Sympathetic Innervation of the Ductus Arteriosus in Relation to Patency*

Donald E. Cassels, M.D., F.C.C.P.,** and Robert Y. Moore, M.D.†

The adrenergic innervation of the patent human ductus arteriosus was studied using the Falck-Hillarp fluorescent histochemical method for the localization of monoamines. Few adrenergic terminals were demonstrated in the muscular layers of the patent ductus arteriosus in 14 specimens examined. The innervation to the adventitia was sparse to moderate. The possibility that a developmental abnormality in the sympathetic innervation may contribute to patency of the ductus arteriosus is discussed.

Increased incidence of patency of the ductus arteriosus (PDA) in infants of mothers contracting rubella in early pregnancy was noted in the first reports of congenital malformations associated with maternal rubella. Further study of the problem provided evidence of a greater range of abnormalities and confirmed the frequent involvement of the cardiovascular system. The literature on this subject has recently been reviewed by Monif, who notes an incidence of congenital heart disease of 50 percent in the overtly affected infant.

The association of patent ductus arteriosus with neuroectodermal defects in rubella suggested that an anomaly of ductus innervation might be responsible for failure of closure. Such an anomaly could also occur independently of intrauterine infection. It is the purpose of this paper to review the innervation of the ductus briefly, emphasizing its sympathetic supply and to report observations on the sympathetic innervation of the human patent ductus arteriosus examined using a specific and sensitive histochemical method, the Falck-Hillarp technique.

**Materials and Methods**

Human ductus arteriosus material was obtained at surgery during division and closure of patent ductus in 14 cases and in repair of four cases of coarctation of the aorta. The specimens of patent ductus usually included a complete ring of ductus tissue. The specimens from the coarctation cases included a segment of aorta and either a ductus ligament or, in one instance, a segment of patent ductus entering the aorta. The tissue was obtained as rapidly as possible following removal and frozen in liquid propane cooled to the temperature of liquid nitrogen. The interval between removal and freezing varied from about ten minutes to two hours. Nearly all specimens were frozen within 30 minutes of removal. After freezing, the tissue was placed in a freeze-drier and prepared for histochemical study using the Falck-Hillarp technique as described by Falck and Owman. The formaldehyde vapor treatment was carried out at 80°C for one hour using paraformaldehyde, which had been maintained at a constant humidity of 70 percent. In each freeze-drier containing ductus specimens normal animal material containing known sympathetic innervation was included as a control for the success of the histochemical method. Microscopic study of serial sections from each specimen was carried out using a Zeiss fluorescence microscope equipped with an Osram HBO-200 mercury vapor lamp, Schott BG-12 excitation filters, and barrier filters at 470 and 510 nm.

**Results**

The clinical details, age and hemodynamic data for each patient included in the study are shown in the Table. Fluorescence microscopy on the Falck-Hillarp material demonstrated specific catecholamine fluorescence in each specimen. This fluorescence...
ence, green to greenish yellow in color in the microscope, only developed after the formaldehyde vapor treatment of the Falck-Hillarp method and faded markedly with prolonged exposure to ultraviolet light. Pieces of some specimens were carried through the steps of the histochemical method, with the exception of the formaldehyde vapor treatment. In these, specific catecholamine fluorescence failed to develop. Descriptions of fluorescent material will refer to specimens carried through all steps of the method and will refer to specific catecholamine fluorescence. On the basis of the extensive experience with this method, it can be assumed that the fluorescent material demonstrated in ductus specimens is noradrenalin in synaptic terminals of sympathetic nerves. In peripheral tissues the method can be used to demonstrate sympathetic innervation.

In the patent ductus arteriosus specimens the amount of catecholamine fluorescence varied. Examples are shown in Figures 1-5. Some specimens exhibited only occasional fluorescent structures in the adventitia, and none in the muscular layers. No innervation was ever noted in the intima. Most specimens showed a significant innervation in the adventitia, usually about blood vessels. Some patent ductus also showed fluorescent structures within the media. These were scattered, and some were associated with blood vessels. Others had no clear relation to vasculature and there were a number of apparent terminals larger than the usual sympathetic ending and distorted in shape. The size and appearance of these suggested abnormal endings.

Pieces of ductus and ligament from cases of coarctation of the aorta all showed fluorescent structures in the adventitia and media, and the

<table>
<thead>
<tr>
<th>Patient, No.</th>
<th>Age</th>
<th>Date of Surgery</th>
<th>Diagnosis</th>
<th>Qp/Qs Flow</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>9/12</td>
<td>11-3-64</td>
<td>PDA</td>
<td>1.3:1</td>
</tr>
<tr>
<td>2</td>
<td>3 mos</td>
<td>12-21-64</td>
<td>PDA</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>1 yr</td>
<td>4-13-65</td>
<td>PDA</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>6 6/12</td>
<td>1-19-66</td>
<td>PDA</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>14 yrs</td>
<td>4-27-65</td>
<td>coarctation</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>4 mos</td>
<td>1-18-65</td>
<td>coarctation</td>
<td>1</td>
</tr>
<tr>
<td>7</td>
<td>3 7/12</td>
<td>11-24-70</td>
<td>PDA</td>
<td>2</td>
</tr>
<tr>
<td>8</td>
<td>8 mos</td>
<td>12-10-70</td>
<td>PDA</td>
<td>2.6</td>
</tr>
<tr>
<td>9</td>
<td>3 mos</td>
<td>12-14-70</td>
<td>PDA, coarctation, AS</td>
<td>1</td>
</tr>
<tr>
<td>10</td>
<td>3 4/12</td>
<td>10-27-70</td>
<td>PDA</td>
<td>1.5</td>
</tr>
<tr>
<td>11</td>
<td>5 8/12</td>
<td>9-16-70</td>
<td>PDA</td>
<td>1.9</td>
</tr>
<tr>
<td>12</td>
<td>6 2/12</td>
<td>10-23-70</td>
<td>PDA, VSD</td>
<td>2.9</td>
</tr>
<tr>
<td>13</td>
<td>1 yr</td>
<td>2-19-71</td>
<td>PDA</td>
<td>5.1</td>
</tr>
<tr>
<td>14</td>
<td>1 3/12</td>
<td>2-24-71</td>
<td>PDA</td>
<td>3.7</td>
</tr>
<tr>
<td>15</td>
<td>1 1/12</td>
<td>2-23-71</td>
<td>PDA</td>
<td>1.5-2.5</td>
</tr>
<tr>
<td>16</td>
<td>2/12</td>
<td>2-22-71</td>
<td>coarctation and ligament</td>
<td>2</td>
</tr>
<tr>
<td>17</td>
<td>4/12</td>
<td>3-24-71</td>
<td>PDA</td>
<td>4.3</td>
</tr>
<tr>
<td>18</td>
<td>6 2/12</td>
<td>3-19-71</td>
<td>PDA</td>
<td>2.5</td>
</tr>
</tbody>
</table>
SYMPATHETIC INNERVATION OF DUCTUS ARTERIOSUS

The nerve supply to the ductus arteriosus has been the subject of considerable study. Boyd reviewed the literature and published his observations, chiefly on the rabbit. The discussion related to pressor-receptor sensory innervation and the methods available allowed only speculation that the finer fibers to the media were afferent and motor and sympathetic. Barnard described a structure in position between the ductus and pulmonary artery in association with nerves, some of which appeared to be large branches of the vagus.

The innervation of the human ductus arteriosus was studied in detail by Allan, and the literature was briefly reviewed. He noted that information related to man was incomplete, and dissection of 15 full-term human infants was undertaken. The most frequent and only constant source was the inferior cervical sympathetic cardiac nerve. Branches of the vagus were small and few.

Beginning with Barcroft, Kennedy and Mason and Kennedy and Clark adrenalin and noradrenalin were observed to cause contraction of the ductus in animals, and Born et al, Kovalcik and Dawes also reported the same findings. There are no negative reports of absence of contraction of the ductus in response to noradrenalin. Boreus and colleagues studied the reaction of the human

Discussion

The nerve supply to the ductus arteriosus has
ductus to noradrenalin and acetylcholine. The ductus responded to both, but the aorta reacted only to acetylcholine. The absence of sensitivity of the aorta to noradrenalin correlates with the absence of catecholamine fluorescence in the aorta.

There has been uniform observation that the ductus in the fetus and newborn respond to oxygen by constriction and to hypoxia by dilatation, as originally observed by Kennedy and Clark. This is the opposite effect of the response of pulmonary vessels, which constrict with hypoxia and dilate with oxygen. In the reactive pulmonary vessels the site of stimulus is not known since it is not in the alveolus and not in the circulating blood but probably somewhere in the tissue between. In the ductus arteriosus the site or mechanism of oxygen stimulation for contraction likewise is not known, although Fay suggests that increased availability of oxygen to the terminal cytochrome component has been implicated.

As noted by Hornblad, high arterial oxygen saturation is the only cause of closure in animals in which this occurs almost simultaneously, as in the guinea pig and rabbit, the onset of very effective respiration would have to occur a few seconds after birth.

A major problem related to the study of the ductus arteriosus is the restriction of study to physiologic closure of the normal ductus, since no laboratory model exists of the patent ductus. It has not been demonstrated that physiologic constriction occurs at any stage of persistent patency or even that anatomic occlusion cannot occur without constriction. A phase of constriction followed by anatomic occlusion occurs in the normal ductus arteriosus, but it is not known whether patency is dependent on absence of previous physiologic constriction. Studies have been restricted to sequence of events in animal material, although there are some clinical inferences.

Anatomic facets of the patent ductus problem include the size of the patent ductus in relation to the normal ductus in newborn infants. The ductus at surgery can be (1) much smaller than the average in the newborn infant, or (2) about the same size, but commonly funnel-shaped and smaller at the pulmonary end, or (3) very large, 15 to 25 mm in width and associated with increased size in relation to the ductus in newborn infants.

If patency is directly related to constriction, the small ductus had incomplete constriction. It is not known whether catecholamine fluorescence varies with the size of the patent ductus arteriosus.

Since newer techniques for identification of sympathetic and vagus activity have become available, innervation and related aspects of the ductus requires review. Brundin, Norberg and Soderlund reported briefly the absence of catecholamine fluorescence in patent ductus tissue removed at surgery in six patients. Holmes, for instance, studied the rabbit ductus for the presence of cholinesterase and found ductus tissue was negative for cholinesterase, with a well defined boundary between the ductus and the positive cholinesterase tissue of the pulmonary artery and aorta. In the lamb, however, the ductus arteriosus, as well as the aorta and pulmonary artery, has a rich cholinesterase supply.

In addition to the histologic aspects of patency, it is now necessary to add information related to innervation and catecholamine activity. It seems likely that patency is associated with an anatomic abnormality of the ductus wall itself.

ACKNOWLEDGMENTS: We are grateful to Mrs. Frances Karapas for her skilled technical assistance and to Dr. Peter V. Moulder and Dr. Robert L. Reploge and their associates for providing the surgical specimens that form the basis for this report.

REFERENCES

2. Gregg NMcH: Further observations on congenital defects in infants following maternal rubella. Trans Ophthalmol Soc Aust 4:119, 1944
3. Swan C: Rubella in pregnancy as an aetiological factor in congenital malformation, stillbirth, miscarriage and abortion. J Obstet Gynaecol Br Commonw 41:1 (no. 3) 41:24 (no. 4) 1949
14. Born GVR, Dawes GS, Mott JC, et al: The constriction of...
SYMPATHETIC INNERVATION OF DUCTUS ARTERIOSUS

the ductus arteriosus caused by oxygen and by asphyxia in newborn lambs. J Physiol (Lond) 132:304, 1956
15 Kovalcik VJ: The response of the isolated ductus arteriosus to oxygen and anoxia. J Physiol (Lond) 169:185, 1963
16 Dawes GS: Foetal and Neonatal Physiology. Chicago, Year Book, 1968, p 102, 164-167

ANNOUNCEMENTS

22nd Congress, International Association for Bronchial Study (AIBE)

The 22nd Congress of the Association Internationale pour l'Etude des Bronches will be held in Marseille, France, June 21-23, 1973. Official languages are French, English, German, Spanish, Italian, with simultaneous translation. For information, please write the secretary: Dr. G. Chauvin, Hopital Salvator, 13009 Marseilles, France.

Training Course: Inhalation Therapy for Children and Young Adults

A one-week training course for physicians, nurses, inhalation therapists, and physical therapists in inhalation therapy for children and young adults will be held at the Pediatric Pulmonary Center, Lovelace Foundation for Medical Education and Research, Albuquerque, June 18-22. For information, contact Dr. Roy F. Goddard, Course Director, 5300 Gibson Boulevard SE, Albuquerque, New Mexico 87108.