

Smooth Muscle Cell ex vivo transfection

Mice were sacrificed with an overdose of pentobarbital (60-90 mg/kg) injected intraperitoneally. Thoracodorsal arteries were isolated and placed in RPMI media supplemented with penicillin (2mM)/streptomycin (50 U/mL) (Gibco) and CaCl₂ (2mmol/L). The TDA were then transferred to a cuvette containing 100 µl of Nucleofector solution (Lonza) complemented either with 5 µg of a plasmid containing a Panx1-GFP construct previously described⁷, 10 nmol/L of siRNA targeting Panx1 (Ambion) or 10 nmol/L of control siRNA (Thermoscientific). The cuvette was placed in the Nucleofector cuvette holder and subjected to electroporation (program A033). Arteries were then placed in RPMI supplemented with penicillin (2mM)/streptomycin (50 U/mL) (Gibco), CaCl₂ (2mmol/L) and BSA 1% in an incubator for 14 to 18 hours. After incubation, TDA were cannulated and pressurized at 40 mmHg for 15 minutes and then at 80 mmHg for 15 minutes before performing dose response to PE.