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Light patent ductus arteriosus
oxygen photochemistry

Patent Ductus Arteriosus: A New Light on an Old Problem

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Summary

It has been suggested previously that delayed closure of the ductus arteriosus in premature infants is related to an ineffective constriction in response to an increase in arterial PO_2. The contractile effects of increased PO_2 and excess K^+ were studied in rings of ductus arteriosus from early (70 ± 4 days, $n = 9$) and late (137 ± 3 days, $n = 11$) gestation fetal lambs. Studies were performed in a laboratory using overhead fluorescent lighting or in a dark, enclosed box. Room light relaxed the oxygen-induced contraction in immature vessels but had no significant effect on the K^+-induced contraction. Room light did not alter either the oxygen or K^+-induced contractile responses in mature vessels. When comparing oxygen induced contractions in room light in immature vessels (0.27 ± 0.13 g, $n = 9$) vs. mature vessels (0.82 ± 0.06 g, $n = 11$) there appeared to be an increased response to oxygen with advancing gestational age. However, when the oxygen-induced responses of immature (0.59 ± 0.15 g, $n = 9$) and mature (0.82 ± 0.06 g, $n = 11$) vessels were performed in an environment excluding room light, no significant gestational difference was observed. The role of oxygen in delayed closure of the ductus arteriosus of premature infants will need further evaluation.

Speculation

The difference in sensitivity to photorelaxation between rings of ductus arteriosus from immature and mature lambs may be associated with biochemical differences in vessels between early and late gestation.

In contrast to full-term infants in whom functional closure of the ductus arteriosus occurs within the first 24 hr after birth, preterm infants frequently have delayed spontaneous closure (3). The exact mechanism responsible for the constriction of the ductus arteriosus at birth and for its delay in closure in preterm infants is as yet unknown. Numerous observations have drawn attention to the importance of the postnatal increase in arterial oxygen pressure (PO_2) for muscular closure of the ductus arteriosus (6, 11, 13, 14). Rudolph (18) has suggested that the higher incidence of patent ductus arteriosus in preterm infants might be due to immaturity of ductal smooth muscle. Several *in vitro* studies have suggested that delayed closure of the ductus arteriosus in preterm infants is related to an ineffective constriction in response to increases of PO_2 (10, 14, 15). This development of responsiveness to O_2 has been attributed to maturation of either specific receptors for oxygen (15) or vascular smooth muscle contractility (10). The results reported below identify a third possible explanation for this *in vitro* developmental response of the ductus arteriosus to oxygen. Several years ago Furchgott *et al.* (9) observed a photoactivated relaxation of smooth muscle in isolated strips of rabbit aorta. This relaxation was reversible, depended on the pre-exposure level of active contraction, and occurred in the presence or absence of oxygen. We have observed that vessels from immature lambs are very sensitive to photorelaxation by overhead fluorescent lights, used for general illumination in the laboratory, whereas those from older lambs are insensitive. We suggest that the lack of responsiveness to oxygen of immature vessels *in vitro* may be secondary to the effects of overhead laboratory lights on immature vessels and that there may be minimal gestational differences in contractile response to oxygen.

MATERIALS AND METHODS

Time-dated fetal lambs, between 56 and 145 days of gestational age (term is 150 days), were delivered by cesarean

section and rapidly killed by exsanguination. The ductus arteriosus was dissected free from adventitial tissue and divided into 2 mm thick rings which were placed in separate 150 ml isolated Lucite plastic organ baths (fluid removed by draining). The rings were suspended under an initial load of 1 g between two stainless steel hooks, in a solution containing 127 mM NaCl, 5 mM KCl, 2.7 mM $CaCl_2$, 1.27 mM $MgCl_2 \cdot 6H_2O$, 5.5 mM glucose, and 50 mM Tris·HCl, pH 7.39 at 37°. Isometric responses were measured by a Grass FT 03C force transducer and recorded on a Grass polygraph. Small samples of the bathing solution were withdrawn and pH and PO_2 were measured using Radiometer electrodes and blood gas meter.

General illumination in the laboratory was kept constant throughout the duration of the studies. The laboratory was illuminated from recessed ceiling fixtures containing F40 CW Cool White General Electric fluorescent lamps which were filtered through a 2 mm thickness of acrylic plastic. Overhead lighting was 132 cm above the suspended tissues. The illumination reading at the organ bath, as recorded on a light meter (Weston Electric Instrument Corporation, Newark, NJ) was 77 footcandles (12).

The bathing solution in each bath was bubbled with 100% N_2 (to a PO_2 of 14–20 torr) and the rings allowed to equilibrate for 1–2 hr until a steady tension was developed. The rings were exposed to 100% O_2 (to a PO_2 of 680–720 torr) for 30–50 min, allowing the tension to achieve a new plateau. The vessels then were allowed to relax in 100% N_2. The organ baths were placed in the dark by an enclosed box and the tissues allowed to reach a new steady tension. A second oxygen response was obtained in the dark and the vessels subsequently were allowed to relax in the dark in 100% N_2. The box then was removed exposing the tissues to room light and, after a steady tension was achieved in 100% N_2, a third oxygen challenge was performed. The sequence of light exposures was randomized among the different vessels so that in some vessels the order of oxygen challenges were done in the dark followed by light followed by dark.

The effects of light on K^+-induced contractions were examined in some tissues. Rings of ductus arteriosus were suspended in the above high Na^+ solution and allowed to reach a steady tension in 100% O_2 in room light. The bath solution was rapidly exchanged with a new high K^+ solution (42 mM NaCl, 90 mM KCl, 2.7 mM $CaCl_2$, 1.27 mM $MgCl_2 \cdot 6H_2O$, 5.5 mM glucose, and 50 mM Tris·HCl, pH 7.39, PO_2 680–720 torr, 37°) and allowed to reach a steady tension. The solution bathing the tissue was changed four times with high Na^+ solution and the tissue allowed to relax to a steady tension at 100% O_2. The organ baths then were placed in the dark in an enclosed box. After a steady tension was reached the vessel was challenged again with the high K^+ solution in the dark. The sequence of light and dark exposure was randomized among the different tissues.

The paired Student's *t*-test was used to compare mean tensions.

RESULTS

The response of the ductus arteriosus in room light exposed to high PO_2 was similar to that reported previously (16). The response in room light to changes in PO_2 increased with gestational age from a questionably significant increase in tension (0.27 ± 0.13 g, $n = 9$) in the immature fetus to a significant contraction (0.82 ± 0.06 g, $n = 11$) in the mature one (Fig. 1). However, when the O_2-induced contraction was performed in a dark, enclosed box, no significant difference was observed in the developed tensions of immature and mature vessels, 0.59 ± 0.15 g ($n = 9$) and 0.82 ± 0.06 ($n = 11$), respectively (Fig. 1) ($P < 0.05$).

As can be seen in Figure 1, room light relaxed both the steady tension developed in low PO_2 as well as diminished the O_2-induced contraction in the immature vessels. No significant relaxant effect of light was observed in the mature vessels on the tensions in either low or high PO_2.

Increasing the K^+ concentration of the bathing solution induced a contraction in the vessels of both immature and mature gestational ages above the increased steady tension developed

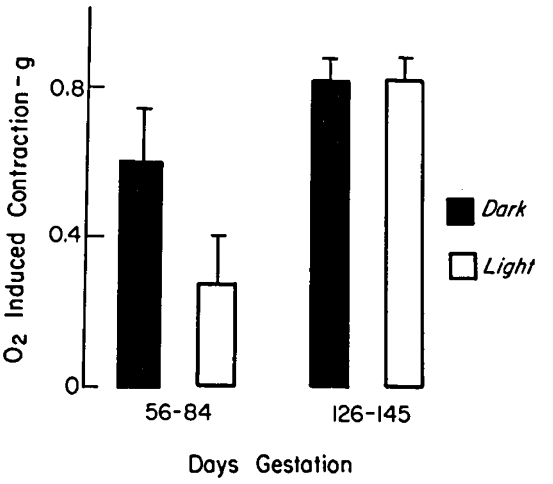

Fig. 1. Effects of light on oxygen-induced contraction in the ductus arteriosus from fetal lambs of different gestational ages. Rings of ductus arteriosus from immature lambs, 56–84 days (mean = 70 ± 4 days, $n = 9$), and mature lambs, 126–145 days (mean = 137 ± 3 days, $n = 11$), were suspended in a low PO_2 (14–20 torr) solution. Some rings were exposed to general room illumination (light) and some were kept in a dark, enclosed box (dark). The initial steady tension of rings from immature vessels in low PO_2 was 0.98 ± 0.06 g in the dark and 0.80 ± 0.08 g in the light ($P < 0.005$, paired *t*-test). The initial steady tension of rings from mature vessels in low PO_2 was 1.16 ± 0.13 g in the dark and 1.13 ± 0.13 g in the light (no significant difference). The bar values represent the increase in tension produced by exposing the tissues to high (PO_2 (680–720 torr) ± SEM. The oxygen-induced contraction is diminished in immature vessels exposed to room light ($P < 0.01$, paired *t*-test), but not in mature vessels.

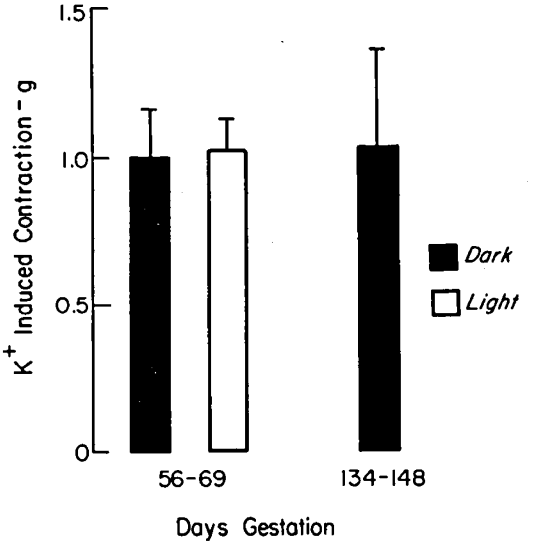

Fig. 2. Effects of light on K^+-induced contraction in the ductus arteriosus from fetal lambs of different gestational ages. Rings of ductus arteriosus from immature lambs ($n = 6$) and mature lambs ($n = 4$) were suspended in a high PO_2 solution (680–720 torr). Some rings were exposed to general room illumination (light) and some were kept in a dark, enclosed box (dark). The bar values represent the increase in tension produced by exposing the vessels to a solution with excess KCl ± SEM. There is no significant difference between the K^+-induced contraction in either immature or mature vessels or immature vessels exposed to room light.

with high PO_2 exposure. Room light had no significant effect on the K^+-induced contraction in either age group (Fig. 2).

DISCUSSION

Furchgott *et al.* (9) observed that smooth muscle strips of rabbit aorta, placed in a state of active tonic contraction by the addition of a stimulating drug, relaxed during exposure to light. The relaxation was reversible and depended on the preexposure level of contraction. The relaxation did not require the presence of oxygen. The action spectrum reached a peak at 310 nm with relatively little effect at wavelengths above 450 nm or below 280 nm. The extent of relaxation produced by a standard exposure was independent of the nature of the stimulating drug used to produce contraction (histamine, norepinephrine, serotonin), however, the relaxation was significantly smaller when the contraction was produced by excess KCl.

General laboratory illumination caused photorelaxation of the ductus arteriosus from the immature lamb fetus. There was a decrease in the maximum oxygen-induced tension developed in room light. There was also no significant effect of room light on contractions induced by excess KCl. The sensitivity of immature rings of lamb ductus arteriosus to photorelaxation appeared to be much greater than that of rabbit aorta. A significant fall in tension in the rabbit aorta was produced by radiation from a xenon arch lamp with an intensity of approximately 100 $\mu W/cm^2$ at 350 nm (9), whereas a significant photorelaxation of the immature lamb ductus arteriosus was produced by overhead fluorescent light of less than 5% of this equivalent intensity (17). In fact, Furchgott (8) has reported that overhead fluorescent lamps do not have any significant effect on the smooth preparations he has studied.

The mechanism through which photorelaxation of the lamb ductus arteriosus occurs is unknown. Whether light activates some photosensitive material in the smooth muscle cells which leads to an alteration of the ionic permeability characteristics of the membrane (2, 4), to a change in cyclic nucleotide metabolism (1), to a change in cytochrome oxidation state (5), to an inactivation of surface receptors (19), to a change in prostaglandin metabolism, or to photooxidation of myoglobin (7) needs to be evaluated in the future. However, the difference in sensitivity to photorelaxation between rings of ductus arteriosus from immature and mature lambs may be associated with biochemical differences in vessels between early and late gestation.

We observed that preparations of rings of ductus arteriosus from mature lamb fetuses *in vitro* are not significantly relaxed by general room illumination. Similarly we observed no significant difference in the oxygen-induced contraction of immature and mature vessels kept in a dark environment. On the basis of these results we would suggest that former experiments demonstrating a diminished contractile response to PO_2 in rings of ductus arteriosus from immature lambs may be secondary to the significant photorelaxation produced by general laboratory lighting rather than to an immature developmental response to oxygen.

Whether the diminished oxygen-induced contraction in *in vitro* preparations of ductus arteriosus from immature guinea pig is due also to a similar mechanism remains to be seen (10, 15). Fay (6) has reported a photorelaxation of the guinea pig ductus arteriosus. There is a diminished response to both oxygen- and norepinephrine-induced contractions in the immature vessels (10), whereas there is no difference in the K^+-induced contractions of immature and mature vessels (15). These results are not inconsistent with the hypothesis stated above. Further experimental work will be necessary to clarify whether there is a maturation of the oxygen-induced contraction of the ductus arteriosus and whether this has a role in the persistent patency of the vessel in premature infants.

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