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L. G. ELTHERINGTON, J. STOFF, T. HUGHES and KENNETH L. MELMON


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INTERACTION BETWEEN OXYGEN AND BRADYKININ

By L. G. Eatherington, M.D., Ph.D., J. Stoff, B.S.,
T. Hughes, M.B., Ch.B., and Kenneth L. Melmon, M.D.

ABSTRACT
Experiments on perfused segments or isolated rings of human umbilical artery showed that the magnitude of their contraction in response to bradykinin, epinephrine, or serotonin depended on the \( P_{O_2} \) of the perfusing or bathing solution. Constriction to bradykinin varied directly with the \( O_2 \) tension and was greatest when the \( P_{O_2} \) was approximately that of umbilical arterial blood during the transition from the fetal to the neonatal period. Catecholamine beta-receptors were not demonstrated (not present or not functional) in the umbilical artery, since there was no vascular response to either isoproterenol or to propranolol. Alpha-receptors were not required for \( O_2 \) or bradykinin constriction since these agents constricted the umbilical artery after enough phentolamine had been given to block constriction by norepinephrine. Because of the interaction between \( O_2 \) and vasoconstrictor agents the \( O_2 \) environment of umbilical vessels must be rigidly controlled in experiments measuring vascular reactivity. Sensitization by \( O_2 \) to the effects of chemical substances endogenous to the fetus or mother may play an important role in circulatory adjustments essential for neonatal life.

ADDITIONAL KEY WORDS
beta-receptors epinephrine histamine serotonin perfusion
plethysmograph oxygen sensitization alpha-receptors

Among the essential adjustments to extrauterine life are constriction of the ductus arteriosus and umbilical vessels and dilation of pulmonary arterioles. Mechanical alterations in the vessels and elevation of the oxygen tension (\( P_{O_2} \)) of arterial blood have been thought to bring about the changes in the pulmonary circulation of newborn lambs (1), but these two factors are not sufficient to account for the total responses in the umbilical vessels and the ductus arteriosus. For example, umbilical vessels of lambs are not constricted by oxygen in vivo (2). Attempts have been made to discover possible mechanisms that would explain the circulatory changes occurring at birth. With the exception of histamine, vasoactive amines known to exist in the fetus do not have pharmacologic properties which mimic the effects of birth on all vessels critical to circulatory adaptation of the newborn. Therefore a single substance cannot account for all the vascular changes measured at birth. Furthermore, reports that summarize data on humoral mediators of neonatal circulatory changes contain no information relating the release or action of vasoactive substances, including polypeptides, to either the circulatory changes at birth or to the increase in the \( P_{O_2} \) of arterial blood (3, 4).

Bradykinin, a potent endogenous vasodilator in man (5), dilates the pulmonary arterioles of fetal lambs (6, 7); constricts the ductus arteriosus of lambs, calves, and guinea pigs (8); and constricts human umbilical cord arteries (9). Because oxygen and bradykinin act qualitatively the same on the fetal blood vessels that are affected most in the

From the Departments of Medicine and Pharmacology, Division of Clinical Pharmacology, and the Cardiovascular Research Institute, University of California San Francisco Medical Center, San Francisco, California 94122.
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circulatory adjustments of birth, we have measured and evaluated the in-vitro relationships between oxygen and bradykinin on human umbilical cord vessels. The results show that (1) bradykinin or oxygen elicits vasocostriction of human umbilical arteries and that the response is directly proportional to the \( P_{O_2} \) of the tissue bath or arterial perfusate; (2) of three drugs tested, bradykinin (on either a milligram or molar basis) is the most potent constrictor drug; and (3) the oxygen and bradykinin receptor(s) of the umbilical arteries may be identical and differ from those for epinephrine and serotonin. Such results provide the basis for studies to determine whether bradykinin may be one of the mediators of circulatory changes in the fetal-neonatal period.

**Methods**

**Perfusion Studies**

Eighty-seven human umbilical cords were used within 3 hours of full-term vaginal delivery. A 15-cm segment, free of gross irregularities, was cut from the middle-to-placental portion of the cord. This particular section of cord was used because it is said to be nerve free (10). One artery was cannulated at each end and placed in a glass plethysmograph (Fig. 1) filled with fluid. This fluid and that used for perfusion of the artery were a modified Krebs bicarbonate solution that contained electrolytes in concentrations found by Newman (11) to approximate most closely those in maternal plasma during the third trimester. The solution was composed of (in mEq/liter) \( Na^+ \), 139.5; \( K^+ \), 3.97; \( Ca^{2+} \), 4.69; \( Mg^{2+} \), 1.47; \( HCO_3^- \), 23.2; \( Cl^- \), 104.2; and \( HPO_4^{2-} \), 1.82. The \( Vo_2 \) of the plethysmograph solution was that of room air and remained stable (by measurement) throughout the perfusion period. The carbon dioxide tension (\( P_{CO_2} \)) of the solution was not measured nor was any attempt made to keep this constant. The inflow cannula to the arterial segment was connected to a constant-flow Sigma motor pump; the outflow was discarded. Flow was always in the direction of fetus to placenta. The \( P_{O_2} \) of the perfusion solution (bubbled with 7% \( CO_2 \)) was measured with a Severinghaus electrode and ranged from 49.3 to 56.7 mm Hg. The \( P_{O_2} \) of the fluid in the reservoirs varied from 15 to approximately 720 mm Hg; it was monitored with a Clark electrode. The temperature and pH of the perfusion solution varied between 36.5 and 37.5°C and 7.17 and 7.24 respectively, approximating values reported in human umbilical artery blood (12). Inflow pressure to the cannulated artery was measured with a Statham pressure transducer. Attachment of a vacuum pump to
SENSITIZATION OF UMBILICAL VESSELS BY OXYGEN

one of the two side arms of the plethysmograph allowed rapid and reproducible distention of the arterial segment (9, 13). The amount of subatmospheric pressure necessary to produce a consistent vascular constriction varied from one preparation to another. A steady state was defined as a reproducible increase in the pressure perfusing the umbilical artery in response to a standard subatmospheric pressure. If a steady state was not achieved within 4 hours of beginning perfusion, the preparation was discarded. When it was obtained, drugs were administered either as a single injection (Fig. 1) or as a constant infusion by a second pump; when a pump was used, the volume of the tubing resulted in a delay time of 4 minutes (transit time was measured by a blue dye).

Perfusion was begun with a solution whose \( P_{O_2} \) was 15 mm Hg. As the artery gradually relaxed, flow was increased from 0 to 25 ml/min, at which it was stable for the remainder of the experiment.

RINGS OF UMBILICAL ARTERY

Human umbilical arteries were gently and rapidly dissected free of connective tissue. Two rings of artery 1.0 to 1.5 cm long were suspended, in tandem, in a 10-ml muscle chamber (Fig. 2). The rings gradually relaxed in the next 45 to 60 minutes. The rings were connected to a Phipps and Bird linear motion transducer with a resting load of 4 g. For 1 to 10 hours, each exposure to increased \( P_{O_2} \) or drugs resulted in increased response. Once reproducible responses were obtained to test stimuli, a steady state of sensitivity was maintained for 5 to 10 additional hours, although 45 to 60 minutes were usually required between drugs before reproducibility could be achieved. The Krebs-Ringer bathing medium was prepared according to the method of Umbreit et al., modified to contain 5.5 instead of 11.0 mEq/liter calcium (14). We reduced the calcium concentration and changed from the perfusion solution to a Krebs-Ringer bathing medium in an unsuccessful attempt to shorten the period of delay before reproducible responses were obtained and to reduce spontaneous constrictor activity. The solution was maintained at a pH of 7.35, and the temperature varied between 36.5 and 37.5°C. Throughout the experiment, 7% CO\(_2\) and 2% to 93% O\(_2\) bubbled through the muscle chambers. In all experiments on rings and on segments of arteries, peak responses were measured. All stimuli were applied in random order.

DRUGS

Bradykinin, epinephrine chloride, 5-hydroxytryptamine creatinine sulfate (serotonin), isoproterenol hydrochloride, phenolamine methanesulfonate, and propranolol hydrochloride were prepared fresh for each experiment, and concentrations were calculated as free base. The concentrations of the drugs are recorded later in the paper.
The direct relationship between PO2 of the perfusion solution and inflow pressure to the umbilical artery. Magnitude of response (slope of the line) was greatest over the PO2 range of 15 mm Hg to 120 mm Hg, even though a PO2 of 700 mm Hg caused significantly greater constriction (P < 0.05 by t-test) than 120 mm Hg. The means ± 1 so of 4 experiments are shown.

Bradykinin was much more effective in constricting vessel segments perfused with a solution at a PO2 of 120 mm Hg than with one at 15 mm Hg (Fig. 5). Bradykinin was a

and in Figure 6. In addition, appropriate volumes of diluent or vehicle (as in the case of phentolamine) were used as controls.

**Results**

**PO2 AND DRUG EFFECTS**

Figure 3 shows the rise in inflow pressure produced by a constant infusion of bradykinin through a segment of an umbilical artery. The pressure returned to control values in spite of the continued injection of bradykinin.

Of particular interest was the response of the umbilical artery to oxygen. Figure 4 shows that inflow pressure increased as the PO2 increased from 15 to 120 mm Hg. The peak pressure was not sustained at any given PO2. Increasing the PO2 of the perfusion solution to approximately 700 mm Hg resulted in a significantly greater increase in inflow pressure (P < 0.05), over that obtained at a PO2 of 120 mm Hg.

Bradykinin was much more effective in constricting vessel segments perfused with a solution at a PO2 of 120 mm Hg than with one at 15 mm Hg (Fig. 5). Bradykinin was a
more potent constrictor on a molar basis than epinephrine or serotonin ($P < 0.001$) tested on the same arteries (Fig. 6).

In 125 experiments designed to characterize umbilical artery drug receptors, neither phentolamine ($5 \times 10^{-8} \text{ M}$) nor propranolol ($1 \times 10^{-3} \text{ M}$) altered the response of 25 isolated umbilical artery rings to $93\%$ $O_2$ or to bradykinin ($1 \times 10^{-4} \text{ M}$). This concentration of phentolamine was sufficient to inhibit, but not reverse, the response to epinephrine. Propranolol, a beta-receptor blocking agent, did not modify the epinephrine response. Even when the artery rings were previously constricted by $O_2$ or bradykinin, they did not respond to high concentrations of isoproterenol ($1 \times 10^{-3} \text{ M}$) in the presence or absence of a beta-receptor antagonist. No attempt was made to block the serotonin effect.

**Discussion**

This study demonstrates that bradykinin and $O_2$ elicit in-vitro constriction of human umbilical arteries. The magnitude is directly proportional to the $O_2$ of the perfusion fluid. At a $O_2$ of 15 mm Hg, which some authors state occurs in the human umbilical arterial blood in vivo (12), the vessels were relatively unresponsive to all drugs tested. This evidence suggests that the three vasoactive compounds tested in these studies and that occur naturally in the fetus (or may be administered to the mother and cross the placenta) would be unlikely to decrease fetal placental perfusion in vivo. We have been unable to demonstrate vaso-dilation with isoproterenol but have not eliminated “silent” or functionless beta-receptors capable of binding catecholamines (15). The apparent absence of functioning beta-receptors in human umbilical arteries suggests that any release of epinephrine from the fetal adrenal medulla during birth could act only to reduce fetal blood flow to the placenta, especially in the presence of gradually increasing oxygen tension in the umbilical arterial blood. In the human, decreasing placental blood flow as determined by umbilical vessel constriction provides effective blood volume homeostasis during the period between delivery of the baby and of the placenta. Whether bradykinin is involved in human umbilical artery constriction remains for future studies.

An important limitation to the extension of the in-vitro data to in-vivo conditions is implied by the very low flow rates obtained in the perfusion studies. These vessels were obtained after they had become constricted during normal childbirth. They remained constricted, so that almost no umbilical blood flow would have occurred if they were in the same condition in vivo. Driving pressures of 25 mm Hg were needed to cause a flow of 25 ml/min in a 15-cm segment of one artery. This segment represents less than one-third the usual cord length and of course does not include resistances created by vessels in the chorionic villi and fetal liver. Arterial pressure in the fetus of 50 to 60 mm Hg may drive as much as 500 ml blood (not saline)/min through the fetal circulation. Therefore, the in-vitro test system has 1% to 2% of the “conductance” in vivo. At first glance, it might be necessary to postulate another mechanism independent of increased $O_2$ or bradykinin, epinephrine, or serotonin release to produce closure of umbilical arteries. However, such substances may cause an initial and irreversible constriction of the vessels. Such constriction might even be independent of vascular viability. Despite the limitations of an in-vitro test system and the alteration and perhaps irreversible constriction, the vessels were responsive to the three drugs tested and to stretch. The isolated rings also responded to drugs in the same qualitative way as the vessels used in perfusions. What physiologic importance oxygen and bradykinin have in in-vivo constriction of cord vessels could only be assessed in studies done in vivo or on unconstricted vessels of species closely resembling the human.

There is no evidence that the response of the umbilical artery to $O_2$ and to bradykinin is mediated by the same receptors. It is known that extracts of human umbilical arteries free from blood contain at least one vasoconstrictr substance (16). This substance has
not been identified, but does not appear to be bradykinin. Whether bradykinin is produced in the fetal-neonatal period and whether it could be an important mediator in neonatal vascular changes are discussed elsewhere (17). Regardless of whether bradykinin is a mediator of such changes, this study makes it evident that the human umbilical vessels in vitro are sensitized by \( \text{O}_2 \) to the effects of epinephrine, serotonin, and bradykinin. Sensitization may play an important in-vivo role in the circulatory adjustments essential for normal human newborn life.

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**References**