Effect of Hypoxia on Ion Homeostasis of Human Coronary Artery Smooth Muscle Cells (HCASMCs)

Background: Metabolic vasodilation of the coronary artery is an important mechanism where blood flow is increased to meet enhanced oxygen demand of the heart. However, its precise mechanisms remain unresolved (1). Previous studies suggested that hypoxia causes K^+ channel activation that hyperpolarizes artery smooth muscle cells, causing a fall in intracellular Ca^{2+} ($[Ca^{2+}]_i$) and relaxation. In particular, K_{ATP} channels, activated by a decrease in cellular ATP/ADP ratio, have been implicated (2). Here we evaluated the effects of hypoxia and metabolic inhibitors on $[Ca^{2+}]_i$ and membrane potential using HCASMCs with fluorescent probes.

Methods: Fluo-4 was loaded as an AM form to report intracellular Ca^{2+} changes. DiBAC4(3), which re-distributes following the charge across cell membrane, was used to measure change in membrane potential. Fluorescent signals were detected using a confocal microscope (LSM510) with a capability to regulate CO_2, humidity, temperature, and environmental O_2.

Results: Vasoconstrictors (PDGF-BB, PGF2α and U46619) induced/increased calcium oscillations in HCASMCs. Exposure to hypoxia (1% O_2) reduced calcium oscillations induced by vasoconstrictors (Table 1). Hypoxia appeared to decrease oscillation frequency in high K^+ (60 mM) but not significantly. Application of 10 μM glibenclamide and 25 μM $BaCl_2$, blockers of K_{ATP} and inward rectifier K (K_{ir}) channels respectively, increased DiBAC4(3) signal by 54.94±8.18% (n=37) and 69.95±7.46% (n=26), suggesting depolarization. Both metabolic inhibitors (rotenone, antimycin) and hypoxia decreased DiBAC4(3) signal suggesting hyperpolarization. The effects of metabolic inhibitors were abolished by glibenclamide and high K^+ (60 mM). Although extracellular high K^+ also blocked the effect from hypoxia, the application of a single K^+ channel inhibitor didn't completely abolish the effect.

Table 1 Effect of hypoxia on calcium oscillations (*P<0.05; **P<0.01; ***P<0.001)

	Amplitude (A)		Frequency (/hr, F)		AxF		Area under curve	
O_2 (%)	**20%**	**1%**	**20%**	**1%**	**20%**	**1%**	**20%**	**1%**
PDGF (n=7)	1.34 ±0.23	0.78** ±0.16	22.86 ±2.86	17.14 ±2.76	31.20 ±4.90	12.47** ±2.96	0.53 ±0.08	0.46 ±0.11
PGF2α (n=6)	1.99 ±0.49	1.37** ±0.53	15.56 ±2.22	17.67 ±3.40	29.31 ±6.40	17.83 ±2.44	0.76 ±0.22	0.47** ±0.40
U46619 (n=17)	0.83 ±0.28	0.65* ±0.13	25.89 ±2.58	16.68*** ±3.25	33.74 ±12.40	13.98* ±5.02	0.43 ±0.08	0.21*** ±0.05
60 K^+ (n=3)	0.19 ±0.28	0.17 ±0.01	22.00 ±2.00	14.67 ±3.53	4.08 ±0.28	2.47 ±0.52	0.05 ±0.003	0.04 ±0.003

Conclusion: K_{ATP} and K_{ir} channels play important roles in regulating resting membrane potential of HCASMCs. Metabolic inhibitors cause vasodilation through, in part, the activation of K_{ATP} channels. Hypoxia vasodilation is also partly through the activation of K^+ channels.

***References*:**

(1) Duncker DJ et al. (2008). *Physiol Rev* **88**: 1009-86.
(2) Flagg TP et al. (2010). *Physiol Rev* **90**: 799-829.