

Interaction of Three Factors in the Closure of the Ductus Arteriosus (37319)

MASANAO IKEDA,¹ EDUARDO H. RUBINSTEIN,² AND RALPH R. SONNENSCHNEIN

Department of Physiology, UCLA School of Medicine, Los Angeles, California 90024

Patency of the ductus arteriosus in the mammalian circulation during fetal life assures an appropriate tissue oxygenation because part of the placental blood is distributed to the periphery through the right-to-left shunt constituted by the open ductus.

Immediately after birth, several mechanisms apparently trigger the powerful localized vascular smooth muscle contraction that seems to be required in the first stage of the process of ductus closure.

The important role of the increase in arterial P_{O_2} with the initiation of breathing and its effect on the vascular tone of the ductus has been repeatedly confirmed (1, 2) since the reports of Kennedy and Clark (3).

The marked hemodynamic changes that occur following lung inflation, evidenced by the increased systemic arterial pressure and a decrease in pulmonary arterial pressure, will certainly modify the extent of tension in the vascular wall of the ductus. These changes in stretch could activate a local myogenic response in the form of contraction following an increase in intraluminal pressure (4).

Recent morphological and pharmacological studies have indicated the presence of an adrenergic and a cholinergic innervation of the ductus arteriosus, which suggest autonomic control as another factor determining the timely closure of the aortopulmonary connection (5-9).

The present study was designed to analyze the relative importance of the innervation in the responses of isolated rings of ductus arteriosus in relation to the other contractile

factors: oxygen tension and degree of stretch.

Methods. The ductus arteriosus was obtained from fetal guinea pigs (80-120 g) that were delivered by cesarean section after anesthetizing of the pregnant mother with pentobarbital sodium (20 mg/kg), supplemented with local anesthesia (2% Xylocaine). After removal of the fetus, its chest was opened before expansion of the lung and the ductus carefully dissected, removed and placed into a beaker containing a modified Krebs bicarbonate medium kept at 37° and gassed with a low-oxygen mixture.

The composition of the supporting solution was (in grams per liter):

NaCl, 7.14	NaHCO ₃ , 2.15
KCl, 0.39	MgSO ₄ ·7 H ₂ O, 0.3
CaNa ₂ EDTA, 0.01	CaCl ₂ ·2 H ₂ O, 0.23
dextrose, 2.0	ascorbic acid 0.2 mg/ml

The pH was adjusted to 7.4 and the oxygen concentration was kept at the fetal level of 28 mm Hg by using a gas mixture of 4% O₂, 5% CO₂, and 91% N₂. When required, higher O₂ concentrations were readily obtained by altering the proportions of the gasses flowing through three separate flowmeters of an anesthesia machine.

A 2-3 mm long ring of ductus was mounted between a stationary stainless steel rod (0.2 mm o.d.) and a similar rod attached to a Statham model UC2 isometric transducer coupled to a Grass Model 5 polygraph. The preparation was kept inside an air bath at 37° and superfused at 5 ml/min with the Krebs bicarbonate solution.

The ring was usually stretched to a resting tension of 400 mg, but this level could be modified readily by adjusting the degree of stretch with a micrometer.

Two platinum wire electrodes (0.2 mm

¹ Current address: Department of Cardiovascular Surgery, Kenritsu-Amagasaki Hospital, Amagasaki, Hyogo-ken, Japan.

² Send reprint requests to Dr. Eduardo H. Rubinstein.

diameter) were placed tangentially on opposite sides of the vascular ring, to deliver transmural electrical stimulation. Square-wave pulses of 0.02 msec duration at 2 to 10/sec were derived from a Grass Model 5 stimulator that triggered a variable voltage constant current dc power supply.

For some experiments the ductus was depleted of catecholamines by the intraperitoneal injection to the fetus—still inside the uterus—with approximately 100 mg/kg of 6-hydroxydopamine hydrobromide. After 30 min the fetus was delivered by cesarean section and the ductus excised and mounted as described above. The effectiveness of the depletion of adrenergic neurotransmitter was corroborated by injecting a fetus of the same litter in a similar manner and quickly freezing the ductus immediately after excision and preparing the tissue by the fluorescence histochemical method (10). Microscopic observation of the ductus and heart from the treated fetus showed no evidence of the specific fluorescence of adrenergic nerves. In some cases predelivery depletion was supplemented by addition of bretylium tosylate (10^{-7} g/ml) to the superfusate.

Other drugs utilized in the study were: 1-norepinephrine bitartrate (Levophed, Winthrop), tetrodotoxin (Sankyo), and tyramine hydrochloride.

Results. 1. The exposure of the vascular rings to increasing oxygen concentrations elicited a progressive contractile response. Figure 1 represents the correlation between the developed tension and the P_{O_2} of the perfusate when the ductus was subjected to three different levels of initial tension at P_{O_2} of 0 mm Hg. The ductus developed increasing amounts of tension when exposed to graded concentrations of O_2 above 0 mm Hg. The developed tension at any P_{O_2} was greater when the initial tension (at 0 mm Hg P_{O_2}) was high.

2. The role of the initial tension in the contractile response to increased O_2 was analyzed in more detail, in the transition from 28 to 100 mm Hg P_{O_2} , namely, the physiological range. Figure 2 represents the results of experiments where the change in tension in response to the increase in oxygen

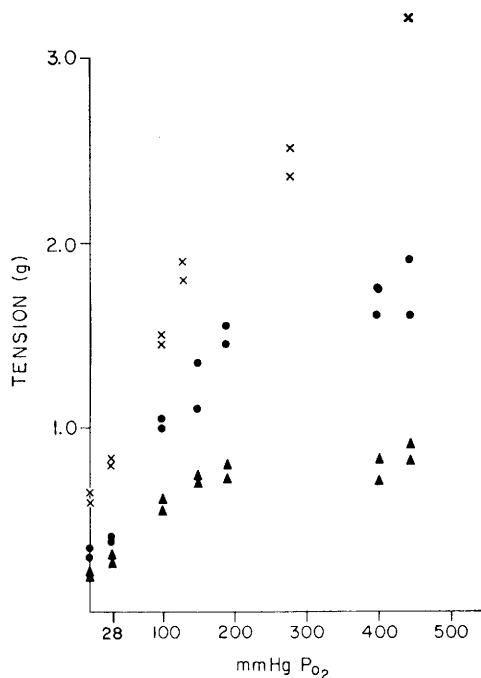


FIG. 1. Relationship between the developed tension and the O_2 content of the superfusing solution. Results of two ductus tested at three levels of initial tension (at P_{O_2} of 0 mm Hg). Triangles: low initial tension (200 mg). Circles: intermediate initial tension (300–380 mg). Crosses: high initial tension (600 mg).

was plotted as a function of the initial tension. The diagram shows that at an initial tension of around 400 mg the contractile response is maximal. This tension would correspond to 15–40 mm Hg transmural pressure, as calculated from the simplified LaPlace relationship, $T = Pr$ ($r = 0.5$ – 1.0 mm; total initial tension = 400 mg applied over a 2-mm length of vessel, *i.e.*, 200 mg/mm).

3. Transmural electrical stimulation was utilized to excite intramural nerve fibers in order to imitate the effect of neurogenic discharge. However, the tension developed varied continuously from threshold without two distinct points that could be ascribed to the indirect (neurogenic) or direct excitation of vascular smooth muscle. In order to evaluate the neurogenic contribution to the contractile response two approaches were attempted:

(a) Transmural stimulation was performed before and after adding to the superfusate

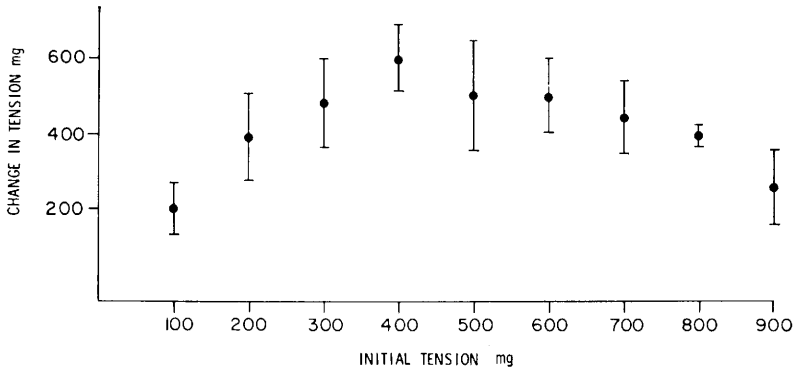


FIG. 2. Mean change in tension developed in response to exposure to 100 mm Hg P_{O_2} . Results are expressed as a function of the initial tension, set when P_{O_2} was 28 mm Hg. Results of three ductal rings expressed as mean change in tension \pm standard deviation.

tetrodotoxin, which blocks the generation of nerve action potentials (11). The appropriate concentration of the neurotoxic agent was estimated by applying test transmural stimulations at 10-min intervals during the exposure of the ductus to different concentrations of tetrodotoxin. Figure 3 represents the results of that experiment and shows that concentrations of the agent between 10^{-8} and 10^{-7} g/ml decreased the response (after 30 min) to a similar extent with no apparent decrease thereafter. In contrast, a concentra-

tion of 10^{-6} g/ml rapidly decreased the contractile response and the decrement continued as a function of time of exposure. In essence, tetrodotoxin in concentrations of 10^{-8} – 10^{-7} g/ml diminished the contractile response to transmural stimulation to approximately 70% of the control value.

(b) Tyramine was added to the superfusate to evoke a contractile response secondary to its neurotransmitter releasing effect (12). The effective dose of the agonist (maximal dose without direct vasoactive effect) was

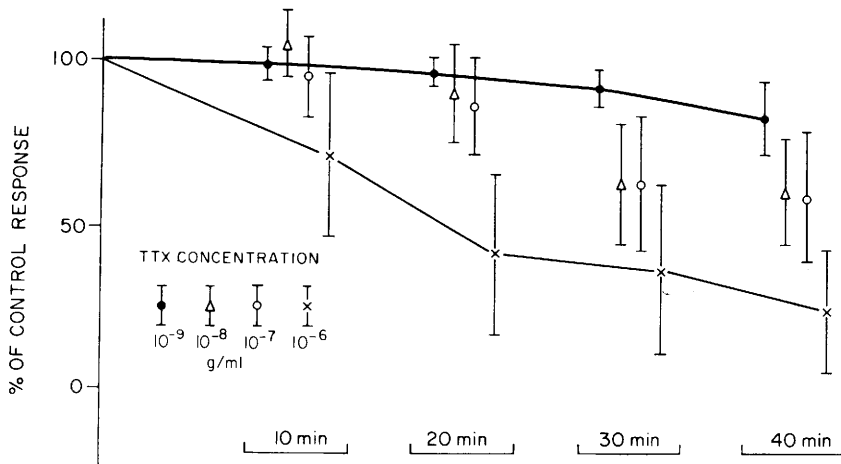


FIG. 3. Decrement in the response to transmural stimulation after progressive exposure of the ductus arteriosus to different concentrations of tetrodotoxin (TTX) from 10^{-9} to 10^{-6} g/ml. Each point represents the mean \pm standard deviation of contractile responses obtained in 3 ductus. Initial tension 400 mg; P_{O_2} 28 mm Hg. Transmural stimulation (0.02 msec, 5 Hz, 30 V) was applied for 10 sec at 10, 20, 30, and 40 min after application of TTX, at the specified concentrations.

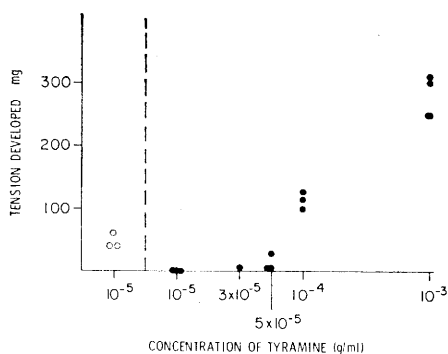


FIG. 4. Contractile response of the ductus arteriosus to increasing concentrations of tyramine. To the left in open circles: intact vascular ring. To the right in filled circles: sympathetically denervated vessels (6-hydroxydopamine). Initial tension 400 mg; P_{O_2} 28 mm Hg.

estimated by superfusing, with increasing concentrations of tyramine, the ductus of fetuses pretreated with 6-hydroxydopamine. Figure 4 displays the results obtained in the catecholamine depleted ductus in comparison with the contractile response of intact vessels. Tyramine seemed to have a direct effect on vascular smooth muscle at concentrations higher than 5×10^{-5} g/ml. Accordingly the dose utilized in the experiments with intact ductus was kept at 1×10^{-5} g/ml. The diagram shows that this concentration of tyramine evoked no changes in the initial tension of the catecholamine depleted ductus. The contractile response to tyramine in the intact vessel varied between 39 and 60 mg.

4. The threshold voltage for transmural stimulation (at a fixed pulse duration of 0.02 msec and 2 Hz for 10 sec) was measured before and after 30-min perfusion with

tetrodotoxin at 10^{-7} g/ml. In four vessels, set at initial tension of 400 mg and 28 mm Hg P_{O_2} , the mean control threshold was 28 ± 2.4 (SE) V, while after tetrodotoxin it increased to 35 ± 2.0 V, the difference being significant at the 5% level.

5. The contractile responses of the ductus to different agents and transmural stimulation were compared while the vessels were superfused at fetal (28 mm Hg) and postnatal (100 mm Hg) P_{O_2} . The results in Table I represent the ratios of the responses elicited by each stimulus at the two oxygen concentrations. A rapid increase in Ca concentration from 1.6 to 4 mM (column IV) elicited a contractile response that was slightly potentiated by O_2 . Norepinephrine and tyramine (columns I and II) showed a greater potentiation. The contractions in response to acetylcholine and transmural stimulation underwent a marked, fourfold enhancement when the vessel was superfused at 100 mm Hg P_{O_2} . An example of this latter potentiation is represented in Fig. 5 which shows the marked response at a higher P_{O_2} ; this could not be mimicked by increasing the initial tension (at 28 mm Hg P_{O_2}) before the stimulation.

6. An additional observation was that spontaneous contractile activity could be triggered by transmural stimulation or by increasing the O_2 concentration. Figure 6 shows two examples of that phenomenon. The first tracing shows spontaneous activity that was triggered by prolonged transmural stimulation (60 sec) at low frequency (2 Hz). Contractions started at a frequency of 2/min, followed later by an increase to 4/min,

TABLE I. Potentiation of Contractile Responses by O_2 .^a

Norepinephrine	Tyramine	Acetylcholine	Calcium chloride	Transmural stimulation
(10^{-11} g/ml)	(10^{-5} g/ml)	(10^{-11} g/ml)	(4 mM)	0.02 msec 2 Hz, 10 sec, 30 V
2.1 ± 0.1^b	2.1 ± 0.2^b	4.0 ± 0.8^b	1.4 ± 0.2^b	4.4 ± 0.6^b

^a Ratio of $\frac{\text{response at 100 mm Hg } P_{O_2}}{\text{response at 28 mm Hg } P_{O_2}}$.

^b $p < 0.05$. Mean \pm standard error of 4 ductus, each tested with the five procedures.

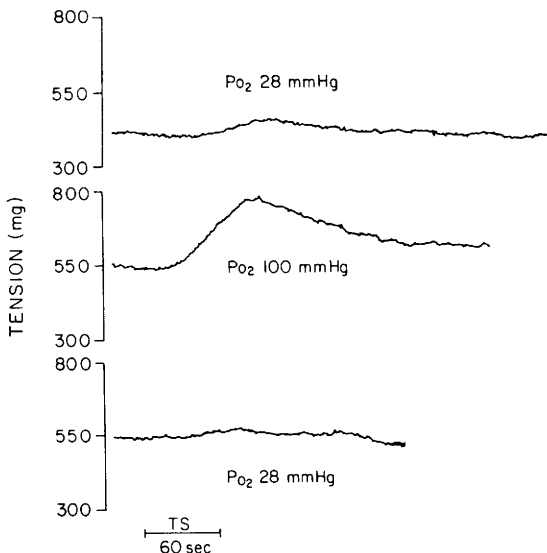


Fig. 5. Contractile response of the isolated ductus arteriosus to transmurial electrical stimulation (TS): 0.02 msec, 5 Hz, 30 V. Top tracing: response to TS at 400 mg initial tension and 28 mm Hg P_{O_2} . Middle tracing: response to TS at 100 mm Hg P_{O_2} ; the initial tension was actively raised to 550 mg by the increase in P_{O_2} . Bottom tracing: response to TS at 28 mm Hg P_{O_2} after passive elevation of the initial tension to 550 mg. The three examples were taken from the continuous record of one ductal ring.

then 9 to 10/min and finally to 25/min. The second tracing shows a comparable phenomenon, this time triggered by exposing the ductus to a P_{O_2} of 100 mm Hg. Low-frequency transmurial stimulation rapidly increased the rate of spontaneous activity until it could not be followed by the recording system.

Discussion. Our results support the concept that change in P_{O_2} , stretch of the vessel, and neurogenic activity may interact in causing the powerful contraction of the ductus arteriosus that initiates its closure after birth. In confirmation of others (1, 2, 3) we observed a progressive increase in tension of the vessel as P_{O_2} was raised from 0 mm Hg through the physiological range (28–100 mm Hg), and on up to 480 mm Hg. The development of tension in the range 0–28 mm Hg indicates that some degree of tone already exists in fetal life. The facilitation by higher P_{O_2} of the spontaneous myogenic activity of the ductus (Fig. 6) suggests that at least part of the enhancing effect of O_2 on tone

of this vessel may be by way of an action on local pacemaker activity.

The response to O_2 is clearly enhanced by elevation of the initial tension (Fig. 1 and 2). This may correspond to the change that occurs after birth when the combination of increased systemic arterial pressure, brought about by hemodynamic and neurogenic effects (13, 14), and lowered pulmonary arterial pressure (15) leads to an increase in intra-ductal pressure at some phase of the cardiac cycle (15).

The role of the innervation in ductal closure is difficult to establish clearly. It appears from the reduction in contractile response to transmurial electrical stimulation after the selected dose of tetrodotoxin that the innervation may account for about 30% of developed tension, *i.e.*, 30–60 mg. This corresponds approximately to the increment in tension induced by tyramine (Fig. 4) which apparently releases an amount of neurotransmitter (NE) similar to that released by neural stimulation. In both cases the resulting developed tension, at P_{O_2} of 28 mm Hg, is small.

When the vessels were superfused at 100 mm Hg P_{O_2} , however, the response to transmurial stimulation and tyramine, and NE as well, was markedly enhanced, as was that to acetylcholine. As shown in Fig. 5, this potentiation did not depend upon change in the initial tension. The enhancement by high P_{O_2} of the neurogenic effect could come about by an increase of transmitter release, of smooth muscle sensitivity to the transmitter, or of "contractility" of the smooth muscle. Our results do not allow a choice among these mechanisms. However, the lack of potentiation by O_2 of the response to increased Ca concentration suggests that O_2 may not increase the availability of Ca to the contractile site, *i.e.*, it may not alter ductal smooth muscle contractility.

The relative roles of the adrenergic and cholinergic innervations are unknown at present. Either of these may be activated by the increased chemoreceptor activity that occurs when the umbilical cord is clamped at birth (15).

It thus appears that three vasoactive factors, O_2 , stretch and autonomic innerva-

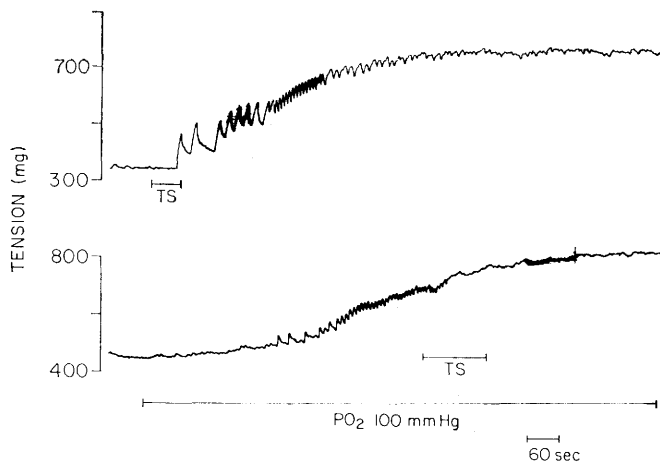


FIG. 6. Effect of transmural stimulation (TS: 0.02 msec, 2 Hz, 30 V) and O_2 on the spontaneous contractile activity of the ductus arteriosus. Top tracing: effect of transmural stimulation (TS) on the mechanical activity of a ductal ring at 28 mm Hg P_{O_2} . Bottom tracing: spontaneous mechanical activity triggered in another isolated ductus upon changing the P_{O_2} of the perfusate from 28 mm Hg to 100 mm Hg. Transmural stimulation (TS) further increased the frequency of the spontaneous contractile activity.

tion, may have a reciprocal potentiating effect in ductal contraction: the stretch that accompanies the rise in intraluminal pressure at birth may enhance the response to O_2 , and the elevation of P_{O_2} after the initiation of breathing may potentiate the neurogenic response.

Summary. Isolated rings of the ductus arteriosus of the guinea pig developed tension in response to changes in P_{O_2} and transmural electrical stimulation. Increasing the initial tension potentiated the response to elevated P_{O_2} . In turn, an increase in P_{O_2} potentiated the responses to transmural stimulation, and also tyramine, norepinephrine and acetylcholine. The three factors, O_2 , stretch of the vessel wall and autonomic innervation, may potentiate each other in their vasoactive effect during the initiation of ductal closure, secondary to the respiratory and cardiovascular changes occurring at birth.

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