Oxygen-Induced Contraction in the Guinea Pig Neonatal Ductus Arteriosus

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SUMMARY We investigated the mechanism of oxygen-induced contractions in ductus arteriosus isolated from neonatal guinea pig. A preparation equilibrated at low Po2 (<40 mm Hg) displayed a steady membrane potential of -54.8 mV. Application of oxygen (Po2 = 300 mm Hg) resulted in: (1) stepwise development of tension coupled to action potentials and (2) sustained membrane depolarization to -32.9 mV associated with tonic contraction. Mechanical sensitivity to oxygen persisted at any [K]o up to 126 mM, and tension was always larger at a given [K], or a given membrane potential with high Po2 than with low Po2. The change in membrane potential per decade change in [K]o was 35 mV at low Po2 and 16 mV at high Po2. Oxygen contractions occurred when the ductal strips were bathed in K-free media or exposed to ouabain. We conclude that oxygen caused a conductance change in the sarcolemma resulting in depolarization, which is coupled to contraction. There is also evidence of a membrane potential-independent contraction mechanism.
and built in the shops of the Johnson Research Foundation. Nitrogen and oxygen gas (with 3% CO₂ vol/vol) was used to achieve a low P₀₂. High P₀₂ was obtained using a mixture of nitrogen gas with an equal proportion. A low P₀₂ was maintained using a mixture of nitrogen gas continuously gassed with appropriate mixtures of nitrogen and oxygen containing 3% CO₂ (vol/vol). Fluid P₀₂ was monitored with a Clark-type oxygen electrode. The functional tip extended into the organ bath within 3 mm of the preparation. The instrument was calibrated with potassium and air-saturated solutions. Fluid P₀₂ was also measured in a blood micro-system analyzer (BMS 3 Mk2; Radiometer Copenhagen). With nitrogen gassing, P₀₂ reached a new level within 60 seconds. High P₀₂ is any P₀₂ equal to or greater than 300 mm Hg. Isometric tension and P₀₂ were recorded on a Gould Brush 220 recorder.

**Electrophysiology Experiments**

The apparatus was designed for simultaneous microelectrode and tension measurements at low and high P₀₂ and during the transition periods. The bath fluid was equilibrated with dithionite and air-saturated solutions. Fluid P₀₂ was also measured in a blood micro-system analyzer (BMS 3 Mk2; Radiometer Copenhagen). With nitrogen gassing, P₀₂ reached a new level within 60 seconds. High P₀₂ is any P₀₂ equal to or greater than 300 mm Hg. Isometric tension and P₀₂ were recorded on a Gould Brush 220 recorder.

**Mechanical Sensitivity to Oxygen**

Isometric responses to changes in bath fluid P₀₂ are shown in Figure 1. As can be seen (Fig. 1A), the isometric tension from isolated strips of ductus arteriosus varied from 350 to 150 mN. The oxygen electrode amplifier, as well as the probe, was designed and built in the shops of the Johnson Research Foundation.
ductal strip adjusted its contractile tone to surrounding P02. A rapid switch from low to high P02 resulted in a mechanical response as shown in Figure 1B. Oxygen-induced contractions were tonic, completely reversible, and reproducible. They developed with a characteristic pattern, i.e., the tension increased progressively before it underwent a fast rise composed of small mechanical steps (Fig. 1C). Figure 2 shows the relationship between steady state tension and fluid P02. Contractile activity is expressed as percentage of the maximal response to oxygen (i.e. zero tension is the tension at low P02). Developed tension reached a half-maximal value at P02 of approximately 75 mm Hg.

**Oxygen-induced Contraction vs. Membrane Potential**

At low P02 (less than 40 mm Hg) the resting membrane potential averaged -54.8 ± 5.5 mV (SD; number of cells = 10), and the cells were electrically quiescent. During the tonic phase of the oxygen-induced contraction, the plasma membrane potential averaged -32.9 ± 3.9 mV (n = 18). Most of our cell penetrations occurred either prior to the oxygen contraction or after a maximal oxygen contraction had occurred. In three runs, however, we were able to make a penetration during development of a contraction. Records from one of these experiments is shown in Figure 3. These data indicate that the step-like development of tension corresponds to repetitive firing of action potentials. The average rate of action potentials in the three experiments was 83/min.

### Potassium-Induced Contraction and Depolarization

We studied effects of altering bathing solution P02 under conditions where [K]o in the bath had been increased in order to define relationships of membrane potential and mechanical tension. Raising [K], at low P02 resulted in a gradual increase in ductus mechanical tension (Fig. 4). Unlike oxygen-induced contraction, K-induced contraction developed smoothly. Contractions achieved at [K], up to 29.5 mM were tonic, whereas the tensions produced with 59 and 126 mM [K], reached a transient maximum and declined slowly to a steady state level. K-induced contractions displayed mechanical sensitivity to oxygen at any [K], tested. Figure 5 shows data from steady state experiments where the effects of [K]o on isometric tension at low and high P02 were investigated. It is clearly seen that the effect of oxygen is to increase the magnitude of any K-induced contraction. At [K], of 59 mM or higher,
the O\textsubscript{2} effect was biphasic with an initial small relaxation followed by contraction.

Figure 6 gives membrane potential data obtained at various [K\textsubscript{o}] at low and high P\textsubscript{O\textsubscript{2}}. Switching from low to high P\textsubscript{O\textsubscript{2}} resulted in further membrane depolarization at all [K\textsubscript{o}] studied, and the effect was completely reversible. A slope of 35 mV per decade was extrapolated from the portion of the curve between 5.9 and 29.5 mM [K\textsubscript{o}] for low P\textsubscript{O\textsubscript{2}} data. At high P\textsubscript{O\textsubscript{2}}, the change in membrane potential averaged 16 mV per decade change in [K\textsubscript{o}].

Effects of Bathing in K-free Media or in Solutions Containing Ouabain

We studied effects of ouabain or K-free media to test the hypothesis that inhibition of an electrogenic Na pump (Hendrickx and Casteels, 1974; Hermemeyer, 1976) might cause membrane depolarization seen with oxygen induced contractions. Oxygen-induced contractions were always seen during exposure of the ducti to K-free media or ouabain (10^{-5} M). The magnitude of these contractions were not significantly different from control (99.5 ± 4% of control in K-free media, n = 5; 92 ± 4% in 10^{-5} M ouabain solution). After a long exposure to either solution, the time course of the contraction was prolonged. Figure 7 illustrates the resistance of oxygen contractions to exposure to a K-free solution.

Oxygen reactions could not be completely reversed by low P\textsubscript{O\textsubscript{2}} with strips in these solutions. Whether this was related to a change in intracellular electrolyte activity or to another effect was not determined in this study. During maintenance of K-free high P\textsubscript{O\textsubscript{2}} tension, readmission of K ions to the bath resulted in an initial relaxation followed by a secondary contraction (Fig. 7). This finding is similar to data described by Bonaccorsi et al. (1977), using vascular smooth muscle, and has been attributed to an initial hyperpolarization resulting from a hyperactive electrogenic Na pump, followed by depolarization as the activity of this pump falls to normal as [Na] approaches normal levels.

Discussion

The present paper describes a preparation from neonatal guinea pig ductus arteriosus that displays the characteristics of oxygen sensitivity as previously reported by Fay (1971), i.e., similar pattern of contraction development and similar P\textsubscript{O\textsubscript{2}} requirement for half-maximal tension. The dissection used here, ductus cut open and tied as opposed to intact vessels, did not seem to impair much of ductus intrinsic properties and provided us with a preparation suitable for electrophysiological investigation. The resting membrane potential of the ductus arteriosus cells in a low P\textsubscript{O\textsubscript{2}} environment is similar to the values observed in other quiescent vascular muscles studied under oxygenated conditions (Sonoly et al., 1969; Hermemeyer, 1976a; Casteels et al., 1977; Harder and Sperelakis, 1978). The slope of the curve relating the membrane potential to the logarithm of [K\textsubscript{o}] is less than expected if the mem-
brane behaved like a K-electrode. Such low slope has been reported for other vascular smooth muscles (Hermansmeyer, 1976a; Harder and Sperelakis, 1978) and may not represent the maximal slope of K-induced depolarization because of the participation of an electrogenic pump in this low range of [K]₀ (Droogmans et al., 1977).

The major finding of our investigation is that membrane depolarization accompanies the oxygen-induced contraction. More precisely, data indicate that application of oxygen to a ductal strip results in transient firing of action potentials during step-like development of tension and sustained membrane depolarization during tonic contraction. An oxygen-induced depolarization has been reported previously in goat ducti cells (Noel et al., 1973).

The sustained depolarization seen during O₂ contraction appears not to be due to inhibition of an electrogenic ouabain inhibited Na pump. This follows from the finding of normal amplitude O₂ contractions with the strips in ouabain or K-free media. Our data implicate a change in ionic conductance of the sarcolemma as a cause of the O₂-induced depolarization. The finding that oxygen reduced the ability of K to depolarize the cell membrane suggests an increase in the ratio of sodium or chloride conductance to K conductance. Further work is required to delineate the nature of these conductance changes.

Our data imply a role of plasma membrane depolarization in generation of oxygen contractions. The finding that O₂ contractions were seen in experiments where [K]₀ was elevated as high as 126 mm argues there may also be a membrane potential-independent component. The plot shown in Figure 8 indicates that, at high P₀₂, there are larger mechanical tensions at the same membrane potential than seen during low P₀₂. This finding is not inconsistent with the finding that at the same [K]₀, going from low P₀₂ to high P₀₂ caused further depolarization. At [K]₀ of 29.5 and higher, it is unlikely that any further membrane depolarization on switching from low to high P₀₂ is linked to contraction, since near maximal K contractions occur at about [K]₀ of 29 mm and a membrane potential of about −30 mV. Increasing [K] may have effects on contractility in addition to the effects on the membrane potential, and it is possible that high K turned on a membrane potential-independent mechanism sensitive to oxygen. We cannot completely exclude the possibility that increasing P₀₂ increased amplification of a given membrane potential change so that oxygen contractions are still completely dependent on membrane depolarization. Evidence related to this possibility for drug contractions has been discussed previously (Coburn, 1979). The concept that there is a membrane potential-independent mechanism, as well as a membrane potential-dependent component, is consistent with work on other vascular smooth muscles (Droogmans et al., 1977).

The mechanisms of the O₂ effect on the electrical properties of the sarcolemma remain to be elucidated. There is evidence that cytochrome a₃ is an “O₂ sensor” coupled to tension in this tissue (Fay, 1971, 1972). The mechanisms by which alteration in energy production or the cell oxidation-reduction state can alter the sarcolemmal electrical properties should be investigated further.

Synthesis of prostaglandins or other arachidone by-products is believed to be involved in O₂ contractions in lamb ductus (Cocceani et al., 1976). The sensitivity of the neonatal guinea pig ductus to O₂ persists in the presence of 10⁻⁶ M indomethacin and indomethacin does not contract ductus during low P₀₂ (Roulet and Coburn, unpublished data). It seems unlikely to us that arachidonate cyclooxygen-
ase by-products are involved in the basic mechanisms of oxygen-induced contractions in ductus from this species.

Acknowledgments

We gratefully acknowledge the advice and assistance of Dr. A.V. Somlyo.

References


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doi: 10.1161/01.RES.49.4.997

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