within a single family of Na_V, $Na_V1.X$ and four isoforms of genes (*Scn1b-4b*) encoding β subunits have been also detected.

The electrophysiological and pharmacological properties of Na_V in murine *vas deferens* smooth muscle cells were investigated using patch-clamp techniques. In whole-cell configuration, a fast, transient inward current was evoked in the presence of Cd^{2+}, and was abolished by TTX (K_d = 11.2 nM), mibefradil (K_d = 3.3 μM) and external replacement of Na^+ with monovalent cations (TEA^+, $Tris^+$ and $NMDG^+$). The fast transient inward current was enhanced by veratridine, an activator of voltage-gated Na^+ channels, suggesting that the fast transient inward current was a TTX-sensitive I_{Na}. The values for half-maximal (V_{half}) inactivation and activation of I_{Na} were -46.3 mV and -26.0 mV respectively. The molecular identity of the TTX-sensitive pore-forming subunits was revealed using RT-PCR analysis, *in situ* hybridization and immunohistochemistry. RT-PCR analysis revealed the expression of *Scn1a*, *2a* and *8a* transcripts, whilst *Scn1b* was only detected. The *Scn8a* transcript and the α subunit protein of $Na_V1.6$ were detected in smooth muscle layers. Furthermore, using $Na_V1.6$-null mice ($Na_V1.6^{-/-}$) lacking the expression of the Na^+ channel gene, *Scn8a*, I_{Na} were not detected in dispersed smooth muscle cells from the *vas deferens*, whilst TTX-sensitive I_{Na} were recorded in their wild-type ($Na_V1.6^{+/+}$) littermates. This study demonstrates that the molecular identity of the Na_V responsible for the TTX-sensitive I_{Na} in murine *vas deferens* myocytes is primarily $Na_V1.6$. (Zhu *et al*., 2008), co-expressed with β1 subunits.

Zhu HL, Aishima M, Morinaga H, Wassall RD, Shibata A, Iwasa K, Nomura M, Nagao M, Sueishi K, Cunnane TC, Teramoto N (2008) *Biophysical Journal* **94**, 3340-3351.