

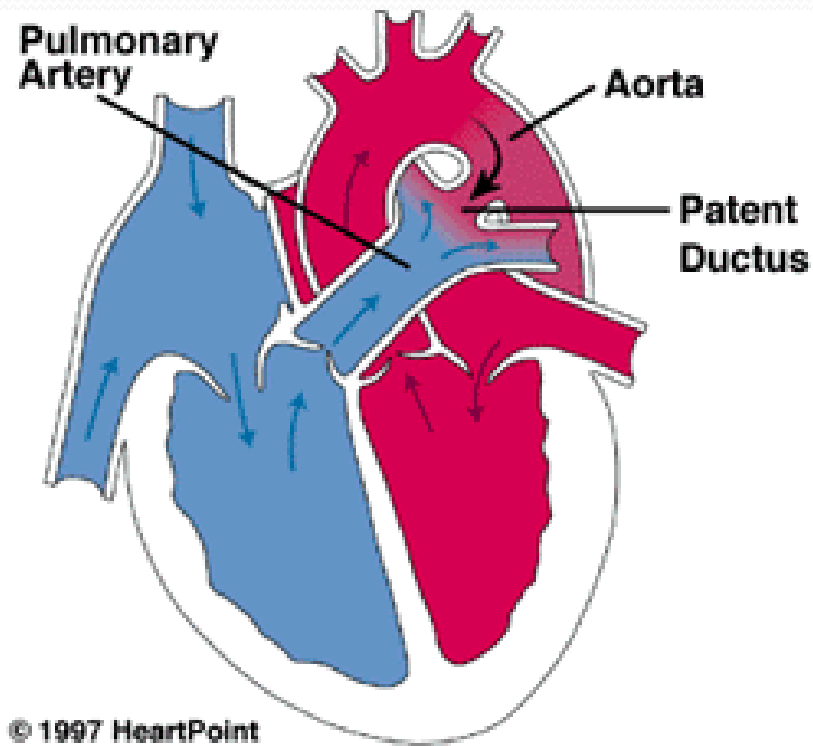
Hypoxic Effects in Contraction of the Ductus Arteriosus in the Chicken Embryo

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The Ductus Arteriosus

- The ductus arteriosus (DA) is a fetal blood vessel
- Diverts blood from the right ventricle → aorta → body → fetal gas exchanger
- Closure of the ductus occurs during the first few hours after birth
- Inability to close results in a patent DA

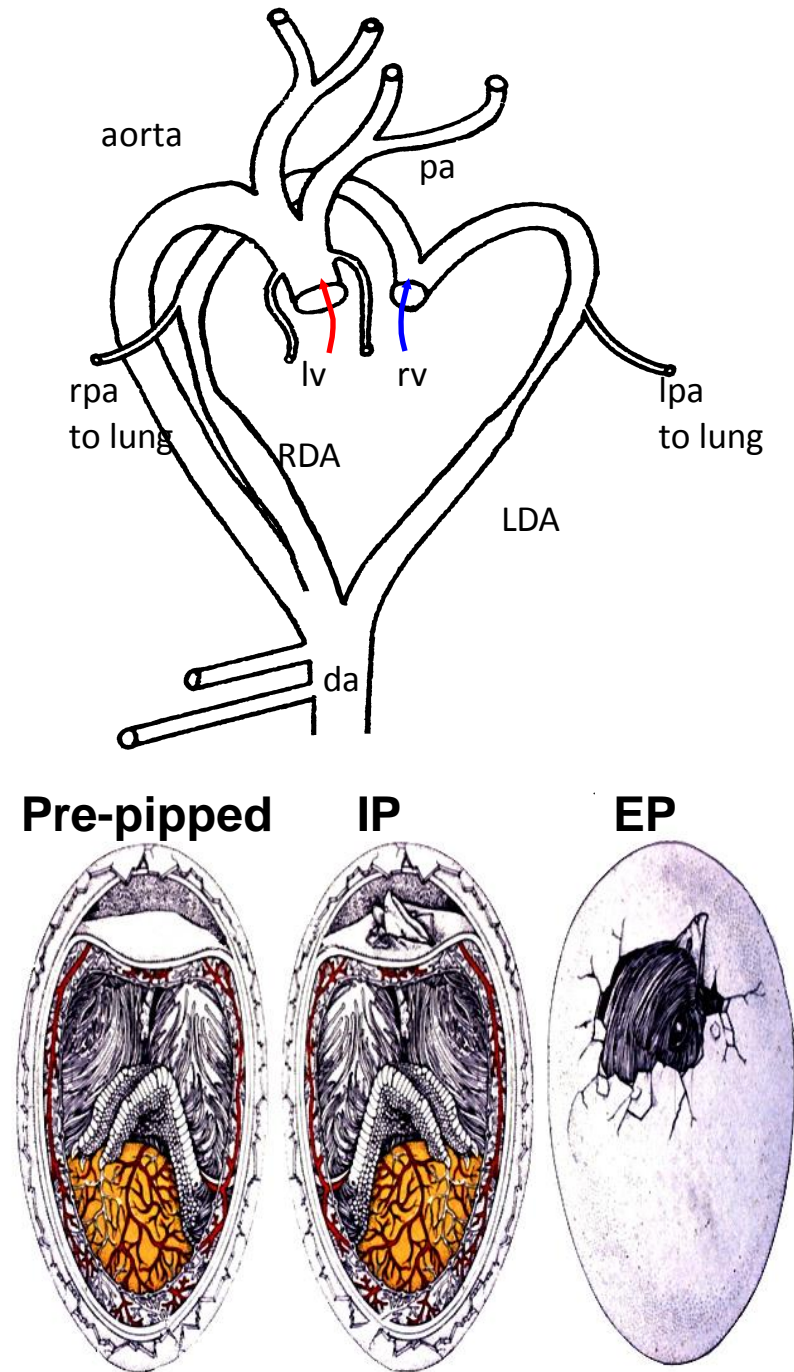
Fig. 1 Fetal Heart Diagram



The Avian DA

- Two distinct DA
- Hatching differences
 - Pre-pipped (Day 18)
 - Internally pipped (IP)
 - Externally pipped (EP)
- Interested in DA changes during hatching

Fig. 2 Chicken DA and Developmental stages



Hypoxia

- **A link between human development in chronic hypoxia and the incidence of PDA in preterm infants (26 - 32 weeks gestation) was found (Rakza et al. 2007)**
- **Incidence of PDA significantly higher in children born at higher altitudes (3847-4533m) in comparison to children born at lower altitudes (1650-2835m or 2792-4360m; Chen, et al., 2008)**
- **60% of preterm infants with intrauterine growth restriction (IUGR) developed PDA**
- **15% of preterm infants without IUGR developed PDA**

Mechanism of Contraction

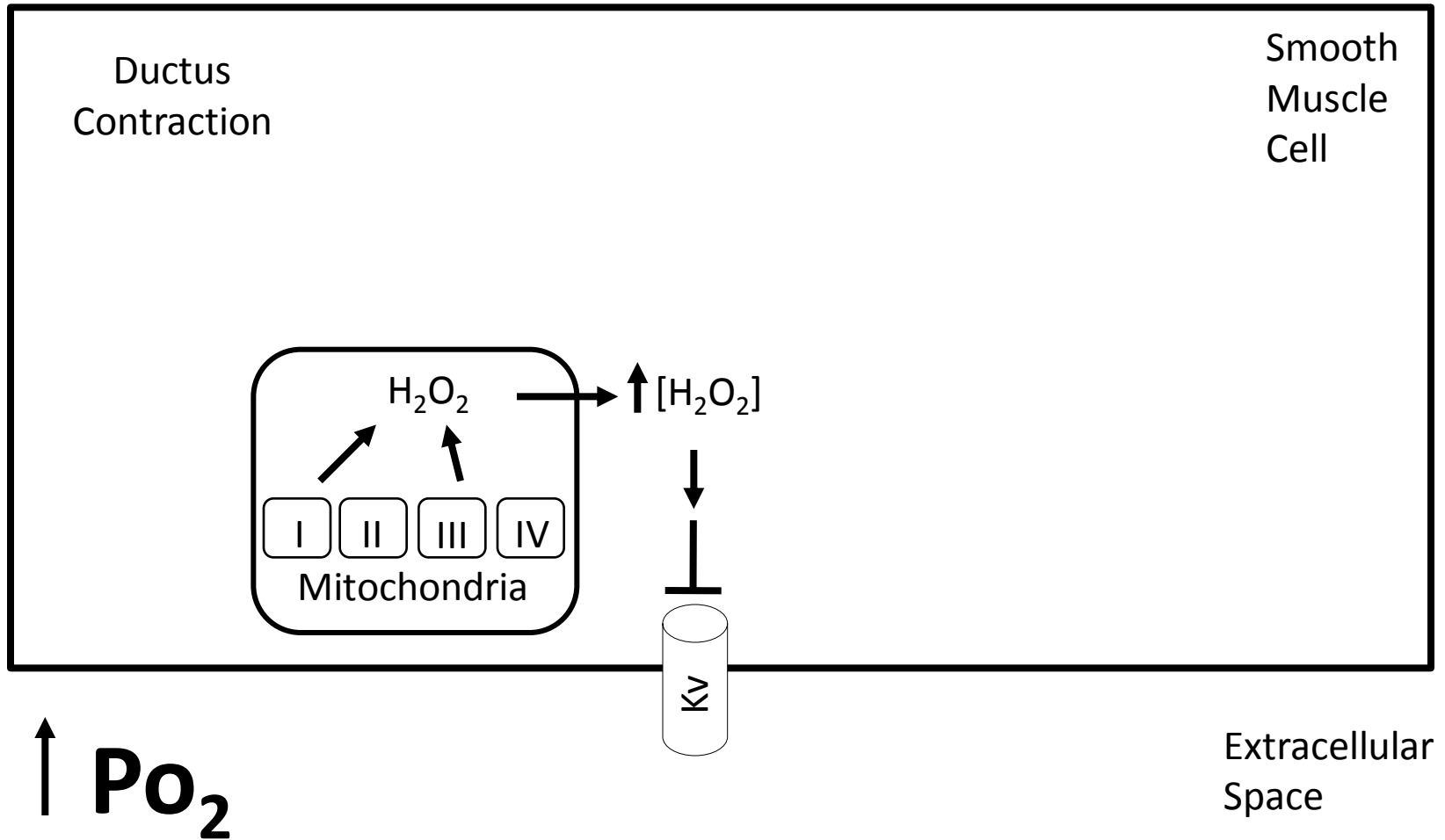
Ductus
Contraction

Smooth
Muscle
Cell

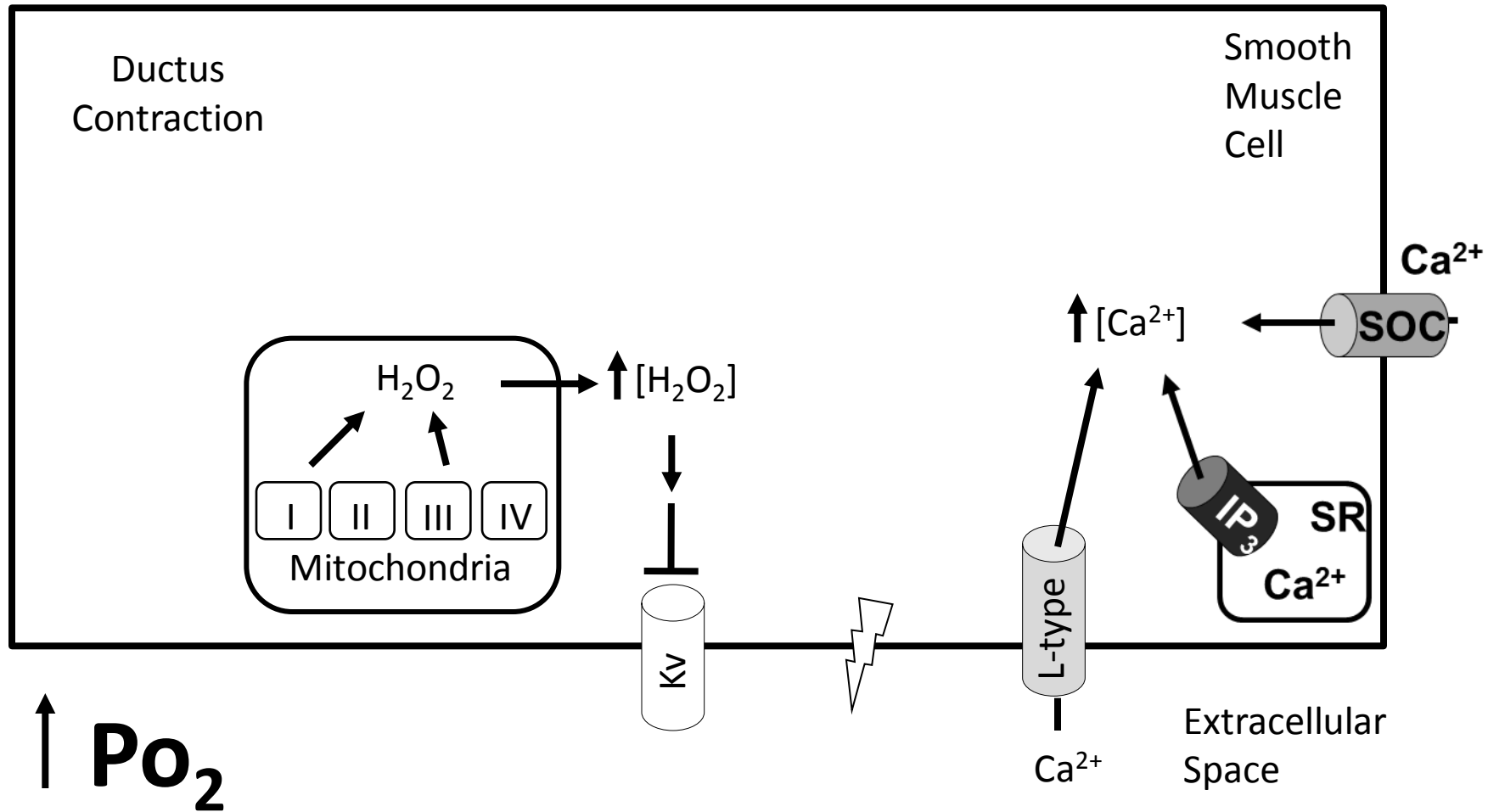
↑ **Po₂**

Extracellular
Space

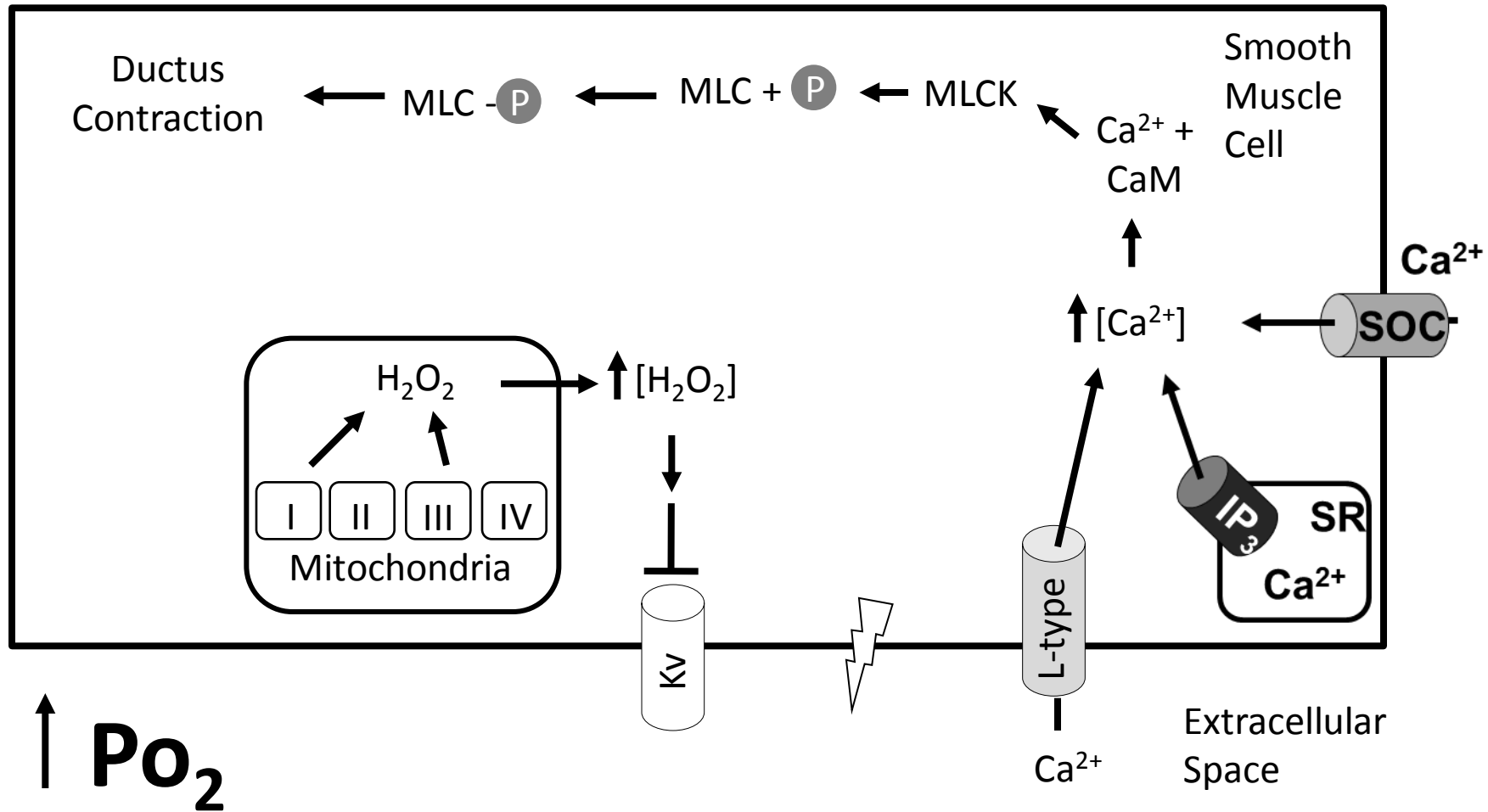
Mechanism of Contraction



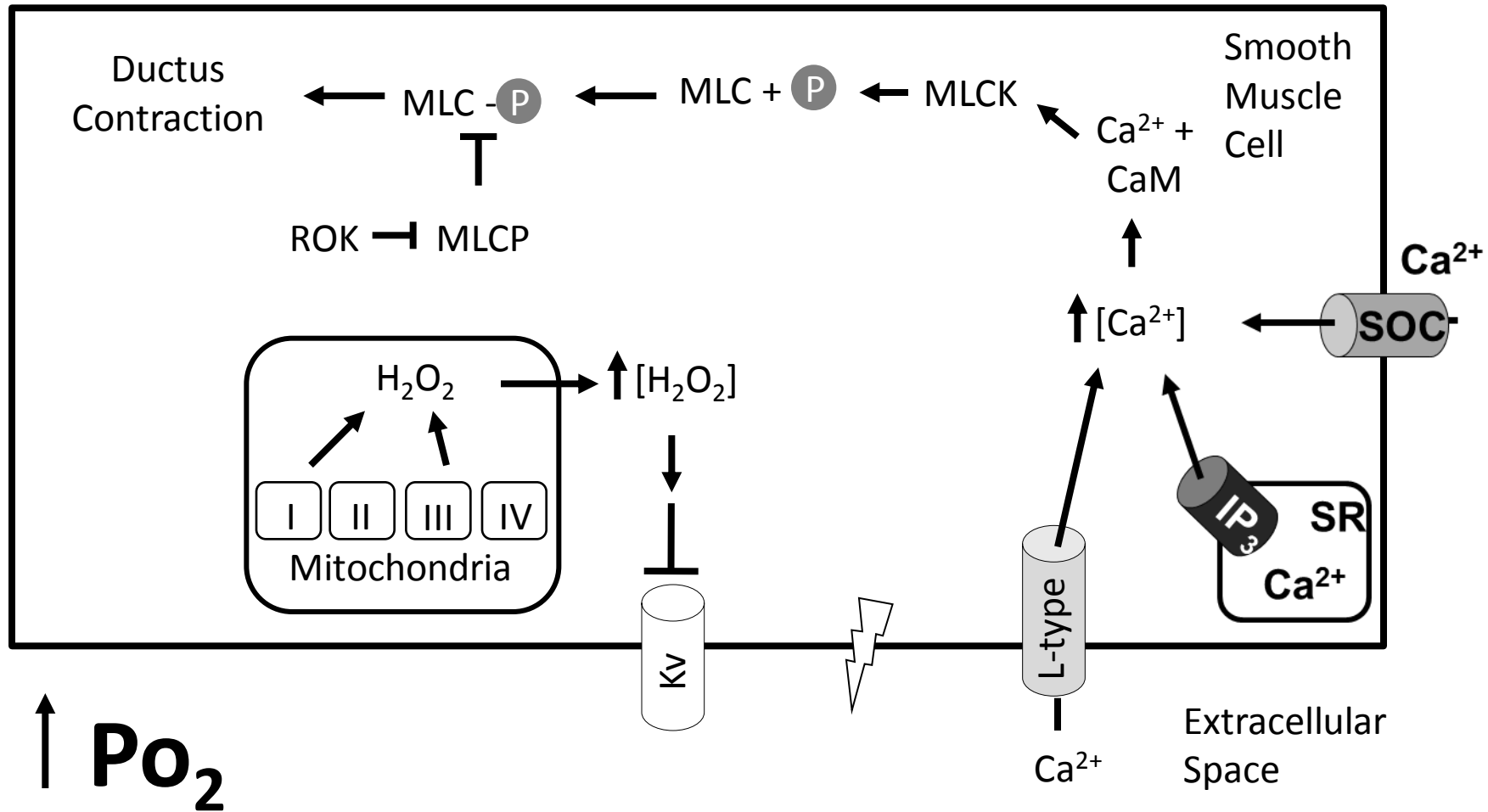
Mechanism of Contraction



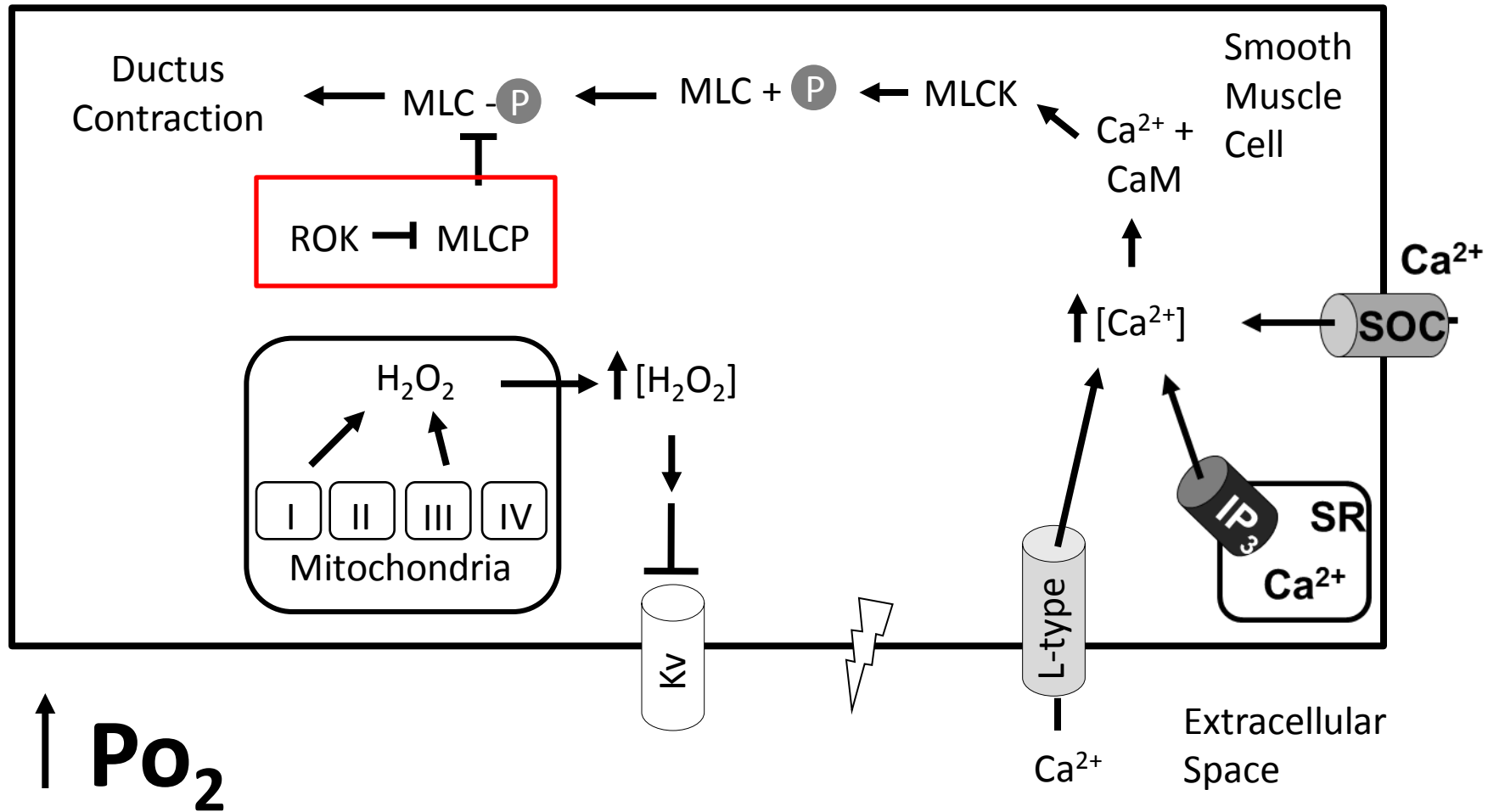
Mechanism of Contraction



Mechanism of Contraction



Mechanism of Contraction



Hypothesis

- **Hypoxia delays maturation in the Rho-kinase pathway**
- **Hyperoxia accelerates the maturation of the Rho-kinase pathway**

Materials and Methods

- White leghorn chicken eggs were incubated at 37.5°C and relative humidity of 70%
- Eggs were incubated in three groups:
 - Normoxia
 - Hypoxia (15% O₂)
 - Hyperoxia (30% O₂)
- Excised Day 18, IP, EP vessels
- Blocked Rho-kinase pathway with Y-27632

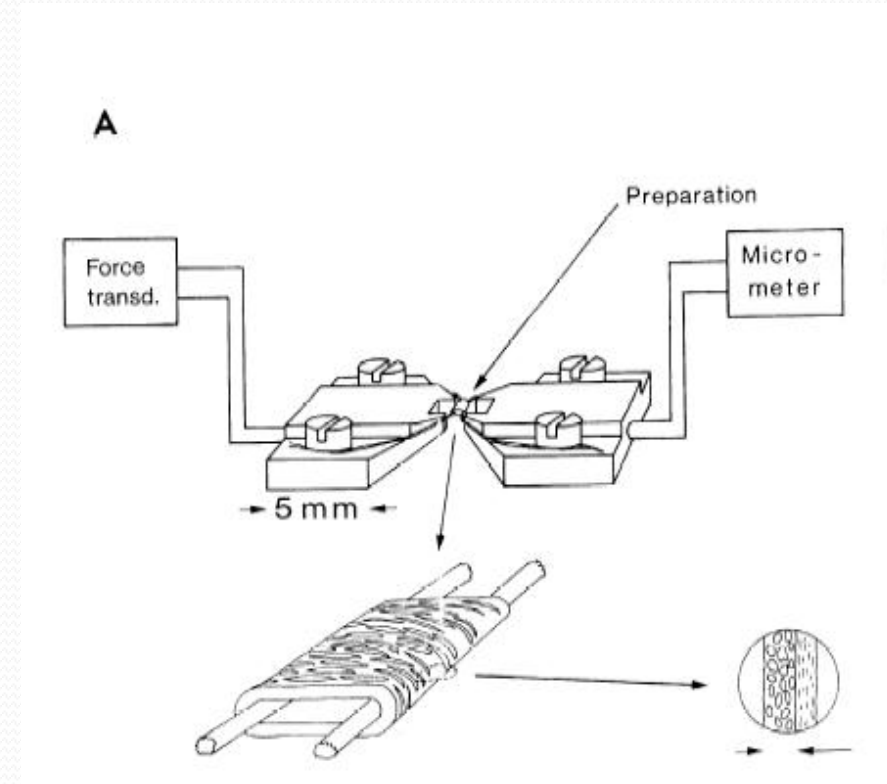


Fig. 3 Myograph Diagram Adapted from (Mulvany and Aalkjaer, 1990)

Experimental Protocol

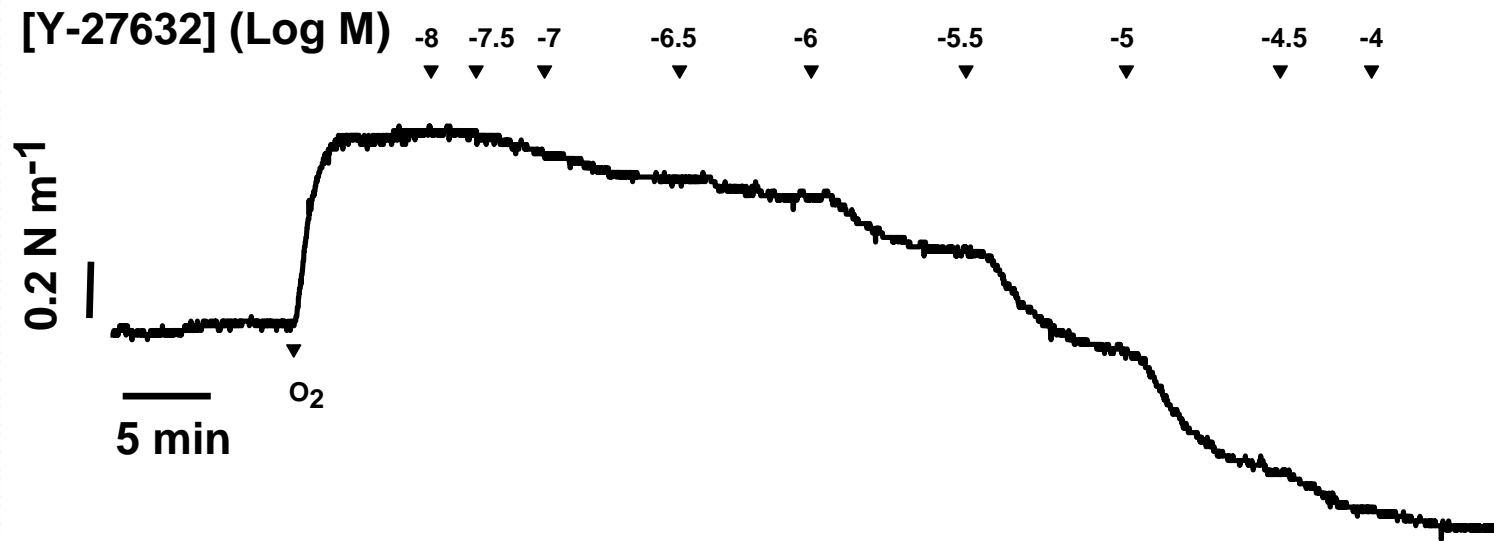
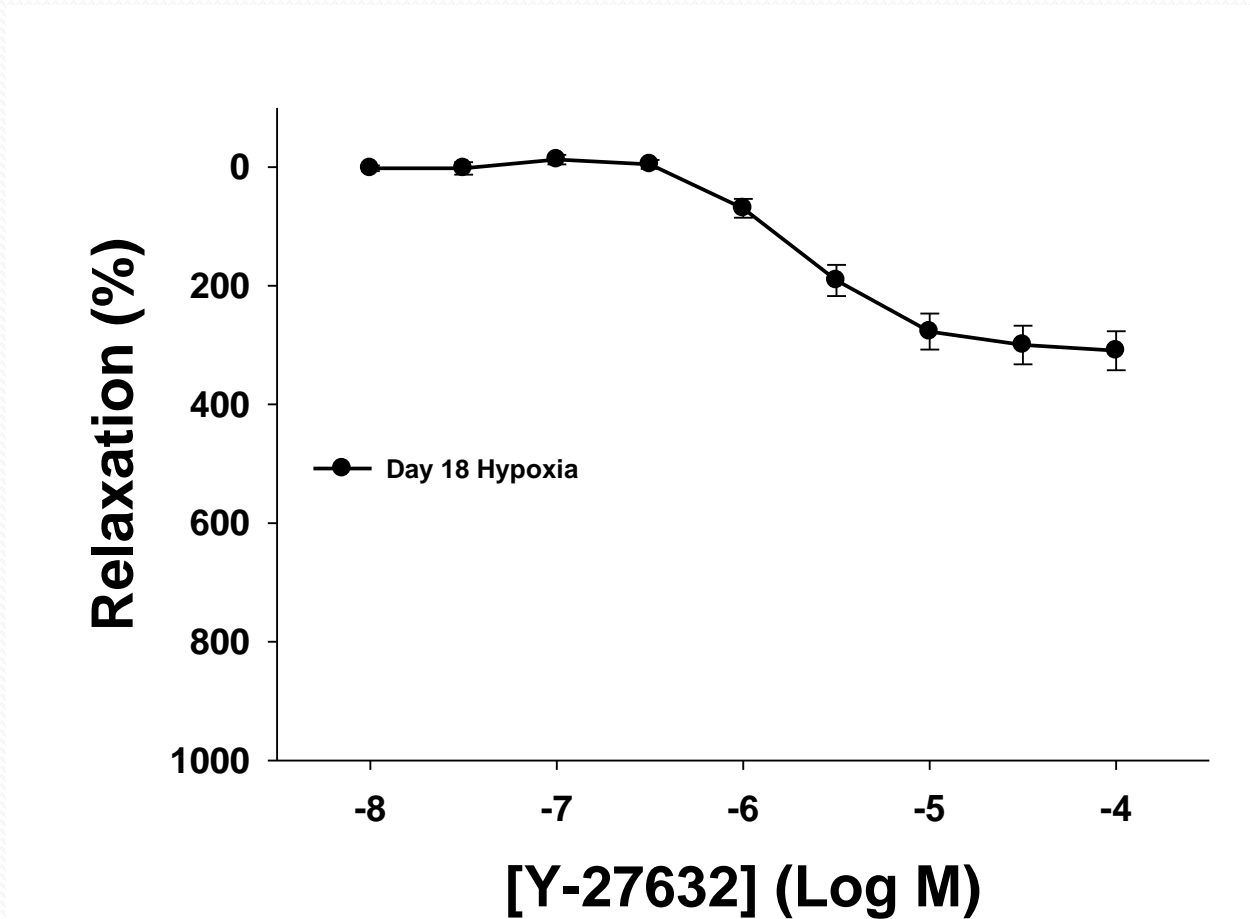
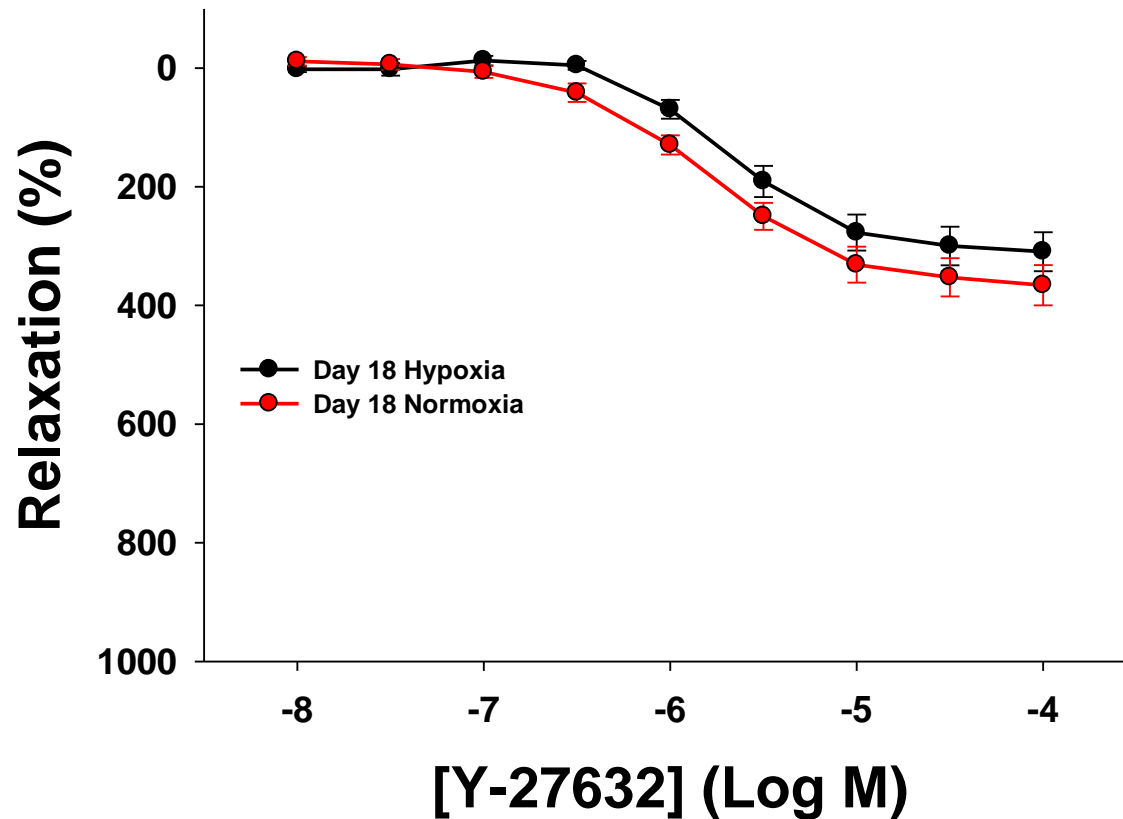


Fig. 4 Exposed to a step-wise increase in the Rho-kinase inhibitor Y-27632 (10^{-8} to 10^{-4} M)

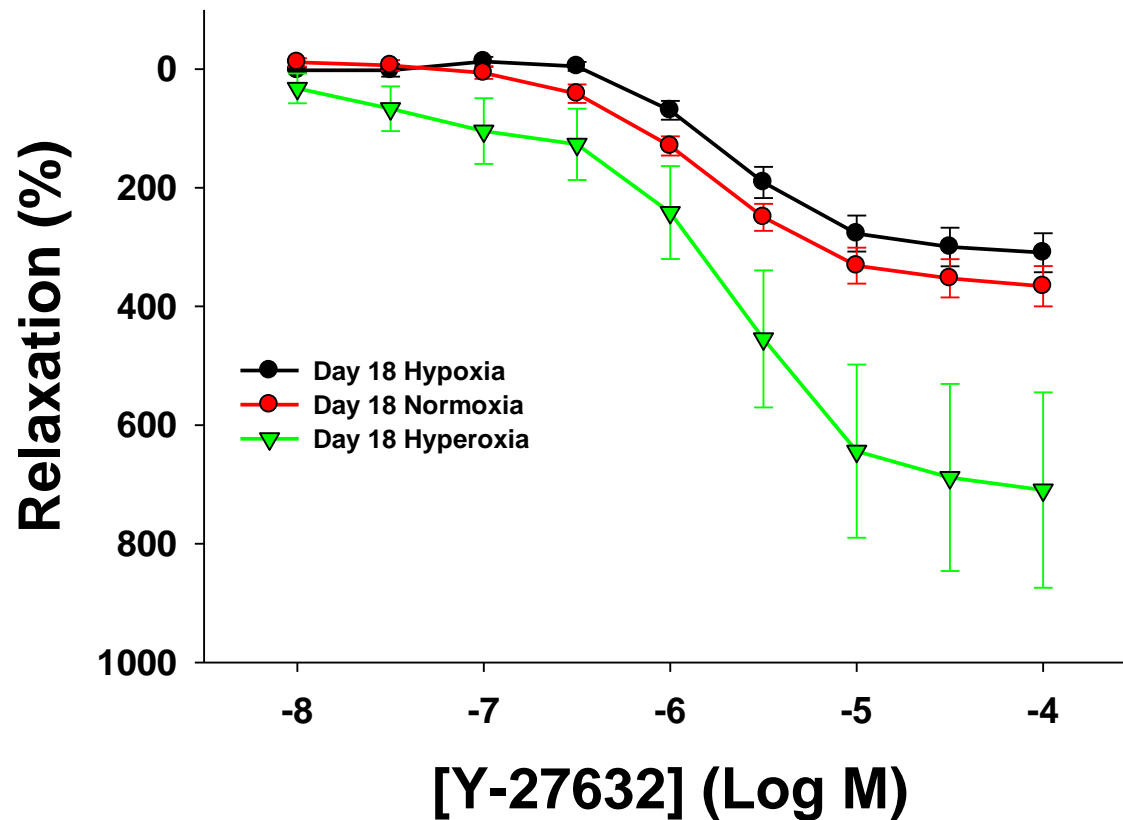
Relaxation Response in Day 18 Embryos



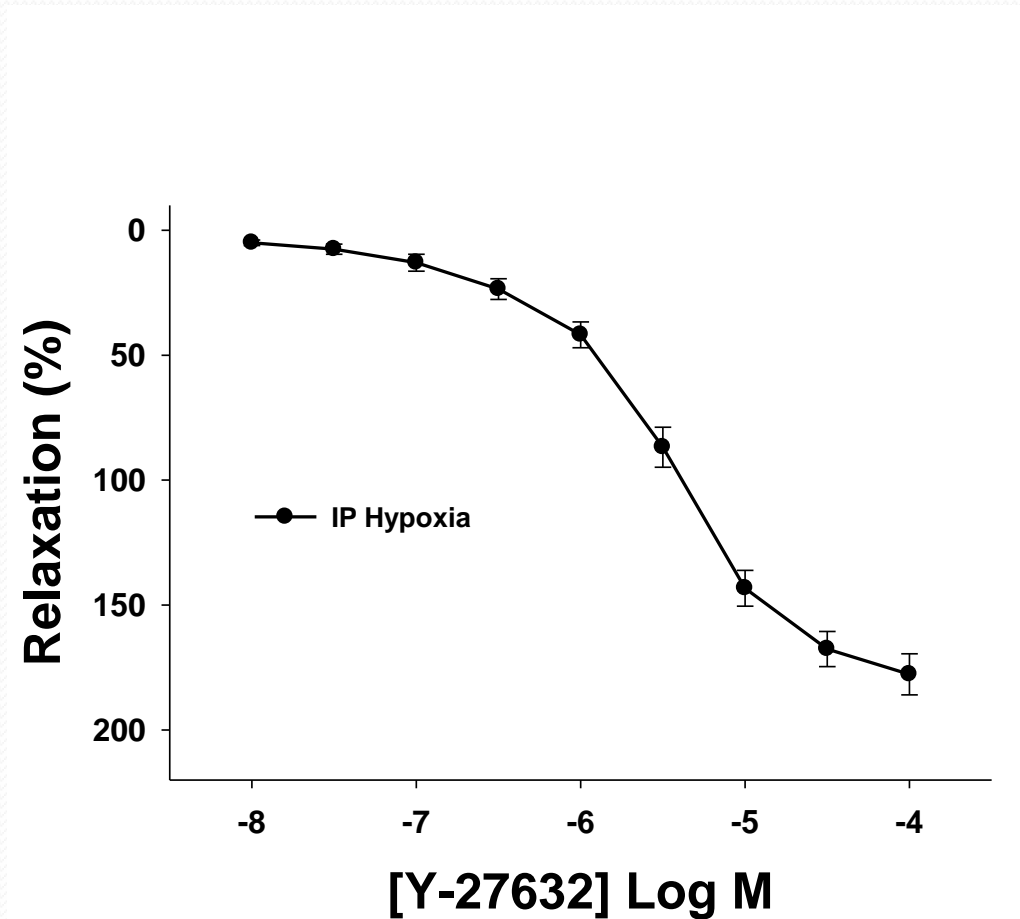
Relaxation Response in Day 18 Embryos



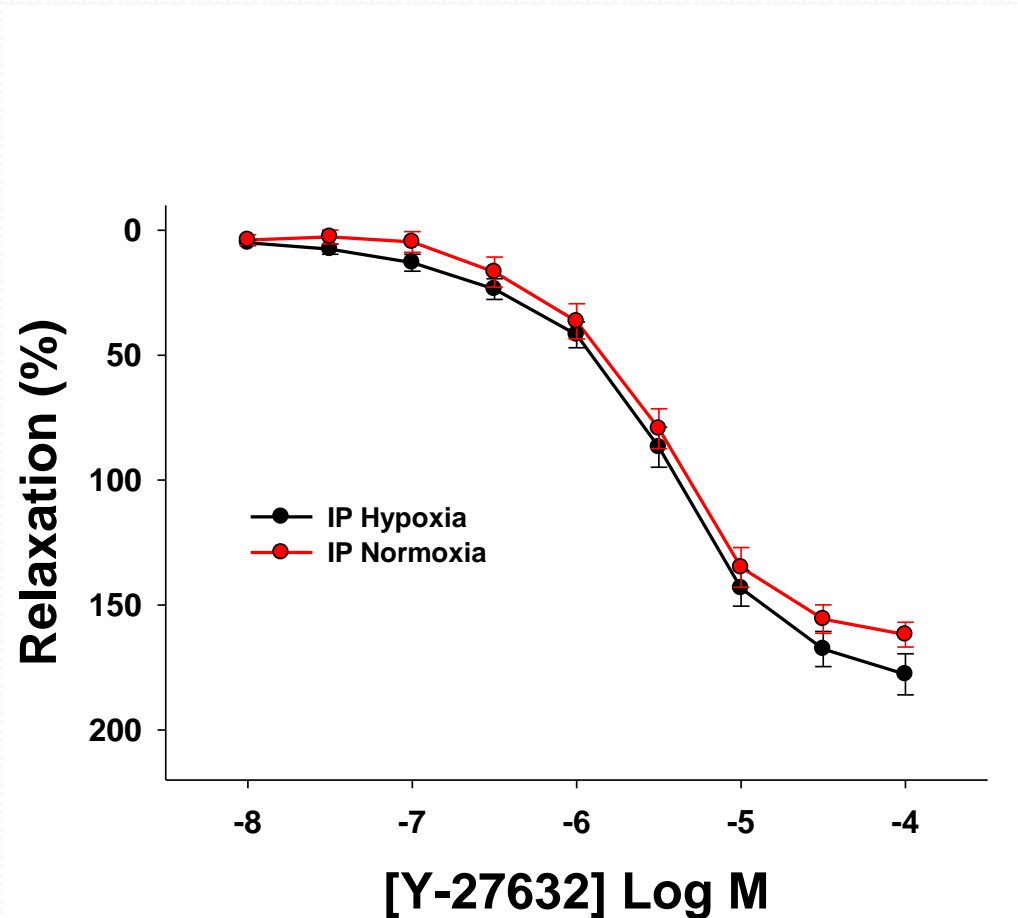
Relaxation Response in Day 18 Embryos



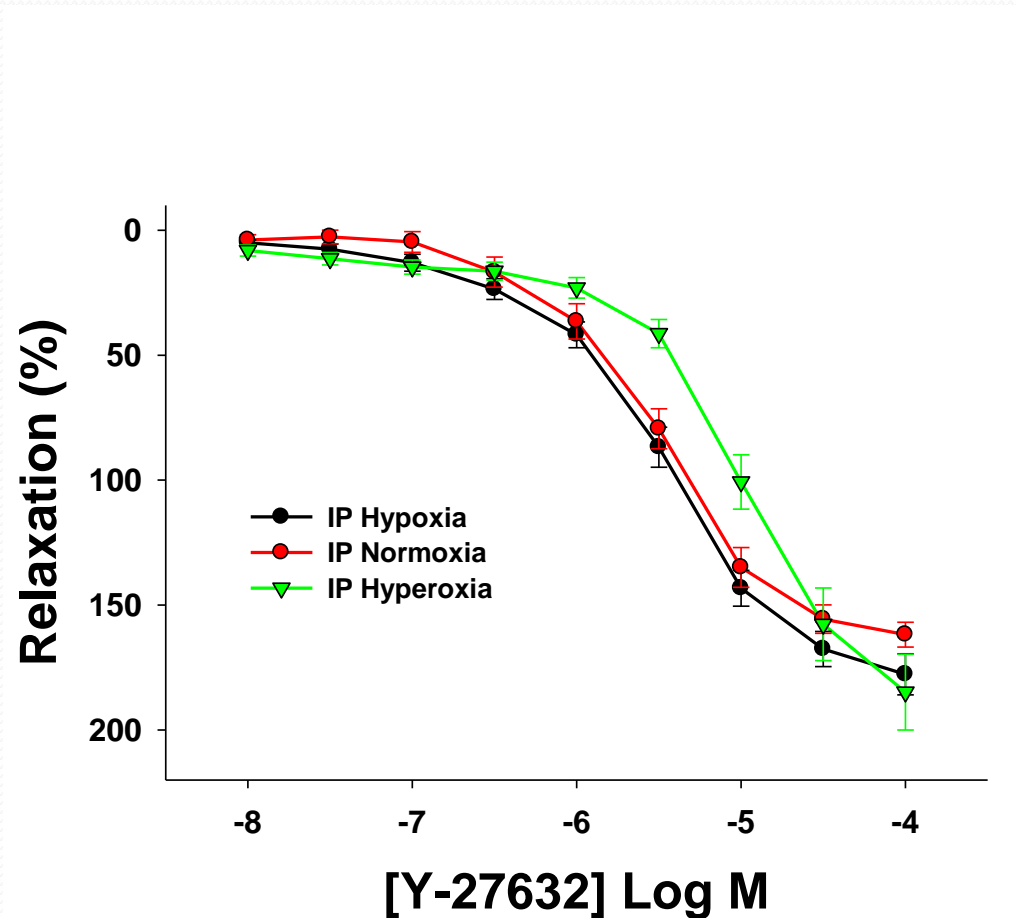
Relaxation Response in IP Embryos



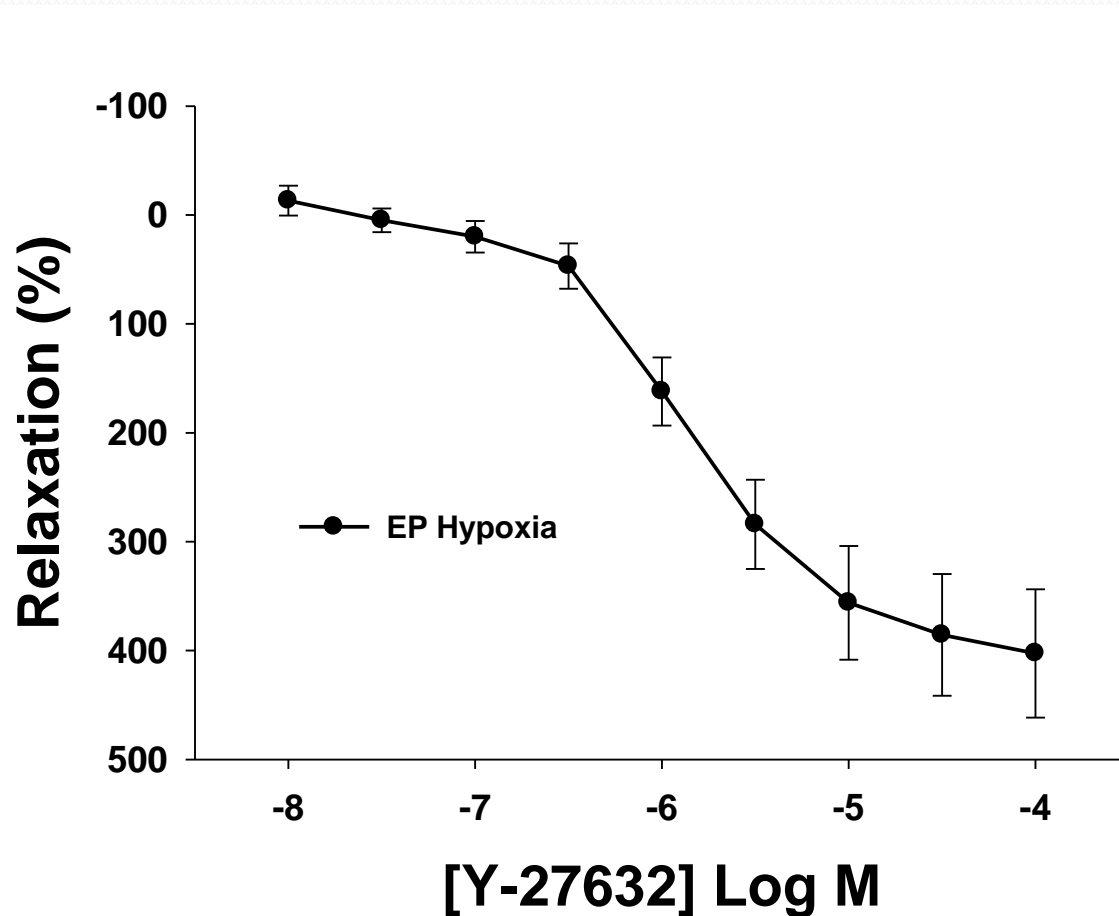
Relaxation Response in IP Embryos



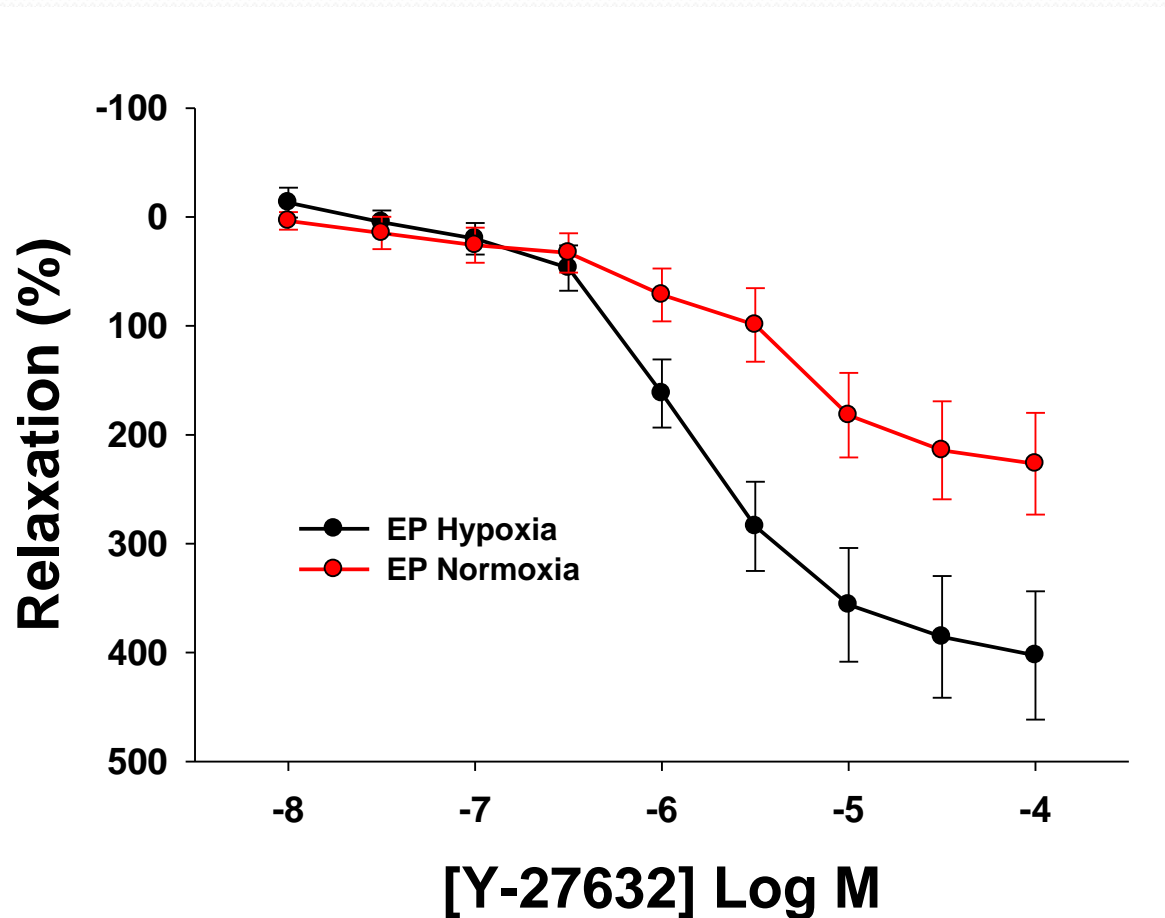
Relaxation Response in IP Embryos



Relaxation Response in EP Embryos



Relaxation Response in EP Embryos



Conclusions

- **Y-27632 inhibited the Rho-kinase pathway, allowing significant relaxation of the DA in all stages and all incubation environments.**
- **Day 18 hyperoxic DA showed a greater relaxation than day 18 hypoxic and normoxic DA, suggesting a higher Rho-kinase activity in eggs incubated in hyperoxia.**
- **Incubation under chronic hypoxia or hyperoxia did not affect the Y-27632 induced relaxation when compared to normoxia during IP.**

Conclusions Cont.

- **Because the onset of IP and EP in embryos incubated under chronic hypoxia and hyperoxia is delayed or accelerated, further experiments will characterize the response to Y-27632 in relation to the length of development.**
- **Given the differences observed during Day 18 and EP, we are currently examining expression levels of Rho-kinase in hypoxic, normoxic, and hyperoxic incubated embryos to gain a better understanding on their role in DA contraction.**
- **The Rho kinase pathway is important for the contraction of the chicken DA.**

Acknowledgments

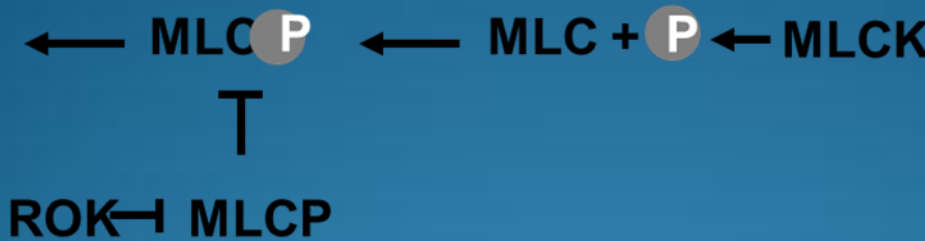
- **This study was supported by an American Heart Association South Central Grant to Dr. Dzialowski**
- **The Dzialowski Lab**
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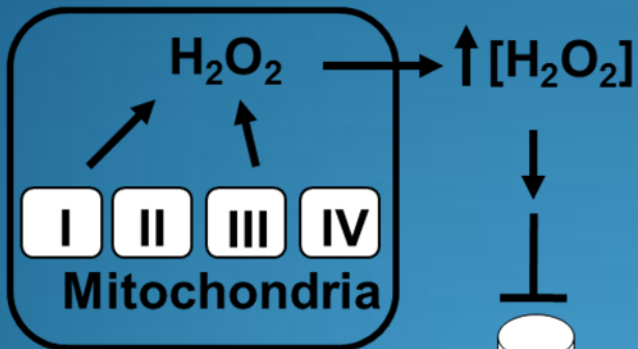
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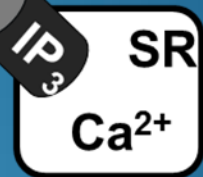
Ductus
Contraction



Smooth
Muscle
Cell



Ca²⁺



Extracellular
Space

Ca²⁺



↑ [Ca²⁺]



Ca²⁺ +
CaM

↑
P_O₂