Constriction of the Fetal Ductus Arteriosus Induced by Oxygen, Acetylcholine, and Norepinephrine in Normal Dogs and those Genetically Predisposed to Persistent Patency

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SUMMARY
Functional closure of the isolated perfused ductus arteriosus was studied in normal dog fetuses and in fetuses receiving various known proportions of their genes from dogs with patent ductus arteriosus (PDA). Constriction of the ductus in response to O₂, acetylcholine (ACh), and norepinephrine (NE) was evaluated by plotting changes in input conductance against time. Ductuses from the control (normal dog) fetuses constricted in response to high PO₂, ACh, and NE, indicating that in the dog the response of the ductus arteriosus to these agents is qualitatively similar to that in other mammals. Hypoxia relaxed the O₂ constricted ductus. After two to three cycles of oxygenation and hypoxia, the ductus became refractory to O₂ but remained responsive to ACh and NE. Ductuses from PDA-related fetuses tended to be more widely patent when hypoxic and did not respond to O₂, ACh, and NE to a degree comparable to the control fetuses. Both the proportion of subnormal ductal responses and degree of abnormality in the PDA-related group increased as the degree of relationship to dogs with PDA increased. These experimental results are consistent with the polygenic mode of inheritance previously demonstrated for canine PDA. It is suggested that the liability to defective ductal closure in the dog results from a quantitatively inherited inability of the ductus to constrict.

Additional Indexing Words:
Congenital heart disease Input conductance Polygenic Hereditary disease
Patent ductus arteriosus Functional ductal closure

The ductus arteriosus normally closes within hours after birth as a result of constriction of its muscular wall. Anatomic obliteration of the lumen occurs over a longer period of time following extensive morphologic changes in the ductal wall. During the last 35 years, beginning with the investigations of Barcroft, Kennedy, Mason, and Clark, the functional stage of ductal closure has been studied in an attempt to define its initiating stimulus and role in the hemodynamic adjustments of the neonatal period. Of the species examined, the fetal guinea-pig and lamb have been studied most thoroughly. The ductus arteriosus in these species constricts in response to several agents normally present in the body, but functional closure seems most closely related to the increase in arterial O₂ tension which occurs following the onset of breathing. Constriction of the ductus arteriosus with O₂ has also been reported in the pig, rabbit, cat, monkey, and human. Early studies of the physiologic closure mechanism in the dog demonstrated constriction of the ductus arteriosus and a marked decrease in ductal blood flow by 12 hours after birth. Other investigators have reported that ductus closure can be delayed in the dog by rearing newborn pups in a low O₂ environment. However, in more recent studies, no response of the fetal ductus arteriosus to O₂ was found in the dog, and it

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was suggested that the dog may differ from other species in its physiologic closure mechanism.\textsuperscript{14}

Although the normal mechanisms involved in closure of the ductus arteriosus have been extensively studied, as yet there is little insight into the cause and pathogenesis of the common anomaly, patent ductus arteriosus (PDA). The recent recognition that PDA in the dog is a genetic trait and the subsequent development of a strain of dogs having a predictable incidence of PDA\textsuperscript{15, 20} have made it possible to investigate the physiologic closure mechanism in fetal dogs that have a high risk of defective ductal closure.

This paper reports results of experiments designed to describe in vitro ductal responses of the normal fetal dog to O\textsubscript{2}, acetylcholine, and norepinephrine and to compare them with the ductal responses of fetal dogs genetically predisposed to defective ductal closure. The differences observed are offered as new information on the pathogenesis of PDA.

\textbf{Materials and Methods}

\textbf{Source of Dogs}

Twenty fetuses were obtained from six matings of two breeds. These fetuses were expected to have normal ductuses and were used as controls for comparison with the PDA fetuses. Fifteen were purebred coonhounds with no known relationship to dogs with PDA. Five fetuses in one litter were from a strain of beagles in which PDA has not been observed in more than 500 pups. In addition, the natural prevalence of PDA in coonhounds and beagles has been shown to be low.\textsuperscript{18}

Twenty-eight fetuses receiving 25–100\% of their genes from dogs with PDA were obtained from nine matings. These pups were partially or completely of miniature- or toy-poodle ancestry.

\textbf{Surgical Procedures}

Pregnant, near-term bitches were premedicated with \% mg atropine sulfate and morphine sulfate (approximately 2.2 mg/kg body weight of fetus) subcutaneously. A cesarean section was performed under epidural analgesia using 2\% xylocaine (Lidocaine). Only one fetus was delivered and studied at a time. Ductuses were obtained from as many as six fetuses from one bitch over a 14-hour period. Analgesia was maintained with additional injections of xylocaine made through an indwelling catheter in the lumbosacral space. Additional morphine was occasionally given.

\textbf{In Vitro Perfusion of the Ductus Arteriosus}

Each fetus was vigorous at the time of delivery. Breathing was prevented by immediately covering the head with a saline-filled glove. The chest was then opened and the heart and lungs removed. Tissues were moistened with Tyrode's solution during the 15–20 min required to complete the dissection.

The ductus was rinsed of all blood prior to suspension, without tension, in a recirculating bath of Tyrode's solution maintained at 37\degree C (fig. 1). It was then perfused via a polyethylene cannula (1.67 mm id x 2.42 mm od) tied into the cut end of the pulmonary trunk. The pulmonary arterial branches and aortic arch and its branches were ligated. The perfusate exited into the bath through a 1.5 mm long, 14-gauge stainless-steel cannula which ensured a fixed lumen size at the junction of the thoracic aorta and ductus. Inlet pressure at the ductus was measured with a strain-gauge transducer (Statham SP37) connected to a polyethylene catheter (0.76 mm id x 1.22 mm od) tied into a pulmonary artery or connected at right angles to the perfusing cannula within 2 mm of the pulmonary artery. The conductance of the cannula alone at maximum pump output (165 cc/min) was six times larger than any value calculated for any ductus, thus placing little restriction on the full range of ductal responses.

The bath could be gassed with either 95\% O\textsubscript{2} or 95\% N\textsubscript{2} in 5\% CO\textsubscript{2} (fig. 1). The gasses were mixed and analyzed commercially (Liquid Carbonic Corp.). Gas tensions and pH of the bath were measured periodically using microelectrode cuvettes (Radiometer Copenhagen).

The ductus was perfused with a roller pump (Model 3500 LF, Sarns, Inc.) made nonpulsatile by insertion of a short segment of high-compliance tubing (Windkessel effect) (fig. 1). The volume of the bath and perfusion system was approximately 250 cc in each experiment. Inlet pressure to the ductus was monitored with a digital meter and controlled by manual adjustment of the pump speed. The pump output was linearly related to the current drawn by the motor. Signals from the pressure transducer and pump motor were processed by a hybrid computer system. Every 30 sec the time-average pressure (mm Hg), flow (cc/min), and input conductance (IC = flow/input pressure in cc/min/mm

\textbf{Diagram of experimental system used for monitoring changes in lumen size of the excised, perfused ductus arteriosus by plotting the input conductance (IC) against time.}

\begin{figure}[h]
\centering
\includegraphics[width=0.4\textwidth]{figure1.png}
\caption{Diagram of experimental system used for monitoring changes in lumen size of the excised, perfused ductus arteriosus by plotting the input conductance (IC) against time.}
\end{figure}
CONSTRICTION OF FETAL DUCTUS ARTERIOSUS

Hg) were printed by teletype. To aid evaluation of results during the course of an experiment, input conductance was graphed at 30-sec intervals by an X-Y plotter.

Drugs

The ductal responses to the following drugs were tested by injecting them directly into the bath. In terms of the concentrations of their salts in the bath-perfusion system, they were: acetylcholine bromide 1.5–10.0 \( \mu \text{g/cc} \); atropine sulfate 1.5–4.0 \( \mu \text{g/cc} \); levarterenol bitartrate (Levophed) 6.5–16.0 \( \mu \text{g/cc} \).

Experimental Protocol

The ductus was first perfused for 20–40 min in the N\(_2\) gassed bath. During this period of hypoxia, the ductus was gradually diluted by slowly increasing the flow until a stable input conductance was reached. Within the limitations imposed by the ductus, we attempted to maintain a steady pressure within a physiologic range (30–50 mm Hg). If the maximum pump output was reached while the ductus was still dilating, the input conductance was established at a lower intraluminal pressure. After the conductance had stabilized, the gas was abruptly switched to \( \text{O}_2 \). As conductance decreased, the flow was proportionately lowered so the ductus contracted against a reasonably steady intraluminal pressure. Usually the N\(_2\)–\( \text{O}_2 \) cycle was repeated one or two times. If \( \text{O}_2 \) failed to elicit a response, the viability of the ductus was tested by adding ACh or NE to the bath (fig. 2). The response to both drugs was frequently evaluated by reversing the effect of ACh with atropine before adding NE. Once added to the bath, a drug continued to circulate for the duration of the experiment.

Variations in Fetal Body Weights

There was a real weight difference (\( P < 0.001 \)) between the control (326 ± 69 g) and PDA fetuses (214 ± 76 g). Since lumen size of the ductus presumably varies in proportion to body size, individual and group comparisons were made by calculating conductance for each ductus per 100 g of body weight.

Quantification of Ductal Responses

The evaluation of ductal vasomotor responses was based upon the relation for steady flow expressed in terms of conductance. It is well known that under these conditions conductance is directly proportional to the fourth power of the radius (Poiseuille-Hagan equation). Since conductance can be calculated easily by measuring the flow and pressure variables, an indirect estimate of radial change is possible. So that a more direct correspondence to radius could be made, the input conductance adjusted for body weight is expressed as the fourth root (IC\(_4\)).

Use of this relationship rests upon three conditions: (1) In the dog fetus, ductal length is maximally only two to three times its diameter, and in defective ductuses the relative length is even shorter. Length is effective only as a first-power factor under steady-flow conditions, and consequently small variations in ductal length make a minor contribution to changes in conductance. (2) The pressure downstream from the ductus is equivalent to the height of the bath fluid above it (2 cm) and is constant, in which case the perfusing pressure is immediately determinable from the input pressure. (3) All changes in vessel size occurred slowly so that the vessel was in a quasistatic situation with respect to the flow.

Results

Control Ductal Responses

The responses characteristic of the control ductuses are illustrated in figure 2. Oxygen, ACh, and NE were capable of causing a precipitous fall in input conductance. The response usually began within 30 sec to 4 min, and generally sooner for ACh and NE than for \( \text{O}_2 \). Constriction in response to raising the bath p\(_{\text{O}_2}\) was reversed by gassing the bath with \( \text{N}_2 \). The ductuses became partially or completely refractory on the second or third exposure to high p\(_{\text{O}_2}\) but were still viable, since they could be made to constrict when ACh or NE was added to the bath. Though the control ductuses consistently responded to a rise in p\(_{\text{O}_2}\), the degree of constriction was variable and often less than obtained with ACh or NE (see figs. 4, 5). Atropine reversed the response to ACh but did not block the response to NE (fig. 2). Unlike the gradual relaxation during hypoxia, atropine caused a rapid rise of input conductance in the ACh-constricted ductus (fig. 2). The effect of atropine on the response to \( \text{O}_2 \) was not specifically investigated. However, in the one instance when a ductus was pretreated with atropine, the anticipated decrease in input conductance followed a rise in p\(_{\text{O}_2}\).
Comparison of Control and PDA Ductal Responses

When first placed in the bath, most ductuses behaved as though they were partially constricted. Generally, the intraluminal pressure required to dilate the ductus and at which a steady state was eventually reached during hypoxia was higher for the control group. A stabilized input conductance was achieved at or above 30 mm Hg (range 19–76) in 65% of the control ductuses but in only 32% (range 5–50) of the PDA ductuses.

Effect of Hypoxia

The IC1 of 10 of the 28 hypoxic PDA ductuses (35%) was above 2 standard deviations from the mean of the control group (fig. 3). Though the number of observations is small, the lumen size under these conditions appears to increase with the proportion of genes derived from dogs with PDA. This was particularly apparent for the PDA fetuses receiving 75% or more of their genes from PDA dogs.

Response to Oxygen

Input conductance decreased in all but two PDA ductuses following a rise of bath pO2 over 500 mm Hg (fig. 4). In most instances, the drop was greater for the control ductuses, though some individuals in this group changed little. In 17 of the 28 ductuses from the PDA-related group (59%), the response to O2 was slight, falling more than 2 standard deviations beyond the mean of the control group. The percentage of ductuses with diminished responsiveness and the degree of impairment became greater as the proportion of the genome derived from dogs with PDA increased (fig. 4). Only 30% of ductuses from fetuses receiving all of their genes from dogs with PDA showed responses within 2 standard deviations of the mean of the control group.

Patency of the ductus arteriosus (expressed as the input conductance adjusted for body weight) during the initial, stabilized period of hypoxia preceding exposure to O2 or vasoactive drugs. Each dot marks a single ductus. The horizontal line represents 2 standard deviations above the mean (1.052 ± 0.182) for the control group. The PDA ductuses are grouped according to the proportion of the fetal genome derived from dogs with PDA. The control ductuses, having no relationship to dogs with PDA, are grouped at 0. (These conventions are repeated in figures 4, 5.)

Patency of the ductus arteriosus at the point of maximum constriction in response to bubbling 95% O2–5% CO2 in the organ bath. The horizontal line represents 2 standard deviations above the mean (0.737 ± 0.164) for the control group. (See fig. 3 for details.)
Though the control ductuses maintained a decreased lumen size as long as the bath was gassed with O2, six PDA ductuses which had constricted underwent a gradual, progressive dilatation despite continued high bath pO2. Thus some PDA ductuses also showed an inability to sustain the limited constriction.

Response to Acetylcholine, Atropine, and Norepinephrine

The responsiveness of both the control and PDA-related ductuses to ACh or NE was greater than to O2 alone (fig. 5). In most instances, the lowest input conductance was attained after one or the other of these drugs had been added to the bath. Two control ductuses which previously had constricted weakly with O2 were highly responsive.

ACh and NE were more effective in constricting the PDA ductuses than was O2, but most responses were less substantial than for the control group (fig. 5). The two PDA ductuses which previously had failed to respond to O2 were stimulated to constrict weakly.

A combination of elevated pO2 and ACh or NE produced the clearest division between the ductal responses of the control and PDA groups. Again, responsiveness decreased as the PDA-derived proportion of the genome became greater (fig. 5). When the proportion was 0.50 or less, 27% of the PDA ductuses exceeded 2 standard deviations from the control mean, while at 0.75 and 1.0 all exceeded 2 standard deviations.

Atropine had the same effects on the PDA ductuses as described for the control group.

Discussion

In these experiments the ductal responses of the normal fetal dog corresponded closely to Kovalcik’s findings in isolated ductuses from fetal guinea pigs and sheep. In addition to O2, ACh and NE were effective constrictors of the ductus arteriosus. However, our results tend to support the view that O2 alone is normally an effective stimulus and operates independently of an intact autonomic nervous system or circulating autonomic agents. All but two of the 20 control ductuses reached a near-maximal constriction when only O2 was added to the bath. In nine (44%) the response could not be incremented with ACh or NE. The independent action of O2, ACh, and NE was supported by two lines of evidence. Once the ductus had become refractory to O2, it was still sensitive to ACh and NE. Also, on one occasion when a ductus was pretreated with atropine, subsequent exposure to O2 and NE was followed by prompt and vigorous constriction, while the response to ACh was blocked. These observations are compatible with arguments that availability of O2 initiates contraction of ductal smooth muscle. The failure of Gillman and Burton to demonstrate constriction of the dog ductus arteriosus in response to O2 is unexplained.

Comparison of the behavior of control and PDA-related ductuses in our experiments reveals two major differences. Ductuses from fetuses genetically predisposed to PDA had larger lumens under the initial conditions of hypoxia than did control fetuses (fig. 3) and had diminished capacity to respond to O2, ACh, and NE (figs. 4, 5). Moreover, these characteristics became more pronounced as the proportion of the fetal genome derived from dogs with PDA became greater. In some ductuses from pups receiving 25–50% of their genome from dogs with PDA, the O2 response was feeble, but substantial constriction was achieved with ACh or NE. In the group receiving 75–100% of their genes
from dogs with PDA, the ductuses were unresponsive to ACh and NE, as well as O2. Although a diminished response to O2 may be apparent first, a general decrease in the capacity of the ductus to constrict is the case rather than a stimulus-specific refractoriness.

The continuous distribution, without evidence of bi- or trimodality, of both the initial lumen size and the ability of the ductus to constrict is consistent with the clinical incidence of PDA and the polygenic mode of inheritance previously demonstrated by genetic studies in the dog.19, 20 The results of the present experiments imply that the liability to defective ductal closure results from a quantitatively inherited inability of the ductus to constrict. The defect becomes more severe as the PDA-derived portion of the genome increases so that the likelihood of PDA also increases. This is consistent with other preliminary observations which suggest that hereditary canine PDA is associated with an abnormality in the amount and distribution of smooth muscle in the ductal wall.21, 22

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References

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