Guinea pig ductus arteriosus. II. Irreversible closure after birth

FREDRIC S. FAY AND P. H. COOKE
Department of Physiology, Harvard Medical School, Boston, Massachusetts 02115, and The Biological Laboratories, Harvard University, Cambridge, Massachusetts 02139

Within hours after birth the ductus arteriosus closes due to contraction of smooth muscle in its wall (2, 4). Closure is thought to be triggered, at least in part, by an increase in arterial oxygen content (1, 2, 4, 6, 14, 15). Evidence for this hypothesis derives in large part from the dilatory effect of hypoxia on the ductus arteriosus of the neonate of various species (2, 21, 25, 26). However, within a few days after birth the dilatory effects of hypoxia are lost and the ductus remains "irreversibly" closed thereafter (4, 7). It is not known if irreversibility, when first observed, reflects a specific loss of the oxygen-sensing mechanism of the smooth muscle or if it results from some mechanical restraint imposed on the closed vessel. This study deals with the mechanism underlying irreversibility of ductal closure after birth. In order to investigate this problem, studies were undertaken to determine the exact time course for the onset of irreversible closure of the guinea pig ductus arteriosus. Parallel studies of the reactivity of ductal smooth muscle to oxygen and studies of the postpartum cellular changes within the vessel were also carried out.

METHODS

General. The ductus arteriosus from postnatal guinea pigs, from 1 hr to 4 days of age, was perfused retrograde via the thoracic aorta and changes in flow conductance (1/flow resistance) were measured as a function of oxygen pressure (P02). The ductus was then cut from the aorta and mounted so that changes in circumferential muscle tension could be measured isometrically. The effects on muscle tension of changes in P02, the addition of acetylcholine to the organ bath, and replacement of normal Krebs-Ringer solution with one enriched in K+ were then studied. At various stages after birth, ducts were fixed in situ or in vitro for light and electron microscopy. Similar physiological and morphological observations were made on ducts from animals reared under conditions of reduced oxygen pressure.

Operative procedure. The time of birth of neonates obtained by spontaneous delivery was known within 6 hr. Neonates delivered by cesarean section were always within a few days of their delivery date (64 days, gestational age) as judged by comparing their birth weights with those reported by Draper (5). No differences were observed between animals delivered surgically vs. spontaneously in regard to postnatal changes in ductal structure or function. At various times after birth the neonates were killed either by a blow on the head or intraperitoneal injection of sodium pentobarbital (300 mg/kg body wt). The thorax was opened and the ductus arteriosus, pulmonary artery, and aorta were cleared of adhering connective tissue. The aorta was cut between ligatures anterior to the junction of the ductus and aorta. The ductus itself was cut at the point of attachment to the pulmonary artery. A polyethylene cannula (0.45 inch x .062 inch) was inserted retrograde into the aorta approximately 1 cm posterior to the level of the ductus and ligated to the vessel. The preparation was then transferred to the organ bath.

Experimental apparatus. The polyethylene cannula was attached to the perfusion apparatus (see Fig. 1A) and the preparation was lowered into the organ bath. The perfusion fluid was equilibrated with gas of known composition (95% O2-5% CO2 or 95% N2-5% CO2) by passing it through silicone rubber tubing within a larger polyethylene tube (Fig. 1A). The gas mixture flowed between the inner...
Fig. 1. A: perfusion apparatus and organ bath. Fluid reservoirs and organ bath are constructed of Lucite. Stopcocks allow for rapid rinse of organ bath with solution from either reservoir equilibrated with 95% O₂-5% CO₂ or 95% N₂-5% CO₂. Perfusion apparatus consists of 10 ft of silicone rubber tubing (.020 inch x .037 inch) inside a polyethylene tube (.085 inch x .128 inch); perfusate is brought to temperature in a 6-inch length of 18-gauge stainless steel tubing connecting silicone tubing to preparation. B: tension-measuring apparatus. Ring of ductus arteriosus is slipped over 2 stainless steel pins (27 gauge stainless steel tubing) and placed into organ bath shown in A. Resting tension under anaerobic conditions is adjusted to about 0.5 g with micrometer; tension is measured isometrically with FT 03 force transducer (Grass Instruments Co; nominal displacement 0.02 mm/g).
response to oxygen and nitrogen had been monitored during perfusion in the organ bath. Sections were cut from the aortic to the pulmonary end of the ductus, i.e., in the same direction as perfusate flow. Several consecutive sections at 0.1-mm intervals along the ductus were stained according to the method of Richardson et al. (24) and examined under the light microscope. Electron-microscope observations were also made on 33 preparations. Thin sections were stained with 2% uranyl acetate and lead citrate (23).

Hypoxic conditions. Seven litters of neonates were raised under conditions of reduced \( P_O_2 \) similar to those employed by Wilcox, Roberts, and Carney (18). A small cage was placed inside a "glove bag" and a gas mixture of known oxygen content (9-11%) flowed through the chamber at a rate (about 1 liter/min) sufficient to replace the oxygen consumed by the neonates as judged by measurements of the \( P_O_2 \) within the chamber. The neonates were fed milk from a medicine dropper while inside the experimental chamber. At various times after birth they were killed and their ducts were taken for light and electron microscopy. In addition, ducts from two animals were prepared for perfusion as described previously.

**RESULTS**

Reversibility of ductal closure as a function of age. Table 1 lists the average flow conductance of 37 perfused ducts obtained from animals from 0 to 96 hr postpartum. In the presence of oxygen all preparations exhibited conductances close to zero, indicating complete obstruction of the lumen. Under anaerobic conditions, however, ducts from newborn animals (0-6 hr) developed high flow conductances, indicating that they were widely dilated. The ability to increase flow conductance in response to anaerobic conditions declined progressively with age and by 36 hr after birth flow conductance under anaerobic and aerobic conditions were statistically indistinguishable.

The decline in mean conductance in response to anaerobic conditions in the first few days after birth was due to: 1) increased frequency of preparations that failed to dilate at all (no conductance change), and 2) lesser magnitude of conductance change in those preparations that did respond. The frequency with which irreversibly closed ducts (no conductance change in response to anaerobiosis) were encountered at different ages is shown in Fig. 2. All preparations obtained from animals 72 hr or older were irreversibly closed.

**Effect of age on oxygen sensitivity of smooth muscle of ductus arteriosus.** The maximum increment in tension of the muscle in the wall of the ductus in response to increasing \( P_O_2 \) from 0 to 680 mm Hg decreased from 2.96 ± 0.44 g (mean ± se) in ducts from newborn animals to 0.98 ± 0.26 g (mean ± se) in ducts from animals 3- to 4 days postpartum. To determine if the decrease in response to oxygen with age represents a failure of the oxygen-sensing mechanism or merely a loss of the contractile capacity of the ductal smooth muscle, the response to oxygen was compared to the response to acetylcholine over the same time period. The maximum contractile response of the ductus to acetylcholine also decreased, from 1.32 ± 0.34 g in ducts from newborns to 0.36 ± 0.10 g in ducts from animals 3-4 days of age. As can be seen in Fig. 3, the ratio of the maximum contractile response to oxygen and acetylcholine remained constant during the first 4 days after birth. The ratio averaged 2.56 ± 0.29 (SEM) in ducts from newborns and 2.47 ± 0.28 in ducts from animals 3- to 4-day-old animals, respectively.

**TABLE 1. Effect of age on flow conductance of perfused ductus arteriosus under anaerobic and aerobic conditions**

<table>
<thead>
<tr>
<th>Age, hr</th>
<th>No. of Exps</th>
<th>Flow Conductance, ( 10^{-3} ) ml min (^{-1} ) mm Hg (^{-1} )</th>
<th>( P )</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>6</td>
<td>16.47 ± 4.65.14 ± .11</td>
<td>&lt;.01</td>
</tr>
<tr>
<td>12</td>
<td>6</td>
<td>5.80 ± 1.84.05 ± .01</td>
<td>&lt;.02</td>
</tr>
<tr>
<td>24</td>
<td>5</td>
<td>3.10 ± 1.07.03 ± .04</td>
<td>&lt;.03</td>
</tr>
<tr>
<td>36</td>
<td>5</td>
<td>.72 ± .41.21 ± .11</td>
<td>&lt;.3</td>
</tr>
<tr>
<td>48</td>
<td>6</td>
<td>.30 ± .16.20 ± .20</td>
<td>&lt;.7</td>
</tr>
<tr>
<td>72</td>
<td>4</td>
<td>.04 ± .02.05 ± .02</td>
<td>&lt;.5</td>
</tr>
<tr>
<td>96</td>
<td>5</td>
<td>.04 ± .02.04 ± .02</td>
<td>&lt;.99</td>
</tr>
</tbody>
</table>

Flow conductance values are means ± se.
Effects of age on morphological characteristics of ductus arteriosus.

A perfusion-pressure record for a ductus obtained from a newborn guinea pig, shown in Fig. 4A, demonstrates the ability of the vessel to dilate in response to anoxia and to constrict so that flow is blocked in the presence of oxygen. Figure 5 shows a cross section of the same ductus near the middle of its length after fixation under anaerobic conditions. The lumen of the vessel is patent. The vascular wall is lined with a typical endothelium. The major part of the wall is a muscular coat which merges gradually at the periphery into the loose connective tissue of the adventitia. The cross-sectional profile of the vessel shown in Fig. 5 is representative of all sections cut along the length of ducts from all newborn guinea pigs studied.

Physiological and morphological features of the ductus of an animal 19 hr of age are shown in Figs. 4B and 6. Physiologically, this ductus is little changed from that of a newborn guinea pig; the ductus still dilated in response to anoxia, although the increase in flow conductance was not as great as seen in the newborn. Morphologically, the vessel shows no significant changes (Fig. 6) except that the lumen is somewhat narrower and the subendothelial region and muscularis are thicker compared to ducts from newborn animals (Fig. 5).

None of the ducts taken from animals during the 1st day of life were irreversibly closed (see Fig 2). Several ducts studied on the 2nd postnatal day, however, were no longer able to dilate in response to anoxia. Figure 4C shows a perfusion record obtained for an irreversibly closed ductus from a guinea pig 45 hr postpartum. When cross sections were cut along this vessel proceeding in the direction of perfusion, i.e., from the aortic end of the ductus, the lumen was found to be patent for approximately 1 mm, and then became occluded. A section through the vessel where the lumen is occluded is shown in Fig. 7. A distinct endothelial layer is no longer present and what was once the area of the lumen is now filled with a mass of pleomorphic cells with large nuclei (Fig. 8). Some cells have vacuolated cytoplasm and pycnotic nuclei. Myelin figures are abundant in the cytoplasm of the cells and in the extracellular spaces. By contrast to this irreversibly closed duct, vessels that were not irreversibly closed but fixed under aerobic conditions so that the lumina were occluded, contain a typical endothelium (Figs. 9A and B). Four irreversibly closed ducts from animals in the 2nd postnatal day were sectioned serially from aortic to pulmonary end. Each one contained an area where the lumen was occluded and replaced by a loosely organized mass of cells similar to that described above.

By the 4th day after birth, ducts from all animals studied were irreversibly closed. Figure 4D shows a typical perfusion record for a duct obtained from an animal 93 hr old indicating constant and low flow conductance under aerobic and anaerobic conditions. The lumen of this vessel was occluded over most of its length. Cross sections (Fig. 10) taken near the aortic and pulmonary ends of the occluded portion of the ductus were similar to those shown in Figs. 7 and 8 for the irreversibly closed duct of a 2-day-old animal. There is
no endothelium and numerous cells containing pycnotic nuclei are present in the central area of the vessel. Necrosis appears to be limited to the central region and innermost layers of the muscularis. Cross sections taken midway along the occluded length of the ductus from this 4-day-old animal (Fig. 11) also revealed the absence of an endothelium and the presence of numerous necrotic cells in the central area. By contrast to sections (Fig. 10) obtained near the ends of the occluded region, however, the cells in the central area of Fig. 11 are more loosely arranged, cell fragments are more numerous, and a number of cells involved in phagocytosis are found (Fig. 12).

Loss of a distinct endothelium and the presence of a central mass of cells showing varying degrees of necrosis were characteristic features of all 12 irreversibly closed ducts from neonates between 24 and 96 hr postpartum which were systematically studied. The results suggested that irreversible closure might be causally related to the presence of this central mass of cells and cellular debris. In order to test this possibility, an attempt was made to prevent or delay these transitions within the inner wall of the ductus. If irreversibility was directly related to the presence of this central plug, then delay in its formation would also be expected to delay the onset of irreversibility of closure.

Postnatal guinea pigs at low Po2. When newborn guinea pigs were reared under hypoxic conditions the ductus remained patent for 3–4 days after birth as judged by histological observations on ducts fixed in situ. Furthermore, the endothelium remained intact over the first 3–4 days after birth and cellular necrosis did not occur (Fig. 13). A typical record of flow conductance for one of these ducts is shown in Fig. 4E. Even though obtained from an animal 79 hr of age, this ductus was fully capable of reversible closure and dilation in response to oxygen and anoxia, respectively. Similar changes in flow conductance were also observed in a littermate after 90 hr under hypoxic conditions. These
reversible changes in flow conductance are to be compared with the behavior observed in normal animals where all ducts obtained after 72 hr were irreversibly closed (see Fig. 3).

DISCUSSION

The time course for irreversibility of ductal closure found in the present study agrees well with that observed by Gilman and Burton (7) who also reported the loss of a dilatory effect of hypoxia 72 hr postpartum in the guinea pig. Angiographic studies on newborn children (21) and swine (25, 26) also indicate a tendency for the ductus to become irreversibly closed within the first few days after birth. Further comparison of the present in vitro results with angiographic studies in vivo is not warranted, however, since ductal flow measured in vivo is a complex function of aortic and pulmonary pressures and flows as well as tension within the ductal wall (20).

Direct measurements of changes in tension exerted by the muscular wall of the ductus indicate that the cells of the muscularis retain their sensitivity to oxygen relative to that of other stimuli during the first 4 days after birth. A general loss of the contractile response to all stimuli was noted between 0 and 4 days postpartum, but this may result in part from the trauma involved in forcing stainless steel pins through irreversibly closed ducts of the 3- to 4-day old animals, and in part from necrosis of smooth muscle of the inner media in ducts from the older animals ((13), also Fig. 10 of the present study). Despite a significant loss of the contractile capacity of the muscle in the wall of the ductus, it is clear that, at a time when the perfused ductus has lost its ability to dilate in response to anoxia, the muscle cells of the vessel are still capable, albeit at a diminished level, of contracting and relaxing in response to high and low PO₂, respectively. Furthermore, papaverine, which causes an even greater reduction in tension than N₂, had no dilatory effect whatsoever on perfused ducts which were irreversibly closed (unpublished observations). All of these findings indicate that irreversibility of closure is not the result of sustained active force within the muscularis of the vessel wall.

The histological observations on ducts from animals of various ages provide some insight as to the mechanism that underlies irreversibility of closure. In all 12 irreversibly closed ducts systematically studied, the lumen was occluded at some point along the vessel. Cross sections through the occluded part revealed rearrangement of cells formerly in the area occupied in a constricted vessel by the endothelium and subendothelium and the apparent intermingling of cells from apposing walls. This appearance is not merely the result of apposition of the walls of the vessel, for no loss of an intact endothelium is apparent when ducts not yet irreversibly closed are fixed under aerobic conditions so that their walls are apposed. Before closure has become irreversible, the only difference between open and closed ducts is in the relative thickness of the endothelium and subendothelium which results from changes in the geometry of the vessel. A number of authors have recently reported similar changes as a result of muscular closure (10, 18).

The apparent death of many of the cells in the central region of the vessel was also a prominent and consistent feature of all cross sections through the occluded region of irreversibly closed ducts. In contrast, in ducts not yet irreversibly closed, necrosis was generally absent and when observed was confined to a few cells. When the onset of necrosis and cellular rearrangement was prevented or at least delayed by raising neonates under hypoxic conditions, irreversible closure was also prevented up to 80 hr postpartum.
The correlation between irreversible closure and cellular necrosis and rearrangement in ducts from normal and hypoxic neonates suggests an explanation for the mechanism underlying irreversible closure in the first few days postpartum. Ducts presumably lose their ability to reopen as a result of the presence of this central plug of cells and cell debris. In order for this central plug to effectively prevent flow through the ductus under anaerobic as well as aerobic conditions, it must somehow restrain movement of the vessel walls. Otherwise relaxation of the ductal smooth muscle in response to anoxia would result in the separation of the vessel wall from the plug and an increase in flow conductance. Alternatively, the plug itself may be elastic and expand during anaerobic conditions as the muscle in the vessel wall relaxes. In either case, the central mass of cells and cell debris must itself be cohesive and in addition must adhere to the inner walls of the ductus. These studies do not indicate what the basis for any “adhesiveness” might be. It is likely, however, that adhesiveness resides less in cell-cell interactions than in the properties of some extracellular material, since the ductus remains closed despite phagocytosis of...
FIG. 11. Cross section taken midway along occluded length of same ductus shown in Fig. 10. Note loosely arranged cells in central area. There are numerous pycnotic nuclei (arrows) and there is no endothelium or lumen. X 382.

FIG. 12. Electron micrograph of a cell from central area of ductus shown in Fig. 11. Cell completely encloses a dense mass of material (arrow) that possibly corresponds to a cell fragment. Cell is surrounded by large amounts of elastinlike intercellular substance (e). X 7,000.

FIG. 13. Cross section showing central area of a ductus obtained from a “hypoxic animal” 54 hr postpartum. Lumen (L) is patent, and endothelium (E), subendothelium (arrow), and muscular coat (M) are intact. Moreover there is no evidence of pycnosis. Ductus was fixed in situ. X 350.

FIG. 14. Electron micrograph of a cell from central area of ductus shown in Fig. 11. Cell completely encloses a dense mass of material (arrow) that possibly corresponds to a cell fragment. Cell is surrounded by large amounts of elastinlike intercellular substance (e). X 7,000.

Kennedy and Clark (13) first noted the necrotic changes that take place in the guinea pig ductus during the first few days postpartum. Mato and Aikawa (17) and Jones et al. (12), using the electron microscope, have recently described similar necrotic changes in the ductus arteriosus of the rat; rearrangement of cells of the intima similar to that observed in the present study has also been recently reported (17, 19). Because necrosis and cell dislocation were limited to the central regions of the vessel, and because they followed muscular closure, several authors (9, 13, 18, 19) have suggested that these processes result from interruption of luminal blood flow. The present results with animals raised under hypoxic conditions provide experimental evidence supporting this suggestion. Ducts from hypoxic animals remain patent for 3-4 days after birth and show neither necrosis nor rearrangement of endothelial and subendothelial cells characteristically seen in ducts from normal animals of similar ages in which luminal flow had ceased. It appears, therefore, that cellular rearrangement and necrosis are triggered by metabolic disturbances associated with the interruption of luminal blood flow which results upon muscular closure of the ductus at birth. Ischemia (8, 22, 29) and direct chemical trauma (11) have also been
shown to produce necrotic changes in the walls of other vessels. Furthermore, detachment and migration of endothelial cells following ischemia or direct mechanical trauma to other blood vessels have also been noted (16, 27). Thus the cellular transitions observed in the inner wall of the ductus appear to be typical for the response of a vascular wall to ischemic trauma. The confinement of necrosis to the outer media and adventitia, which do not undergo the inner wall of the ductus presumably reflects the metabolic dependence of this region on luminal flow. The cells of the outer media and adventitia, which do not undergo necrosis, presumably remain viable because blood flow through the vasa vasorum, located predominantly in the outer part of the vascular wall, is unaltered after birth (3).

Our inability to block closure of the ductus by hypoxia for more than 3 or 4 days prevented any longer tests of the relationship between cell death and rearrangement and the interruption of ductal flow. This inability to prevent closure suggests that, especially in cases of systemic hypoxia factors other than oxygen, perhaps circulating catecholamines as Dawes suggests (2, 4) initiate muscular closure of the ductus.

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Present address of F. S. Fay: Dept. of Physiology, University of Massachusetts Medical School, 419 Belmont Street, Worcester, Mass. 01604. Send reprint requests to this address.

Present address of P. H. Cooke: Dept. of Physiology and Cell Biology, University of Kansas, Lawrence, Kan. 66044.

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