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# COMMENTARY

# CONTROL OF THE DUCTUS ARTERIOSUS—A NEW FUNCTION FOR CYTOCHROME P450, ENDOTHELIN AND NITRIC OXIDE

FLAVIO COCEANI*

Research Institute, The Hospital for Sick Children, Toronto, Ontario, Canada M5G 1X8

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The ductus arteriosus is a large fetal shunt connecting the pulmonary artery to the aorta and allowing most right ventricular blood to bypass the unexpanded lungs. At birth, as the lungs acquire their ventilatory function and blood oxygen tension rises to extrauterine values, the ductus closes, and this event coincides with a fall in pulmonary vascular resistance. Through the years, this vessel has been investigated intensively not only for conceptual reasons, but also for the impact that knowledge of the factors controlling ductal tone may have in the management of the sick newborn. From these studies, the ductus emerges as a special vessel in many ways, and a brief account of its peculiarities provides a useful introduction to the article.

Until the early 1970s, it was thought that the ductus is kept patent *in utero* by the intraluminal pressure rather than a vasomotor action, and the idea of fetal patency being a passive state appeared reasonable in light of the high resistance in the pulmonary vascular bed. Then, in a major development for perinatal physiology, this traditional view was proven wrong and it was demonstrated that patency is instead an active state sustained by an intramural prostaglandin [1]. In other blood vessels, $PGI_2$† is the main, if not the exclusive, prostaglandin causing dilatation. In the ductus, however, $PGI_2$ is virtually ineffective, and instead $PGE_2$ is the active agent [1]. Closure of the ductus at birth has been generally linked to the rise in blood oxygen tension and, accordingly, oxygen has been regarded as the trigger for muscle contraction. Based on this notion, many vasoactive agents, including the eicosanoids, have been considered as mediators for oxygen, but results have been consistently negative. Only recently has a suitable effector of ductus closure been found after demonstrating the potent contractile action of ET-1 [2]. Leaving aside the successful outcome of this search, the screening itself has reaffirmed the peculiarity of this vessel by showing a singular lack of sensitivity to $TXA_2$ [3]. From this premise, it may not cause surprise that an insight into the mechanism of ductus closure has come, rather serendipitously, from unrelated research and that a key event in the contraction sequence has been identified with a process, such as a cytochrome P450 reaction [1], which is conventionally associated with metabolic transformations rather than vasoregulation. In fact, the unusual character of the closure mechanism becomes even clearer if one considers that the cytochrome-based reaction is viewed as a limiting step for the synthesis of ET-1. The potential for regulatory factors in the ductus, however, is unlikely exhausted with these findings. Recent work has documented the formation of NO, and this agent is expected to supplement $PGE_2$ in keeping the vessel patent [4]. Furthermore, theoretical considerations [5] and experimental findings [1] raise the prospect of CO functioning as an additional ductal relaxant.

Taken together, the above data provide a picture of ductal regulation, which, despite the uncommon features, presents a rich framework of possible interactions among many different agents. In that respect, the ductus may serve as a model for other blood vessels. At the same time, however, the study of the ductus affords a meeting point for several new lines of research in the area of vasoregulation. The proposed role for a cytochrome P450 in ductus closure fits well with, and provides support to, the growing evidence of the involvement of these hemoproteins in signalling processes in cells [6]. Conceivably, the ductus is not unique in depending on this mechanism for the expression of contractile tone. Gaseous agents, such as NO and possibly CO, form a new class of biological messengers whose action is intimately linked with hemodynamic control [5, 7, 8]. Equally important is the function being assigned to ET-1 [9], and findings in the ductus are expectedly applicable to other vessels.

With these considerations, the present commentary, dealing with the control of muscle tone in the ductus, appears both timely and of general significance. Its emphasis will be on the novel or controversial aspects of ductal regulation, and special attention will also be directed to the potential interrelationship between agents. Whenever appropriate, the clinical implications of experimental findings will be discussed. For the sake of clarity,

* Correspondence: Dr. Flavio Coceani, Research Institute, The Hospital for Sick Children, 555 University Ave., Toronto, Ontario, Canada M5G 1X8. Tel. (416) 813-5752; FAX (416) 813-5086.

† Abbreviations: $PGI_2$, prostaglandin $I_2$; $PGE_2$, prostaglandin $E_2$; ET-1, endothelin-1; $TXA_2$, thromboxane $A_2$; NO, nitric oxide; and CO, carbon monoxide.

contractile and relaxing mechanisms will be treated separately, though this distinction may appear arbitrary at times.

*Contractile mechanism*

Our observation in the early 1980s that CO potently relaxes the lamb ductus, even when it is constricted by oxygen, has led to a new phase of research on the mechanism of closure of this vessel at birth [10]. In a series of investigations [10–13], it was first demonstrated that CO action does not reflect either tissue damage or interference with the energy supply to the muscle. A cytochrome P450 was then identified as the target for CO and, significantly, this hemoprotein was found to be located in the plasma membrane of muscle cells. Moreover, it was shown that the ductal cytochrome likely belongs to the CYP3A (glucocorticoid-inducible) subfamily. At the same time, the possibility that CO, like NO, might relax the ductus by activating guanylyl cyclase was ruled out by examining the photoreversal spectrum for the relaxation over an appropriate wavelength range [11] and also by noting the presence of a full-fledged relaxation in the methylene blue-treated tissue*. The conclusion from these findings was that a special cytochrome serves as a signal transducer for oxygen in the formation of a constrictor, and that this constrictor functions, in turn, as the effector for ductus closure. The identity of this agent was not evident from the data, though the scheme being proposed pointed to the involvement of the product of a monooxygenase reaction. Our proposal envisaged two new features for cytochrome P450 hemoproteins: an unusual subcellular location and an unconventional function. Nevertheless, the experimental evidence was convincing enough to justify further work.

In the attempt to identify the putative ductus constrictor, our attention was directed first to products of arachidonate monooxygenase. This was a logical step in view of the role played by another eicosanoid, $PGE_2$, in keeping the vessel patent. Indeed, it was quite appealing to think that a single precursor, arachidonic acid, would be converted to products with opposing actions ($PGE_2$: relaxation; epoxide: contraction) and that the relative importance of the two metabolic pathways would change with the degree of oxygenation [10]. Regrettably, as often happens with "appealing" ideas, this scheme did not meet with experimental verification [11]. In a separate investigation, however, we found that ET-1 is an exceedingly, and uniquely, potent constrictor of the ductus [2] and, even though the functional linkage of this peptide with a cytochrome P450-based mechanism seemed improbable, we decided to pursue this lead further. Eventually, as expected from the natural mediator of oxygen, it was demonstrated the ET-1 action is exerted on a population of receptors belonging to the $ET_A$ subtype [9] and that the ductus is endowed with a competent system for the synthesis of ET-1 [14]. In fact, in a departure from the accepted view at the time, it was found that in the ductus ET-1 can be formed outside the endothelium in the muscle layer itself. Hence, the site of ET-1 formation agreed with the notion that ductal muscle *per se* can contract to oxygen [15]. In the end, a causal relationship could be established between the earlier data implicating a cytochrome P450 mechanism in ductus closure and the more recent data pointing to an effector role for ET-1. This was achieved by showing in the isolated ductus that ET-1 reverses the CO relaxation [2], that oxygen and CO have opposing effects on ET-1 synthesis (oxygen: activation; CO: inhibition) [14, †] and that inhibitors of ET-1 synthesis and action curtail both the oxygen contraction [9] and the reversal of the CO relaxation brought about by light at 450 nm*. Potentially germane to our findings was also the observation that glucocorticoids may promote ET-1 synthesis in vascular muscle cells and not in endothelial cells [16].

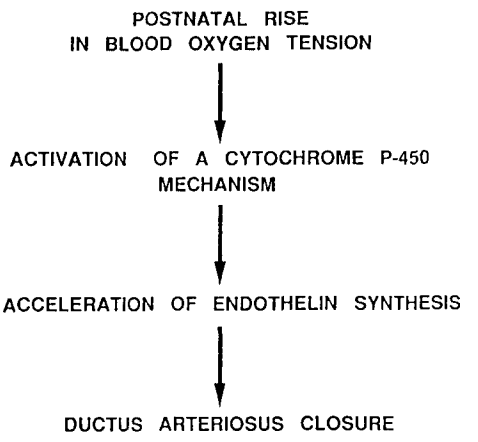


Fig. 1. Schematic diagram of the proposed events in the closing ductus at birth. The cytochrome P450 is thought to condition ET-1 synthesis through a monooxygenase reaction in which substrate and end product are still unknown. Note that cytochrome P450 hemoprotein and ET-1 synthetic system are both confined to muscle cells.

A key question still outstanding in this proposed scheme concerns the mechanism by which cytochrome P450 may control ET-1 synthesis. Theoretically, the hemoprotein could perform this function through a conformational change induced by oxygen or by acting as the catalytic element in a monooxygenase reaction. In actuality, however, the finding that oxygen and CO have opposite effects on ductal tone [10, 11], coupled with the recent demonstration that 1-aminobenzotriazole, a suicide substrate for cytochrome P450-based reactions, inhibits the oxygen contraction but not the contraction to excess potassium,‡ favours the second alternative.

In conclusion, from the convergence of two outwardly distant lines of research a scheme has emerged on the events occurring in the closing ductus at birth. According to this scheme (Fig. 1), closure takes place in a sequence of steps including, in the following order, activation by oxygen, and

* Coceani F, unpublished data.

† Coceani F, Kelsey L and Seidlitz E, unpublished data.

‡ Coceani F, Korzekwa K, Kelsey L and Seidlitz E, unpublished data.

perhaps some appropriate inducer as well (e.g. cortisol, see Ref. 13), of a membrane-bound cytochrome P450 belonging to the 3A subfamily; increased formation of ET-1; and action of the newly formed ET-1 on the muscle. Normally, ET-1 is potent enough to overcome the influence of $PGE_2$ and any other relaxing agent (see below). The actual stimulus for ET-1 synthesis is thought to be provided by a, hitherto uncharacterized, monooxygenase product. In this connection, it is significant that the ductus may also close, albeit slowly, in cyanotic infants with obstructive right heart malformations, that is under a condition where, owing to stress, blood levels of cortisol can be elevated markedly [17, 18]. Conceivably, by acting on the special cytochrome P450 in the ductus, cortisol may compensate for the lack of oxygen stimulation in these sick newborns.

*Relaxing mechanism*

For several years, $PGE_2$ has been viewed as the only agent responsible for prenatal patency of the ductus, and this concept is well founded on experimental and clinical data [1]. Nevertheless, recent developments, specifically the realization of the importance of such gaseous agents as vasoregulators, have provided a new perspective to this problem. We have shown that the ductus, like any other vessel, may generate NO at rest and in response to an appropriate stimulus (e.g. bradykinin) [4]. Furthermore, as expected from the constitutively expressed synthase, NO is formed in the endothelium from where it diffuses to muscle cells and causes relaxation by activating guanylyl cyclase. Compared with $PGE_2$, however, the relaxing action of NO appears weak and of lesser importance in maintaining patency of the vessel. Still, such arrangement could change under certain conditions. Prenatally, NO could become more important as a compensatory factor under the influence of drugs interfering with the formation of $PGE_2$. Relevant to this possibility is an observation in the neonatal pulmonary circulation where, during an extended treatment with indomethacin, blood flow is first reduced and then restored to normal, thanks to a rebound action of NO [19, 20]. Postnatally, the NO mechanism could acquire greater prominence in instances of persistent ductus patency complicating prematurity. In that case, one may envisage the NO system becoming more effective due to a combination of events, including possibly the contribution of the inducibly expressed synthase. The postnatal rise in blood oxygen tension, though inadequate for the full activation of the cytochrome P450/ET-1-based mechanism responsible for ductus closure (see above), may accelerate the synthesis of NO [21]. In addition, the reversal in the direction of blood flow across the patent ductus, with the inevitable shear stress being imposed on the endothelium, may also stimulate the synthetic process [22]. Lastly, and perhaps most importantly, mechanical injury to the vessel wall resulting from transient closure, whether spontaneous or induced therapeutically, may stimulate the inducible NO synthase directly and through the action of cytokines being released from infiltrating blood cells [7, 23]. All these events, singly or in combination, could shift the control of ductus patency towards the NO mechanism.

While there is reasonable evidence to implicate NO in ductus patency, the case for a role of CO remains speculative. However, as suggested by another group [5], the potent relaxant effect of this agent on the ductus (see above), which in our original experiments was deemed pharmacological, may actually reflect a natural phenomenon. Consistent with this possibility is the occurrence of the constitutive isoform of the synthetic enzyme, heme oxygenase-2, in vascular smooth muscle and the growing evidence of CO being another biological messenger [8, 24, 25]. If this is confirmed, CO and NO could complement each other in supplementing the ductus relaxant action of $PGE_2$ thanks to their differences in physicochemical properties (CO: stable; NO: labile), site of formation (CO: muscle; NO: endothelium), and mode of action (CO: primarily inhibition of the cytochrome P450-based mechanism; NO: primarily activation of the guanylyl cyclase-based mechanism). Furthermore, their importance relative to $PGE_2$ could increase in parallel, because synthetic enzymes are amenable to induction by similar factors (e.g. pyrogens) [7, 8]. In fact, considering the characteristics of the two agents, their action could become quite complex and could extend beyond the primary targets mentioned earlier. As NO may also interfere with the cytochrome P450/ET-1 complex, CO may provide a weak activation of guanylyl cyclase [26]. Both NO and CO, on the other hand, may down-regulate NO formation by combining with the cytochrome P450 of the NO synthase [27, 28]. Continuing in this speculation, it is even not too far-fetched to think that cytochrome P450 hemoproteins, whether involved in ET-1 or NO synthesis, may also serve as a substrate for heme oxygenase(s) in the generation of CO [8].

In summary, gaseous agents could act in concert with $PGE_2$ to maintain ductus patency and, at the same time, could afford a broader regulation of muscle tone. These possibilities require experimental verification. If confirmed, the existence of several effectors for patency may, on one hand, be beneficial in providing protection *in utero* from any adverse effect of drugs given to the mother (e.g. nonsteroidal anti-inflammatory drugs) and, on the other hand, may complicate the clinical course of a persistent ductus in the prematurely born infant and may, in fact, explain failures of the indomethacin therapy. In this connection, it is significant that prematures, who have been exposed *in utero* to indomethacin for a threatened labour, present a higher incidence of patent ductus [29].

*Conclusion*

Evidence has been obtained implicating several different agents in the control of muscle tone in the ductus arteriosus. Whereas prenatal patency is thought to be sustained by $PGE_2$ and NO, and possibly CO too, postnatal closure is linked to the combined operation of a cytochrome P450 and ET-1. The action of these agents is conceivably complex, and their interaction is only partially understood. The importance of these studies transcends the

ductus, and findings may be applicable to other blood vessels that cease to exist at birth (ductus venosus, umbilical vessels) [30, 31] as well as to certain vessels of the adult. These data, for example, may bear relevance to the constrictor response of systemic arterioles to oxygen [32]. In this respect, the ductus arteriosus stands out as a useful experimental model for studying mechanisms that are currently at the forefront of research in the area of vasoregulation.

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