Constriction of the Neonatal Aorta by Raised Oxygen Tension

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ABSTRACT

A preparation consisting of the great arteries around the heart from neonatal guinea pigs was perfused at constant flow by physiological saline solutions in a bath in which various gases (95% N₂ + 5% CO₂, air + 5% CO₂, and 95% O₂ + 5% CO₂) were bubbled. The pressure drop across the aorta, including the preductal area, was recorded and the changes in resistance in response to these gas tensions were calculated. The well-known closure of the ductus with increased P₀₂ occurred, but in addition there was a reversible increase, up to 400%, in the resistance of the aortic segment. This was maximal 1 to 2 days after birth, diminishing with age until after 6 days it was very slight. In very young animals (1 to 2 days), responses to air and oxygen were roughly proportional to the P₀₂. Inspection of injection casts of the vessels showed that the constriction was local in the preductal area, closely resembling that of aortic coarctation. Segments of aorta that did not include the preductal area, and from animals of comparable age, showed very slight constriction to raised P₀₂. The neonatal vessels of pigs, rabbits and cats showed similar O₂ sensitivity of different degrees, and with different peculiarities as to time lag and repeatability. Neither the ductus nor the neonatal aorta of dogs appears to constrict to increased P₀₂, and closure of the ductus in this species must be by a different mechanism.

These observations may be interpreted as evidence for the Skodaic theory that infantile aortic coarctation may occur in association and coincident with the closure of the ductus at birth. The administration of high O₂ gases at birth might, in cases of abnormal development, lead to permanent coarctation.

ADDITIONAL KEY WORDS

preductal aorta ductus arteriosus coarctation of aorta

Skodaic theory species difference guinea pigs aortic resistance

Our interest in investigating the response of the neonatal aorta to high oxygen tensions was stimulated by certain features of aortic coarctation and by recent advances in our understanding of the mechanism of the closure of the ductus arteriosus. The remarkable anatomic association of the coarcted segment of aorta with the insertion of the ductus arteriosus has been noted for many years. In order to explain this interesting phenomenon, two theories have arisen. The first theory proposed was that the lesion arose at an early developmental stage. This theory attempted to link the coarctation with some miscarriage in the development of the aortic arch in embryonic life. This was first suggested by Reynaud in 1828 [cited by Friedberg (1)]. This is at present generally held to be the etiology of coarctation. The other view, first proposed by Craigie in 1841, and later popularized by Skoda [cited by Friedberg (1)] has come to be known as the Skodaic hypothesis. This hypothesis states that the aorta becomes involved to some extent in the obliterating process which normally closes the ductus. Although an extensive discussion of the etiology of coarctation is not in order, it can be stated that there are points in favor of both views. The developmental theory does explain the rare instances of coarctation not associated with the ductus. The Skodaic hy-
pothesis does explain very well the anatomic association between the coarctation and the ductus and also explains the general shape of the lesion, which is often as if something on the aortic wall near the ductus had undergone constriction and atrophy (2). Skoda's theory cannot explain fetal coarctation.

As long as the physiological stimulus to the normal closure of the ductus was not known, it was difficult to obtain evidence for the Skodaic theory. However, it now seems quite certain that the stimulus is the rise in oxygen tension at the onset of respiration. Kennedy and Clark (3, 4) showed that closure was associated with the initiation of breathing and that oxygen must be in the inspired gas. Born et al. (5) showed that closure was independent of central nervous control. Assali et al. (6) and Kovalcik (7) independently have shown that contraction can be produced in the isolated ductus when the oxygen tension is raised. Many others have shown in the intact animal that administration of oxygen-rich gas closes the ductus and that the breathing of anoxic gas mixtures can reopen the ductus in the first few days of life. Finally Penaloza et al. (8) found in a survey of those living above 10,000 feet that the incidence of patent ductus was many times greater than at sea level, and that approximately 1% of the population living at high altitude had this disorder.

It seemed to us, then, that the Skodaic theory made it worthwhile to investigate the reactivity of the neonatal aorta to $P_{O_2}$, which is the normal stimulus to contraction of ductal tissue. If oxygen-sensitive tissue similar to that of the ductus existed in the preductal region, it should produce a constriction at that point, when the $O_2$ tension is increased.

**Methods**

**DETECTION OF THE AORTIC RESPONSE**

A quantitative measure of the narrowing of vessels is to perfuse at constant rate of flow from a pump and to use the pressure drop across a given segment, which is proportional to the resistance to flow of that segment, as a measure of vasoconstriction. Burton and Stinson (9) have shown that in this method, the pressure drop is, over a large range, proportional to the "active tension" exerted by the smooth muscle in the wall of the vessel segment. The pump is set to any rate of flow that is convenient to give ade-
quate sensitivity to changes in resistance of the segment, rather than in relation to any physiological rate of flow in the aorta. If vasoconstriction is detected by this method of constant flow, a greater vasoconstriction would be expected to occur in physiological circumstances, when the driving pressure is more likely to remain constant. This is because in our method, vasoconstriction elicits a rise of perfusion pressure, which will increase the transmural pressure and partially counteract the active constriction by producing a passive increase in vessel caliber. While the alternative method of perfusion with constant pressure (6) is more sensitive, it does not provide a good index of the response of the vascular wall (9).

Animal Preparation. Animals of various ages were used; some were delivered before term by cesarian section. Guinea pigs were most frequently used, though experiments have been done with rabbits, cats, pigs and dogs. The chest was opened under ether anesthesia. The pulmonary vessels were clamped and tied at the hilus. The vessels arising from the aortic arch were ligated and cut. The heart was then freed of its attachment to the superior and inferior venae cavae and removed along with the aorta as far distal as the mid portion of the descending part of the thoracic aorta. In some cases the ductus arteriosus was ligated, though in most instances the ductus was tied to a nonfunctioning cannula, which served to occlude it and to stabilize the preparation. The heart was then transected and cannulas were introduced into the proximal aorta and the ductus arteriosus through the aortic and pulmonary valves respectively. Another cannula was inserted into the distal end of the aorta.

Method of Perfusion. The preparation was then mounted on a plastic frame which fitted vertically into a reservoir of perfusion fluid (Fig. 1). The frame held the various cannulas for perfusion, and also a tube terminating in a bubbler, through which various gas mixtures could be driven to envelop the preparation in bubbles of 95% N₂ + 5% CO₂, air + 5% CO₂, or 95% O₂ + 5% CO₂. The whole was mounted in a larger water bath kept constant at 37°C.

The perfusing fluid was a Ringer’s solution, modified by adding both bicarbonate and phosphate buffers so that with any of these gas mixtures the pH was maintained at a constant value of 7.4 (which was monitored). The fluid was drawn by a Sigmamotor pump from the reservoir containing the preparation and circulated through the blood vessels at a constant predetermined flow rate. The drop in pressure across these vessels was directly measured from the difference in height of the two vertical fluid manometers. Since the flow was constant, this difference of pressure was directly proportional to the resistance to flow of the vessels, plus the resistance of the connecting tubes and cannulas. The constant resistance of the latter was obtained by measuring the pressure drop when the cannulas were connected directly, instead of through the vessels of the preparation, and was subtracted from the total resistance to give the resistance of the blood vessels. As explained above, this resistance is linearly related and is approximately proportional to the active tension in the vascular wall (9).

Results

Response to Raised Pₐₒ₂. In the preliminary experiments, we perfused not only the aorta, but by turning taps on the apparatus, the ductus also. The pressure drop across the ductus, from pulmonary artery to distal aorta, rose immediately and very rapidly as soon as O₂ was substituted for N₂. The ductus closed so quickly that no reliable measurements of resistance were possible, even using a lower rate of flow from the pump. Also, since the observations on the ductus merely would verify the work of others, later experiments were confined to perfusion of the aorta.

Figure 2 shows the reversible rise of the transaortic pressure when the gas mixtures were changed; with constant flow this change in pressure is proportional to the change in resistance of the aorta. There was usually a remarkably long lag after changing to O₂ before the resistance began to rise. (The lag time is discussed in detail later.) The full constriction required from 15 to 30 min to develop. Relaxation when N₂ was substituted was usually faster than the constriction to O₂.

Dependence of Aortic Constriction to O₂ on Age. The response to O₂ was greatly affected by the age of the animal. The maximum increase of resistance produced by O₂ was expressed as a percentage increase over the resistance when perfused with Ringer’s equilibrated with 95% N₂ and 5% CO₂. Figure 3 shows the results with age for 34 neonatal guinea pigs (the age of the three delivered by cesarian section is estimated from the fetal weight). There is, as to be expected, considerable scatter, but the sensitivity to O₂ is significantly maximal at one or two days.
Portion of a typical experiment showing the effect of altering the oxygen tension; note the long 'lag' in response.

Change in aortic resistance at different ages due to perfusion with solutions with high $P_{O_2}$. Resistance is recorded as a percent of the basic resistance with 95% $N_2 + 5% CO_2$, which is zero on the ordinate. The lines connect points for litter mates.
after birth. The lines connecting data from litter mates might be expected to be less dependent on individual variation, and these show the trend with age most reliably. By 7 or 8 days, the aortic constriction to O2 is very small and is no greater than the value for adult arteries.

*Effect of Air versus Oxygen.* It might be suggested that the difference in resistance of the aorta in O2 versus N2 represented a dilating effect of anoxia when N2 was used, rather than a constriction due to O2. To test this, in 5 animals the resistance was compared in the same preparation when 95% N2 + 5% CO2 was changed to air + 5% CO2 or to 95% O2 + 5% CO2. Figure 4 shows that the constriction is graded, proportional to the Po2. The dependence of the response on age was much more marked with O2 (confirming Fig. 3) than with air, where there appeared to be little effect of age, in the first 4 days of life.

**Reproducibility.** Generally speaking, the contraction observed following administration of O2 is both reversible and reproducible in guinea pigs. There are considerable species variations in this regard which are discussed later. In the guinea pig, the first contraction is usually the largest and subsequent contractions are more rapid in onset and less vigorous. In very young animals, the magnitude of the second contraction is comparable to that of the first one. With alternation between nitrogen and oxygen, there is a tendency for the nitrogen baseline to remain fairly constant, whereas the oxygen peaks gradually fall (Figure 5, upper part). If the tissue never becomes anoxic, i.e. when air, instead of N2, is alternated with oxygen, the baseline (air) tends to rise, and the oxygen peaks remain more constant or tend to drift only slightly downwards (Figure 5, lower part). At the end of these prolonged experiments, the tis-
sue was demonstrated still to be normally active to norepinephrine.

Time Lag. One of the interesting peculiarities of this smooth muscle reaction is the time lag before the onset of contraction. This period of inactivity after the rise in $P_{O_2}$ varies considerably from animal to animal. In guinea pigs, this time lag is often 20 to 25 min (Fig. 2). The length of the lag does not seem to be due to any known factors such as age, degree of anoxia or oxygenation. It cannot be attributed to delay in rise of $P_{O_2}$ in the apparatus, for tests with an O$_2$ electrode in different places in the circuit showed that even at the end of the circuit, with the lowest rate of flow, the maximum value of $P_{O_2}$ was reached in about 3 min.

The time lag is also a feature of the first oxygen contraction only. Subsequent contractions do not demonstrate this period of inactivity, or do so to an insignificant degree (Fig. 6).

Localization of the Constriction to the Preductal Area. Similar experiments were made using portions of the aorta which did not include the preductal area, i.e. from 5 mm distal to the insertion of the ductus to below the diaphragm. Three animals, ages 2, 3 and 4 days, were used; at this age the constriction in the previous experiments had averaged about 250%. The increases in resistance here when the O$_2$ mixture was used were only $-3\%$, $+20\%$ and $-11\%$, respectively (mean $+2\%$).

Further evidence of the local character of the O$_2$ constriction was obtained by making casts. After the aorta had shown a large constriction, the casting material (Silastic 385 Elastomer) was injected at 100 mm Hg pressure, and the tissue subsequently removed by digestion in alkali. Figure 7 shows two casts of aortas from two animals, one of which had been perfused with the N$_2$ mixture and the other with the O$_2$ mixture for some time before injection.

To obtain quantitative data from these casts, photographs were made in two directions at right angles, and the diameters of the...
FIGURE 7
Two aortic casts. The animals were of equal age (48 hours). The cast on the left was made under anoxic conditions; the cast on the right was made in the presence of high oxygen tension (95%). The latter shows a marked deformity in the preductal region.

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lumen were measured in the ascending aorta, aortic arch, fetal isthmus and descending aorta. The data were expressed as the ratio of this diameter to the diameter of the descending aorta, which was very unreactive to oxygen, but which, of course, changed with age. In spite of a trend with age, the ratios for the preductal area after O₂ were significantly lower than those with N₂. For animals 11 days of age and under, the mean ratio was 0.78 ± 0.023 SEM (13 casts) when N₂ had been used, versus 0.68 ± 0.039 SEM (8 casts) when O₂ had been used. The difference is statistically significant (P < .05). The mean ages of the two groups were comparable (4.1 days for the N₂ group, 3.9 days for the O₂ group), and could not account for the difference in degree of constriction. It is highly probable that this degree of constriction after O₂, from measurements of casts, does not represent the true amount of constriction because of the artifact of relaxation during the process of casting. The casts, however, show convincingly that the constriction is purely local, at just the place where infantile coarctation is most often found.

**Other Species.** Several other species were investigated, showing differences from the results on guinea pigs. Guinea pigs (130 animals) were used most because they were readily available. Compared with guinea pigs, the aortas of rabbits (21 animals used) are much less active. The reactions were about 25% as vigorous as with the guinea pig. The reactivity of the aortic tissue decreased with age in a manner similar to that found in guinea pigs. The contraction of the ductus was much slower than in guinea pigs and did not occlude the lumen. In 13 puppies used, we were surprised to find that the aorta did not react at all to increased P₀₂. Instead, the experiments demonstrated a progressive, slow dilatation of the aorta when P₀₂ was increased. When a cast was made at the conclusion of an experiment on a puppy, the ductus was found to be open in the face of 95% oxygen. Subsequent experiments on resistance to flow also showed that the ductus in puppies appeared to be insensitive to oxygen.

All the dogs tested for flow through the ductus initially appeared to have a “closed” ductus, i.e. at a flow of 9 ml/min, the pressure drop was more than 110 cm H₂O. (These represent the lowest flow rate and maximum pressure which the apparatus used on dogs could handle.) Later in the perfusion, the ductus opened even if the P₀₂ had been raised to that of 95% O₂. The younger the animal, the more quickly the ductus opened in 95% O₂.

Norepinephrine given at the end of the experiment caused contraction of both ductus and aorta. The ductus very often became occluded when 25 μg/liter of norepinephrine was given. Table 1 summarizes our inferences as to species differences based on our experimental evidence (small in some species).

In experiments with kittens, the aorta was found to contract to increased P₀₂. The reaction was often a very vigorous one. After the initial contraction and relaxation in N₂, subsequent contraction with O₂ could not be reproduced in any of the 24 kittens used. At the end of the experiment, the tissue could be demonstrated to be quite responsive to norepinephrine. With kittens, a lag period was generally not observed. After the first contraction, progressive relaxation was generally observed. During the latter part of this relaxation period, the ductus could be shown to be patent. In one animal 12 hours old, the ductus was found to be initially patent and then became occluded with oxygen. Nitrogen was then administered and the aorta was perfused. Soon the ductus was patent again, but, as with the aorta, there was no reaction to oxygen with the second trial. The ductus reacted to norepinephrine, but rather slowly.

The animal most sensitive to oxygen was the newborn pig. In this species, increases in resistance up to 19-fold have been measured. Porcine aortas are usually much more vigorous on the second contraction than the first. The most interesting difference in this animal is the wider distribution of contractile tissue. The aorta consistently showed contraction in the postductal area as well as in the preductal area. However, experiments on different seg-
Inferences as to Species Differences

<table>
<thead>
<tr>
<th>Species</th>
<th>Ductal reaction to PO₂</th>
<th>Preductal aortic response to PO₂</th>
<th>Age at which O₂ sensitivity lost</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Guinea pig</td>
<td>++++</td>
<td>++</td>
<td>Aorta, 8 days</td>
<td>Long time lag on first response, less later</td>
</tr>
<tr>
<td>Pig</td>
<td>++++</td>
<td>++</td>
<td>Ductus, 2 days</td>
<td>Less time lag, more diffuse distribution in aorta</td>
</tr>
<tr>
<td>Rabbit</td>
<td>++++</td>
<td>+</td>
<td>&gt; 3 days</td>
<td>Responds to first stimulus, not to later stimuli</td>
</tr>
<tr>
<td>Cat</td>
<td>++++</td>
<td>++</td>
<td>&gt; 10 days</td>
<td></td>
</tr>
<tr>
<td>Dog</td>
<td>0</td>
<td>0</td>
<td>Probably never sensitive to O₂</td>
<td>Must have mechanism other than PO₂ rise for ductal closure</td>
</tr>
</tbody>
</table>

+++ = marked constriction.
0 = no constriction.

Discussion

Our main purpose in undertaking this study was to determine if, in the aorta itself, tissue may be found which has reactions similar to those found in the ductus arteriosus which cause normal closure of the ductus. The response of the aorta to oxygen that we have found resembles that of the ductus arteriosus in many important respects. The main difference between the two vessels appears to be that the contraction of the aorta is much less than that of the ductus. Both vessels contract in response to O₂ and relax in response to lack of O₂. In our guinea pigs, the ductus opened in response to anoxia until about 72 hours after birth. Beyond this point relaxation may occur in some cases, but it has not been demonstrated. The guinea pig aorta also will contract and relax for many days after birth, though the response is much less after 4 days. The human ductus has been shown to constrict in response to oxygen and to relax in response to hypoxia for up to 27 hours after birth (12).

The fact that the magnitude of the response is to some degree dependent on the magnitude of the stimulus, i.e., on the PO₂ level, probably applies to both vessels. In the uterus, where the PO₂ is about 25 mm Hg, the ductus remains open; after the baby is born and the PO₂ gradually rises to 100 mm Hg (breathing air) the vessel is certainly closed. Pénalozas work on humans at altitude (8) suggests that response depends on the level of PO₂. At levels of oxygen tension between 25 and 100 mm Hg, there may well be a graded response. Assali et al. (6), who worked with sheep, thought this to be the case though their results in vitro may not represent those in life since their graph indicated a brisk flow through the ductus at 100 mm Hg and even at 760 mm Hg. The ductus, in our experiments on guinea pigs and rabbits, was completely closed in 3 min when subjected to 95% oxygen. In the aorta, the contraction to 95% O₂ was always greater than to air.

Our observations on repeated exposure of the aortic tissue to 95% O₂ followed by air showed a "drift" towards a greater degree of constriction, while the relaxation became less. With strips of ductus, Kovalcik observed a similar drift towards greater contraction when O₂ and N₂ were alternated (7).

In the few experiments we have done on ductal tissue, we have failed to observe any appreciable time lag, though it is a marked...
feature of the response of neonatal aorta. However, the lag in the aorta becomes much less during subsequent exposures to increased $P_{O_2}$.

A similarly diminishing time lag in response to raised $P_{O_2}$ has been seen in neonatal retinal vessels (10); the retinal vessels of the neonate will constrict to obliteration if stimulated by high enough $P_{O_2}$, producing retrolental fibroplasia. In studies done on kittens (10), the vessels were seen to begin constriction after 5% hours of exposure to oxygen, and were obliterated after 6% hours of exposure. The animal then breathed air and the vessels relaxed. When oxygen was administered again, obliteration occurred after only 7 min. The third constriction was completed in 5 min.

The localization of the aortic constriction to increased $P_{O_2}$, in guinea pigs shown by our casts, resembles very closely that of infantile coarctation. Glass et al. (11) state that in 90% of fatal cases of coarctation of the aorta when under one year of age, the constriction was localized to the preductal area. We must, of course, assume that our neonatal animals were “normal” and did not have developmental defects. The experiments give evidence of oxygen-sensitive tissue, in the normal neonatal animal, in the aorta in close proximity to the ductus arteriosus. It seems possible that in some of those children that develop infantile aortic coarctation, there has been a developmental defect in which there is an abnormal amount of this oxygen-sensitive tissue in the aorta. Possibly, in those that have patent ductus also, the oxygen-sensitive tissue in the ductus is far less than the normal amount. Even with air, we found evidence of a small degree of aortic constriction, but in normal neonates this may be transient and of trivial physiological consequence.

Despite marked species differences, one thing common to all is that the aorta in each species behaves in a manner similar to the ductus. The sensitivity of the aorta to oxygen appears to be much below that of the ductus and it appears to retain its sensitivity to oxygen a little longer. The latter is very difficult to prove, however, since the ductus is closed irreversibly in many of these animals. Besides pointing out the interesting physiological parallel between ductus and aorta, these studies show that some caution must be exercised in applying principles demonstrated in guinea pigs and sheep to humans.

A practical consideration arises from the experiments. While the administration of $O_2$-rich gases to a normal neonate may have no serious consequences in the aorta (i.e. the aortic constriction may be slight and temporary), in the case of some developmental anomaly in the location of the oxygen-sensitive tissue between ductus and aorta, $O_2$ administration might precipitate permanent aortic coarctation. The experiments certainly do not dispose of this possibility. They suggest that there may be still another hazard of the administration of $O_2$ to the newborn, to be added to the known hazards such as atelectasis and retrolental fibroplasia.

Acknowledgment

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