While the mechanisms of tone regulation in cerebral pial arteries are well-described, much less is known about the vasomotor control of brain parenchymal arterioles (PAs). This is significant given the unique roles of PAs in the cerebral microcirculation, including control of local blood flow and perfusion pressure, and involvement in neurovascular coupling. Further, PAs are considerably more sensitive than pial arteries to increase in intravascular pressure, which causes smooth muscle depolarization and vasoconstriction (myogenic tone). A recently published study from our laboratory indicates that TRPM4 channels couple P2Y purinergic receptor mechanoactivation and myogenic tone of PAs. Based on recent observations that inhibiting protein kinase C has limited effects on PA myogenic constriction, the objective of the present study was to determine the roles of Rho-associated protein kinase signaling in TRPM4-mediated myogenic tone of PAs. Here we report that the Rho kinase inhibitor H1152 robustly inhibited pressure- and P2Y agonist-induced constriction of PAs. In contrast to the typical function of Rho kinase to alter Ca²⁺-sensitivity, we found that H1152 inhibited pressure-induced intracellular Ca²⁺ increases, and reduced UTPγS (P2Y4 receptor)- and UDP (P2Y6 receptor)-initiated Ca²⁺ entry in PA smooth muscle by 61% and 50%, respectively, suggesting that Rho kinase is centrally involved in myogenic depolarization and Ca²⁺ influx in PA smooth muscle. H1152 also reduced basal TRPM4 activity by 61%, and UTPγS- and UDP-activated TRPM4 currents by 75% and 73%, respectively. These results illustrate a novel role for Rho kinase in regulation of TRPM4-mediated depolarization, Ca²⁺ influx and myogenic tone in the cerebral microcirculation.