Maturation of contractile response of ductus arteriosus to oxygen and drugs

S. NOEL AND S. CASSIN
Department of Physiology, University of Florida College of Medicine, Gainesville, Florida 32610

NOEL, S., AND S. CASSIN. Maturation of contractile response of ductus arteriosus to oxygen and drugs. Am. J. Physiol. 231(1): 240-243. 1976. - Contractile responses of rings of ductus arteriosus from fetal and neonatal guinea pigs were studied in buffered Krebs-Henseleit solutions of various oxygen tensions. Oxygen-induced contraction of ductus arteriosus increased with gestational age, peaking at term and attenuating within 24 h after birth. Contractions of ductus in response to potassium were not different in term and preterm fetuses. Maximal contractile response of pre- and postductal aortic rings to oxygen was 8.3% of the maximal oxygen-induced contraction of ductal rings from the same fetuses. Pulmonary artery was quite insensitive to oxygen. Of 12 ductus exposed to bradykinin in the absence of oxygen seven contracted (maximal response was obtained with 15.0 rig/ml). Exposure of ductus to bradykinin in the absence of oxygen enhanced subsequent contractions of ductal smooth muscle rings to air. Atropine failed to inhibit the oxygen-induced contraction of ductus. These data suggest that acetylcholine is not essential for oxygen-induced contraction of the guinea pig ductus arteriosus.

bradykinin; acetylcholine; guinea pig

OXYGEN-INDUCED CONTRACTION of the ductus arteriosus was first demonstrated in situ by Kennedy and Clark in 1942 (12). Kovalcik in 1963 (13) demonstrated oxygen-induced contraction of an isolated ring of ductus arteriosus. Although many studies have demonstrated that oxygen (5, 6, 10, 12, 13, 19, 21) causes contraction of the ductus arteriosus, few have been concerned with maturation of this response during gestation. In one study, McMurphy and Boreus (15) failed to demonstrate oxygen-induced contraction of ductus from human fetuses 12-24 wk of gestational age. However, McMurphy et al. (16) did observe maturation of ductal contraction in response to oxygen in fetal lambs. Attenuation after birth of ductal contraction in response to oxygen was attributed by Sciacci and Condorelli (23) to what they termed an intrinsic process. Moreover, Fay and Cooke (6) suggested that an intimal plug of necrotic tissue and architectural changes in the ductal wall were responsible for loss of oxygen-induced contraction.

Whether oxygen acts directly to effect contraction of ductal smooth muscle or indirectly via release of an intermediary agent has not been definitively established. Several studies have investigated the possible role that epinephrine, norepinephrine (13, 14), bradykinin (13, 15-17), and acetylcholine (13, 15, 21) play as intermediary transmitters in the response of ductal smooth muscle to oxygen.

The objectives of the present study are: 1) to demonstrate maturation of contraction of ductus in response to oxygen as a function of advancing gestation and 2) to evaluate any possible role that acetylcholine (ACh) and/ or bradykinin may have in closure of the ductus arteriosus after birth.

MATERIALS AND METHODS

Ductus taken from guinea pig fetuses of known gestational age and from 24-h neonates were used in the determination of the oxygen dose-response curves. Pregnant guinea pigs were killed by a sharp blow to the base of the skull; fetuses were delivered by Cesarean section within 3 min of maternal death. Fetal and newborn guinea pigs were sacrificed in a similar manner. The thorax was opened and the ductus quickly removed. Segments of aorta and pulmonary artery were excised after removal of the ductus. Sections of pre- and postductal aorta and pulmonary artery were removed from term fetuses only. After removal from the fetuses or neonates, vessel rings were placed in Krebs-Henseleit solution equilibrated with 95% N₂ and 5% CO₂ for 30 min.

Subsequently, vessel rings were suspended in tissue organ baths (50-ml volume) similar to those described by Blinks (1) or those (100 ml) used by Fay and Cooke (6) for an additional 30 min of equilibration and 95% N₂ and 5% CO₂. Each vessel ring was suspended in Krebs-Henseleit buffer with a stainless steel hook and wire in order to measure circumferential muscle tension. Each hook was anchored to a stainless steel rod, which in turn was attached to a metal frame for support. The wire was suspended from a Grass FT03C strain gauge that was connected to the same metal frame by means of a screw clamp. The screw clamp could be adjusted horizontally as well as vertically. The vertical adjustment allowed the suspended wire to exert tension on the vessel ring. A base-line tension of 250 mg was exerted on all vessel rings in 95% N₂ and 5% CO₂. All data were recorded on a four-channel Grass polygraph. Various oxygen and nitrogen mixtures with 5% CO₂ (to maintain pH at 7.4) were bubbled through buffer by means of a sintered-glass disk embedded in the bottom of the tissue organ bath. In the organ bath described by Blinks (1) oxygen-
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An ated solution is circulated from a side arm directly toward the lumen of the ductal ring. Gas mixtures were prepared with a gas mixing pump (Instrumentation Associates, Inc.), stored in a Douglas bag, and analyzed with a Scholander gas analyzer. Unless stated otherwise, Krebs-Henseleit buffer containing 119.0 mM NaCl, 4.00 mM KCl, 2.50 mM CaCl₂, 1.20 mM KH₂PO₄, 1.10 mM Na₂SO₄, 7H₂O, 25.00 mM NaHCO₃, and 11.1 mM dextrose was used in all experiments. The osmolality of this solution (285 mosmol/kg) was determined with a Precision Instrument Co. Osmette A osmometer. The pH (approximately 7.43) was determined with an Instrumentation Laboratory, Inc., 208 pH meter.

Statistics. The Student t test was used to compare two means (3). An analysis of variance and Tukey’s omega test were used to compare more than two means (3). P values less than 0.01 indicate a significant difference between or among means.

Oxygen dose-response curves. All vessel rings were exposed to 0, 20, 25, 30, 35, 44 (95% air, 5% CO₂) and 650 mmHg of oxygen in random order (3). Each vessel ring was exposed to a mixture containing oxygen for 20 min in order to allow sufficient time for contraction. If a vessel ring did not contract within 20 min, it was allowed to relax in 95% N₂ and 5% CO₂ for 15 min. After 15 min the vessel ring was reexposed to the initial oxygen tension. A vessel ring was subjected to three exposures of a particular gas mixture in order to obtain a contraction. The smooth muscle was allowed to remain contracted at a constant tension in the presence of a particular gas mixture for 20 min before measurements were recorded.

Excess potassium. Keatinge’s (11) buffer with and without excess potassium and Krebs-Henseleit buffer were used. Keatinge’s buffer with a physiological potassium concentration contains 133 mM NaCl, 1.38 mM NaH₂PO₄, 4.7 mM KCl, 2.5 mM CaCl₂, 0.11 mM MgCl₂, 16.3 mM NaHCO₃, and 7.8 mM dextrose. Osmolality was 290 mosmol/kg and pH approximately 7.4. Keatinge’s buffer with an excess concentration of potassium contains 3.3 mM NaCl, 16.3 mM KH₂CO₃, 1.38 mM NaH₂PO₄, 89.6 mM K₂SO₄, 2.5 mM CaCl₂, 0.11 mM MgCl₂, and 7.8 mM dextrose. Osmolality was 295 mosmol/kg and pH approximately 7.4.

Vasoactive agents. Viability of each ductus prior to drugs was demonstrated by a contraction of at least 450 mg in response to 95% air and 5% CO₂. Krebs-Henseleit buffer was used as a solvent for the vasoactive drugs. Drug concentrations are expressed as final concentrations in the tissue organ bath.

Bradykinin. Ductus were exposed to bradykinin (Sandoz batch no. 69055; 0.2 ng/ml, 0.4 ng/ml, 0.5 ng/ml, 2.0 ng/ml, 15 ng/ml, and 20 ng/ml) in 95% N₂ and 5% CO₂, bracketed by exposure to 95% air and 5% CO₂ for 20 min.

Acetylcholine block. Atropine (Sigma Chemical Co.) was used to assess involvement of acetylcholine in oxygen-induced contraction of the ductus. Doses of 100, 200, 300, and 400 μg/ml were used. Paired ductus were allowed to contract in oxygen without atropine for the duration of the drug experiments in order to note decay of the preparation with time.

RESULTS

Oxygen dose response. The magnitude of oxygen-induced (Pₒ₂ 144 mmHg) ductal contraction (Fig. 1) of the 48- to 50-day gestational age group was 229% greater than that of the 44- to 46-day group. However, the contractile tension developed in term ductus was 326% greater than that in the 48- to 50-day gestational age group. The P₅₀’s as defined by Fay (5) did not change with advancing gestation. The P₅₀’s were 99 ± 13 mmHg, 80 ± 14 mmHg, and 89 ± 18 mmHg for term, 48- to 50-day gestational age, and 44- to 46-day gestational age, respectively. The contractile response of postnatal (24 h) ductus was greatly attenuated compared with term ductus.

Segments of pulmonary artery (n = 5) immediately adjacent to the ductus, with one exception, failed to contract in response to oxygen tensions as high as 650 mmHg. However, pre- and postdural segments of aorta contiguous to the ductus did contract in the presence of oxygen. The minimal tension of oxygen at which we were able to observe contraction of aortic rings was 144 mmHg, as opposed to 25 mmHg for ductus from the same fetuses. The contractile tension developed by aortic rings exposed to a Pₒ₂ of 144 mmHg was 63 mg ± 22 (SE). This tension was 8% of that developed by ductal rings from the same animals exposed to the same Pₒ₂.

Excess potassium. In contrast to the response to oxygen, the contractile response of ductus to potassium did not change with increasing age. Excess potassium produced (Fig. 2) almost a fourfold increase in contraction of the ductus in comparison to air in 40-day gestational age guinea pigs. The responses of term guinea pig ductus to air and excess potassium were not statistically different from each other.

Vasoactive agents. All ductus used in these studies were from term fetuses. Seven out of 12 ductus exposed to bradykinin (0.4–20 ng/ml) in 95% nitrogen and 5% carbon dioxide contracted. A maximal response was obtained at 15 ng/ml (Table 1). Even after the buffer containing the bradykinin was flushed from the system, a subsequent contraction in response to 95% air and 5% carbon dioxide was augmented. The magnitude of contraction before exposure to bradykinin was 563 ± 74 mg (95% air and 5% O₂) and after exposure to bradykinin

![Fig. 1. Oxygen dose-response curves of ductus from fetal guinea pigs 44- to 46-day, 48- to 50-day, and term gestational age. Values plotted are means ± SE; n = 7.](http://ajplegacy.physiology.org/doi/abs/10.1152/ajpheart.1982.241.1.291)
The present study demonstrates that the contractile response of fetal guinea pig ductus to oxygen matures during gestation. Kovalcik (15) failed to observe any difference in contractile strength in response to oxygen of ductus from immature compared with mature guinea pig fetuses. On the other hand, McMurphy et al. (16) using perfused fetal lamb ductus showed progressively greater increases in resistance to flow in response to oxygen as a function of advancing gestational age. Ductus from lambs of 90- to 125-day gestational age seldom responded to oxygen tensions as high as 700 mmHg. However, from 130 days of gestation to term (147 days) the earliest and greatest response to oxygen was seen in the oldest animals. The suggestion has been made by Rudolph (22) that the higher incidence of patent ductus in immature infants might be due to immaturity of ductal smooth muscle. Failure by McMurphy and Boe (15) to demonstrate contraction of ductus from second-trimester human fetuses concurs with Rudolph's suggestion that fetal immaturity is a cause of ductal patency. The magnitude of contraction of ductus from 40-day-old fetal guinea pigs in response to oxygen was smaller than that in response to excess potassium. In contrast, the size of the oxygen-induced contraction of the term guinea pig ductus is not different from that of the excess potassium contraction in either the term guinea pig or 40-day-old fetus. This difference in magnitudes of contraction indicates that the responsiveness to oxygen and not the number of smooth muscle cells may be the most important factor in determining the strength of contraction of ductus in oxygen as a function of advancing gestation. Fay (5), using term guinea pig ductus perfused with buffer solutions equilibrated with various gas mixtures, noted that the maximum slope of the oxygen dose-response curve occurred between 0 and 140 mmHg oxygen tension. When our data are plotted on Fay's figures (% maximum tension developed versus PO2), the steepest portion of our oxygen dose-response curve is shifted to the right slightly and occurs at a PO2 of 25-144 mmHg. The partial pressure of oxygen at which we have observed a 50% maximal response is 90 + 13 (SE) mmHg in contrast to 80 mmHg observed by Fay (probably an insignificant difference).

Seven of 12 ductus contracted in response to bradykinin in 95% N2 and 5% CO2. Only three of the seven contracted in response to 0.20 and 0.5 mg/ml. All seven ductus responded to 0.40, 2.0, 15.0, and 20.0 mg/ml.

was 825 ± 91 mg (95% air and 5% CO2; P < 0.005). Atropine (400, 300, and 200 μg/ml) in 95% nitrogen and 5% CO2 caused the ductus to contract. However, 100 μg atropine/ml (n = 16) had no effect on ductal contraction in response to 95% air and 5% CO2 (0.05 < P < 0.10; Fig. 3).

DISCUSSION

The present study demonstrates that the contractile response of fetal guinea pig ductus to oxygen matures during gestation. Kovalcik (15) failed to observe any difference in contractile strength in response to oxygen of ductus from immature compared with mature guinea pig fetuses. On the other hand, McMurphy et al. (16) using perfused fetal lamb ductus showed progressively greater increases in resistance to flow in response to oxygen as a function of advancing gestational age. Ductus from lambs of 90- to 125-day gestational age seldom responded to oxygen tensions as high as 700 mmHg. However, from 130 days of gestation to term (147 days) the earliest and greatest response to oxygen was seen in the oldest animals. The suggestion has been made by Rudolph (22) that the higher incidence of patent ductus in immature infants might be due to immaturity of ductal smooth muscle. Failure by McMurphy and Boe (15) to demonstrate contraction of ductus from second-trimester human fetuses concurs with Rudolph's suggestion that fetal immaturity is a cause of ductal patency. The magnitude of contraction of ductus from 40-day-old fetal guinea pigs in response to oxygen was smaller than that in response to excess potassium. In contrast, the size of the oxygen-induced contraction of the term guinea pig ductus is not different from that of the excess potassium contraction in either the term guinea pig or 40-day-old fetus. This difference in magnitudes of contraction indicates that the responsiveness to oxygen and not the number of smooth muscle cells may be the most important factor in determining the strength of contraction of ductus in oxygen as a function of advancing gestation. Fay (5), using term guinea pig ductus perfused with buffer solutions equilibrated with various gas mixtures, noted that the maximum slope of the oxygen dose-response curve occurred between 0 and 140 mmHg oxygen tension. When our data are plotted on Fay's figures (% maximum tension developed versus PO2), the steepest portion of our oxygen dose-response curve is shifted to the right slightly and occurs at a PO2 of 25-144 mmHg. The partial pressure of oxygen at which we have observed a 50% maximal response is 90 + 13 (SE) mmHg in contrast to 80 mmHg observed by Fay (probably an insignificant difference).

The reason for the attenuated magnitude of contraction of ductus from 24-h neonates is not known. However, similar results were obtained by Fay and Cooke (6).

The observation that both pre- and post ductal segments of aorta contract in response to oxygen contrasts with results of Gillman and Burton (8). Gillman and Burton (8) reported that only the preductal segments of the guinea pig aorta contracted in response to oxygen. The reason for this difference is not known but may be due to the fact that the post ductal segments of aorta in their study were removed from farther down the aorta (5 mm distal to the ductal insertion) and from neonatal guinea pigs.
Vasoactive agents. The suggested increase in bradykinin at birth (2, 22) is thought to be involved in the constriction of umbilical vessels (4), vasodilatation of pulmonary vasculature (2, 7, 17), and closure of the ductus arteriosus (16, 17, 22). Perhaps bradykinin sensitizes the smooth muscle of the umbilical vessels, pulmonary vasculature, and the ductus arteriosus to oxygen. Bradykinin concentrations were measured in human umbilical cord blood by Melmon et al. (18) and were found to average 12.8 ± 4.3 ng/ml. According to the data in the present study 12.8 rig/ml would be sufficient to effect constriction of the ductus as well as augment the magnitude of contraction to oxygen in vivo. Neither epinephrine nor norepinephrine produced an increased magnitude of contraction in response to 95% air and 5% CO2. In contrast acetylcholine (20), like bradykinin, did produce an augmented magnitude of contraction in response to 95% air and 5% CO2.

REFERENCES