

# UPREGULATED TRPC3 CHANNEL EXPRESSION AND FUNCTION IN VASCULAR SMOOTH MUSCLE DURING HYPERTENSION



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## Background

Hypertension is associated with profound alteration in calcium homeostasis and smooth muscle cell (SMC) proliferation. Contractility and proliferation of the SMC is reliant upon increase in intracellular calcium concentration. Canonical Transient Receptor Potential (TRPC) Channels are Ca<sup>2+</sup> permeable, nonselective cation channels that have been demonstrated to be important transducers for agonist mediated vascular contractility. Recent studies indicate that expression of TRPC3 (member of TRPC family) is upregulated in pulmonary artery SMC in idiopathic pulmonary hypertension and in monocytes of hypertensive rats and patients. *This study seeks to determine if TRPC3 expression is upregulated in carotid artery in hypertension, and if so, whether it leads to increased vascular contractility.*

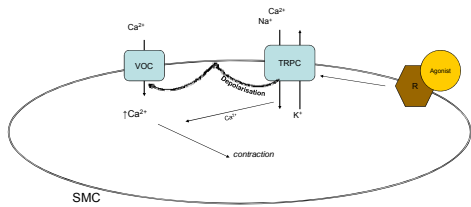


Figure 1: Role of TRPC channels in SMC contraction

## Hypothesis

1. TRPC3 is upregulated in vascular smooth muscle cells of carotid artery during hypertension.
2. Upregulation of TRPC3 renders arteries more contractile to physiological agonists.

## Methods

### Expression of TRPC3 in Rat Carotid Artery (CA)

- RT-PCR Analysis: demonstrate TRPC3 message in the CA from Spontaneously Hypertensive rats (SHR) and the normotensive Wistar-Kyoto (WKY) rats.

- Western Blotting: quantify expression of TRPC3 protein extracted from CA of male SHR and WKY rats.

- Immunohistochemistry: identify cellular location of TRPC3 protein expression within the rat CA.

### Demonstration of TRPC3 Protein Function

- Whole Cell Patch Clamp: evaluate TRPC3 function in freshly isolated CA SMC. TRPC3 currents were elicited by extracellular application of uridine triphosphate (UTP; 60μM).

- Isometric Tension Bath: measure contractions of isolated CA to UTP (1 - 300μM).

## Results

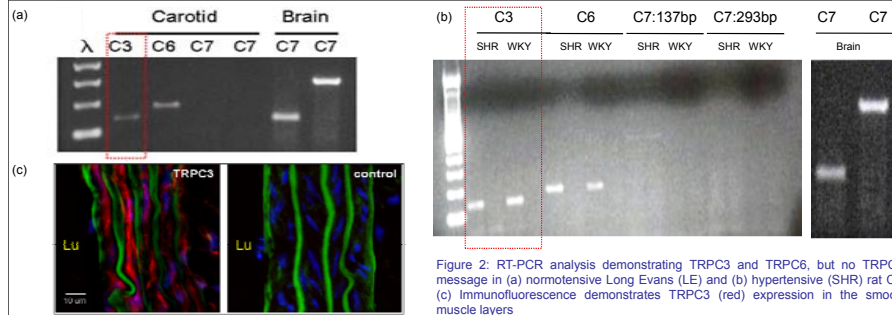


Figure 2: RT-PCR analysis demonstrating TRPC3 and TRPC6, but not TRPC7, message in (a) normotensive Long Evans (LE) and (b) hypertensive (SHR) rat CA. (c) Immunofluorescence demonstrates TRPC3 (red) expression in the smooth muscle layers

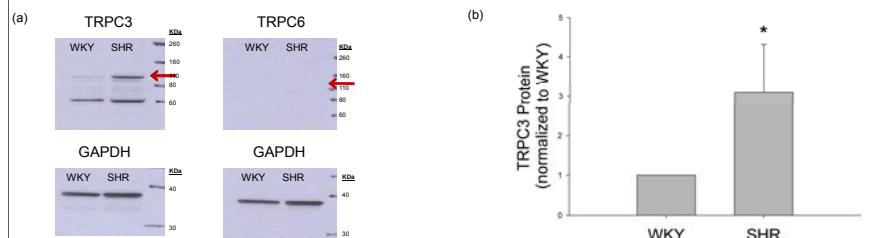


Figure 3: Western Blot showing stronger TRPC3 band (identified by red arrow) in SHR CA while TRPC6 protein was not detectable in the CA of either rats. (b) Densitometry analysis demonstrated that TRPC3 protein expression was 3.1±1.2 times greater in hypertensive compared to normotensive rat CA.

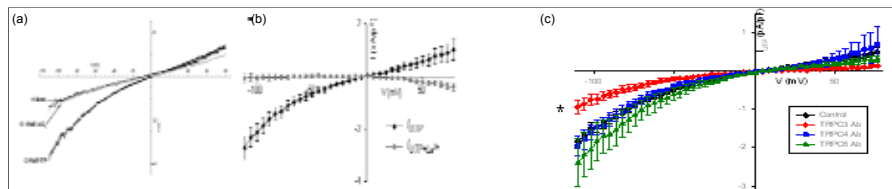


Figure 4: Representative current-voltage (I - V) plots. (a) 60 μM UTP activated a non-selective cation current in freshly isolated SMC of rat CA. (b) Subtracted currents (UTP - basal; I<sub>UTP</sub>) were inhibited by 100 mM La<sup>3+</sup>. (c) I<sub>UTP</sub> was inhibited by anti-TRPC3 antibody but not anti-TRPC4 or anti-TRPC5 antibodies.

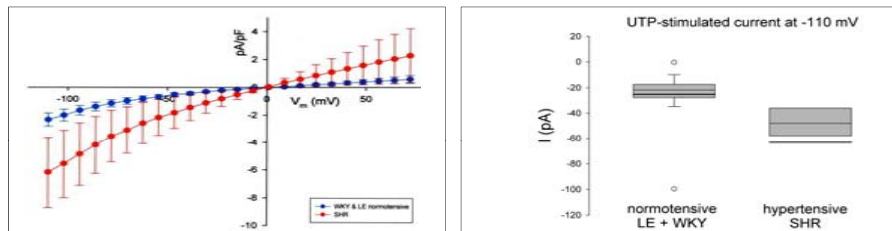


Figure 5: I<sub>UTP</sub> was greater in SHR compared to normotensive WKY and Long Evans (LE) rats

Figure 6: Summary of I<sub>UTP</sub> at -110mV. Peak inward current was significantly greater for SHR than WKY/LE group. Median values were -43 pA (SHR) and -23 pA (WKY/LE). P<0.05 Mann-Whitney Rank Sum Test.

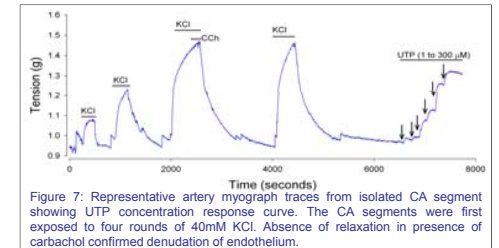


Figure 7: Representative artery myograph traces from isolated CA segment showing UTP concentration response curve. The CA segments were first exposed to four rounds of 40mM KCl. Absence of relaxation in presence of carbachol confirmed denudation of endothelium.

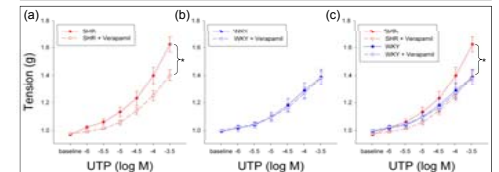


Figure 8: Summary graphs of UTP concentration-tension curves for endothelium-denuded SHR and WKY CA segments. Response to UTP in the absence or presence of verapamil for SHR (a) and WKY groups (b). Panel (c) shows SHR and WKY groups plotted on the same graph. SHR response to UTP is significantly greater than it's own verapamil group or the WKY group. 2 Way RM-ANOVA with Student Neuman Keuls test.

## Summary

- TRPC3 protein expression was significantly increased in hypertensive (SHR) compared to normotensive (WKY) CA
- UTP activated whole cell current (I<sub>UTP</sub>) was significantly increased in SHR compared to WKY and LE smooth muscle
- This I<sub>UTP</sub> was inhibited by a specific TRPC3 antibody
- UTP-mediated constrictions in endothelium-denuded CA were significantly increased in SHR group
- UTP-mediated constriction partially involves L-type Ca<sup>2+</sup> channels in SHR but not in WKY arteries.

## Conclusion

- TRPC3 expression is upregulated in CA during hypertension (Figure 3). Furthermore, this increased expression results in greater TRPC3 channel function in the smooth muscle cells (Figure 5) and greater contractile responses in the intact artery (Figure 8).
- These studies suggest that TRPC3 might be a novel pharmacological target for modulating vascular tone in hypertension.

## References

1. Dietrich A et al. In vivo TRPC functions in the cardiopulmonary vasculature. *Cell Calcium*. 2007
2. Liu D et al. Increased TRPC3 Expression in Spontaneously Hypertensive Rats. *Am J of Hypertension*. 2005
3. Liu D et al. Transient receptor potential channels in essential hypertension. *J of Hypertension*. 2006

## Acknowledgements

Research supported by NIH R01 HL 088435 (PI: Marrelli SP)