Overactive bladder (OAB) is a pervasive and debilitating condition for which effective therapeutic modalities are lacking. As potential novel pharmacological targets for lower urinary tract dysfunction, our recent studies have demonstrated voltage-gated Kv7 channels (Kv7.1-Kv7.5) to be functionally expressed in detrusor smooth muscle (DSM) of the urinary bladder. Nonetheless, the specific roles of individual Kv7 channel subtypes remains poorly understood. Using Western blot, immunocytochemistry, isometric DSM tension recordings, ratiometric fluorescence Ca\(^{2+}\) imaging, and patch-clamp electrophysiology, we demonstrated expression and key physiologic roles for the Kv7.2/Kv7.3 channels in guinea pig DSM using the novel and selective Kv7.2/Kv7.3 channel opener N-(2-Chloro-5-pyrimidinyl)-3,4-difluorobenzamide (ICA-069673). We further sought to pharmacologically target Kv7.4- and Kv7.5-containing channels, which evidence suggests are the prominent subtypes expressed in smooth muscle, to determine their involvement in regulating DSM excitation-contraction coupling. The novel Kv7.4/Kv7.5 channel activator N-(2,4,6-Trimethylphenyl)-bicyclo[2.2.1]heptane-2-carboxamide (ML213) was shown to enhance Kv7 channel currents in isolated DSM cells, hyperpolarize the DSM cell membrane potential, and attenuate the contractile activity of DSM isolated strips via a Ca\(^{2+}\)-dependent mechanism. ML213 exhibited significantly greater potency for inhibition of DSM contractility in comparison to Kv7.2/Kv7.3 channel opener ICA-069673. Using \textit{in situ} proximity ligation assay (PLA), it was further revealed Kv7.4 and Kv7.5 channel \(\alpha\)-subunits co-localize to form heteromeric Kv7.4/Kv7.5 channel complexes in DSM isolated cells. These studies suggest Kv7.4/Kv7.5 channels are functionally expressed in guinea pig DSM, where they critically regulate DSM excitability and contractility. Finally, to ascertain the translational implications of our aforementioned findings from experimental animals, we examined Kv7 channel expression and function in human DSM. Kv7 channel activators and inhibitors were shown to attenuate and potentiate, respectively, human DSM excitability and contractility in normal and OAB samples. Noteworthy, using \textit{in situ} PLA, we confirmed the molecular interaction between Kv7.4 and Kv7.5 channel \(\alpha\)-subunits, suggesting heteromeric Kv7.4/Kv7.5 channel subtype expression at the cellular level in human DSM cells. These findings are consistent with our findings in guinea pig DSM and provide strong support to suggest Kv7.4/Kv7.5 channels are among the key subtypes regulating human DSM function. In conclusion, our combined studies reveal novel insights into the expression, subunit composition, and physiological roles of Kv7 channel in DSM, providing critical information for directing future research efforts concerning the utility of Kv7 channels as therapeutic targets for OAB.