Selective modulation of ion channel subunit expression to probe regional differences in vascular smooth muscle BK_{Ca} function

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β1-subunits enhance the gating properties of BK_{Ca} channels formed by α-subunits. In arterial vascular smooth muscle cells (VSMC) β1-subunits are vital in coupling SR–generated Ca^{2+} sparks to BK_{Ca} activation, affecting contractility and blood pressure. Studies in cremaster and cerebral VSMC show heterogeneity of BK_{Ca} activity due to apparent differences in the β1:α subunit ratio. To define these differences studies were conducted at the single channel level while siRNA was used to manipulate specific subunit expression.

Methods and Results: β1 modulation of the α-subunit Ca^{2+} sensitivity was studied using patch clamp techniques. Significant leftward shifts in BK_{Ca} channel open probability (NP0) *versus* membrane potential (Vm) curves (at $[Ca^{2+}]_i$ from 0.5 to 100μM), were observed in cerebral *versus* cremaster VSMC. As $[Ca^{2+}]_i$ increased from 0.5 to 100μM, the V1/2 values of channels decreased from 72.0 ± 6.1 to −89 ± 9mV in cerebral compared to 101 ± 10 to −63 ± 7mV in cremaster VSMC. Ca^{2+} set points (Ca0) were 12.1 and 5.0μM in cremaster and cerebral VSMC, respectively. Thus, at Vm of −30mV, a mean $[Ca^{2+}]$i of 39μM was required to open half of the channels in cremaster *versus* 16μM $[Ca^{2+}]_i$ in cerebral VSMC. Further, shortened mean open and longer mean closed times were evident in BK_{Ca} events from cremaster VSMC at either −30 or 30mV and any given $[Ca^{2+}]$. Uptake of siRNA into VSMCs was verified by studies of both a fluorescently labeled unrelated siRNA and β-subunit directed siRNA. Further, Western blotting confirmed a decrease in protein subunit expression. siRNA directed at the α subunit caused a decrease in BK_{Ca} function in both cell types. β-subunit directed siRNA decreased the Ca^{2+} sensitivity of BK_{Ca} in cerebral VSMCs and the appearance of STOCs such that the cells more closely resembled the activity seen in cremaster VSMC.

Conclusion: The data are consistent with a higher ratio of β1:α subunit of BK_{Ca} channels in cerebral compared to cremaster VSMC. Functionally, this leads to both higher Ca^{2+} sensitivity and NPo for BK_{Ca} in the cerebral vasculature relative to that of skeletal muscle.