α-Toxin-permeabilised rabbit fetal ductus arteriosus is more sensitive to 
Ca²⁺ than aorta or main pulmonary artery

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Abstract

Objectives: The Ca²⁺ sensitivity of contractile protein-generated tension production was measured in the smooth muscle of the rabbit ductus arteriosus and compared with two neighbouring fetal blood vessels (main pulmonary artery and aorta). The effect of prostaglandin E₂ (PGE₂), 3-isobutyl-1-methylxanthine (IBMX, a phosphodiesterase inhibitor), cyclic adenosine 3',5'-monophosphate (CAMP) and forskolin (an activator of adenylate cyclase) on Ca²⁺-activated force generated by preparations from ductus arteriosus was also examined.

Methods: Strips of smooth muscle from the three vessels were permeabilised using crude α-toxin from Staphylococcus aureus. The relationship between Ca²⁺ and force production was then measured in the three tissues and the effect of PGE₂, cAMP, IBMX and forskolin was examined on submaximal Ca²⁺-activated force (0.3 μM Ca²⁺) in preparations from rabbit ductus arteriosus. Results: Permeabilised smooth muscle from fetal rabbit ductus arteriosus was significantly more sensitive to Ca²⁺ (EC₅₀, 0.20 μM) than its two neighbouring blood vessels aorta (EC₅₀, 0.52 μM) and main pulmonary artery (EC₅₀, 0.72 μM). Submaximal Ca²⁺-activated force (0.3 μM Ca²⁺) was depressed by PGE₂ (1 nM) in the presence of IBMX (10 μM), by cAMP (10 and 100 μM) and by forskolin alone (0.1 μM and 1 μM). Conclusion: PGE₂-mediated depression of Ca²⁺-activated force in the smooth muscle of the ductus arteriosus may play a role in the maintenance of a patent ductus arteriosus in the fetus. The intrinsically high Ca²⁺ sensitivity of smooth muscle contractile proteins may aid the sustained vasoconstriction of the ductus when the PGE₂ levels fall after birth.

Keywords: α-Toxin; Calcium sensitivity; Ductus arteriosus; Prostaglandin; cAMP; Rabbit, vascular myocytes; Rabbit arteries

1. Introduction

The ductus arteriosus is a fetal shunt blood vessel which extends between the main pulmonary artery and the aorta [1,2]. In fetal life, it diverts deoxygenated blood away from the pulmonary circulation to the descending aorta and ultimately to the placenta. At delivery of the fetus, the smooth muscle in the wall of the ductus arteriosus contracts, closing the vessel [2]. Later the lumen closes permanently by necrosis of the vessel wall and the eventual formation of the ligamentum arteriosus.

Prostaglandins, principally prostaglandin E₂ (PGE₂), are implicated in maintaining patency of the ductus arteriosus in the fetus and are produced within the smooth muscle of this vessel [3,4]. Circulating concentrations of PGE₂ (1–2 nM) [5] are in the range which dilate the ductus and are thought to maintain the ductus in the patent state in utero. Circulating concentrations of PGE₂ fall to 10% of fetal levels within hours of birth [5] and increasing oxygen tension increases the sensitivity of the ductus to the dilator effect of PGE₂ [6–8]. Prostaglandins E₁ and E₂ are administered to the neonate in conditions where it is desirable to maintain ductus patency and indomethacin, a cyclooxygenase inhibitor which blocks synthesis of prostaglandins, is the main medical therapy for patent ductus arteriosus in the neonate [9]. It is thought that PGE₂ dilates the ductus through activation of adenylate cyclase, as PGE₂ increases intracellular concentrations of cyclic

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adensine 3',5'-monophosphate (cAMP [10]). Furthermore, the receptor mediating the effects of PGE₂ on the ductus has been identified as the EP₄ sub-type [11]. The receptor has been cloned and when transfected into cultured cells is coupled to adenylyl cyclase [12–14].

Closure of the ductus is in part due to elimination of the effects of PGE₂. However, it is the immediate rise of PO₂ at birth that is thought to mediate principally the ductus closure. One possible mechanism is the inactivation of K⁺ATP channels and subsequent depolarisation of the smooth muscle cells [15,16].

The aim of this study was to examine the Ca²⁺ sensitivity of tension production by the contractile proteins of the smooth muscle from rabbit ductus arteriosus and to compare this with the Ca²⁺ sensitivity of the smooth muscle from two adjacent blood vessels: aorta and main pulmonary artery. Permeabilisation of the smooth muscle by α-toxin from Staphylococcus aureus renders the surface membrane permeable to molecules below a molecular weight of 1000 Da while retaining functional membrane bound receptors and their associated G-proteins and enzyme systems [17–19]. This technique allows examination of the intracellular effects of receptor activation. This study has also examined the effect of PGE₂, cAMP and forskolin on submaximal Ca²⁺-activated force.

2. Methods

2.1. Tissue

At day 28 of their 31-day gestation, pregnant New Zealand White rabbits were killed with an intravenous dose of sodium pentobarbital (Euthatal) followed by exsanguination. The fetuses were rapidly removed by Caesarean section and decapitated before onset of respiration, and the ductus arteriosus, aorta and the main pulmonary artery were quickly dissected. Small strips of circular muscle were dissected from the three vessels (approximately 100 µm in diameter and 2–3 mm in length) and attached to a force transducer (Akers, AE17625; SensoNor a.s., Norway) and a fixed point with snares in a small tissue bath (0.96 ml). Whilst superfused with Tyrodes’ solution (Solution C; Table 1), a small amount of resting tension (0.15 mN) was applied. All diagrams show active isometric tension generated by the preparation. Isolation of the smooth muscle layer from the wall of the vessel allows the smooth muscle responses to be studied in the absence of endothelium-derived factors. The investigation was performed in accordance with the Home Office Guidance on the operation of the Animals (Scientific Procedures) Act 1986, published by HMSO, London.

2.2. α-Toxin permeabilisation procedure

The smooth muscle preparation was permeabilised by superfusing with a mock intracellular solution (Solution A, 37°C). The operation of the Animals (Scientific Procedures) Act formed in accordance with the Home Office. Solutions contained adenosine triphosphate (ATP) and phosphocreatine (PCr) to support contraction of the permeabilised muscle. A range of [Ca²⁺] was obtained from two adjacent blood vessels: aorta and main pulmonary artery. Permeabilisation of the smooth muscle by α-toxin from Staphylococcus aureus renders the surface membrane permeable to molecules below a molecular weight of 1000 Da while retaining functional membrane bound receptors and their associated G-proteins and enzyme systems [17–19]. This technique allows examination of the intracellular effects of receptor activation. This study has also examined the effect of PGE₂, cAMP and forskolin on submaximal Ca²⁺-activated force.

Table 1: Solution composition (in mmol.L⁻¹ except where stated)

<table>
<thead>
<tr>
<th>Solution</th>
<th>A</th>
<th>B</th>
<th>C</th>
</tr>
</thead>
<tbody>
<tr>
<td>K⁺</td>
<td>120</td>
<td>120</td>
<td>5</td>
</tr>
<tr>
<td>Na⁺</td>
<td>40</td>
<td>40</td>
<td>120</td>
</tr>
<tr>
<td>Total Mg²⁺</td>
<td>7.0</td>
<td>7.0</td>
<td>1.0</td>
</tr>
<tr>
<td>Total Ca²⁺</td>
<td>10.06</td>
<td>0.02</td>
<td>2.0</td>
</tr>
<tr>
<td>Free Ca²⁺ (µM)</td>
<td>100</td>
<td>5 × 10⁻⁶</td>
<td>—</td>
</tr>
<tr>
<td>EGTA</td>
<td>10</td>
<td>10</td>
<td>—</td>
</tr>
<tr>
<td>CH₃(SO₃)</td>
<td>100</td>
<td>100</td>
<td>—</td>
</tr>
<tr>
<td>CI⁻</td>
<td>14</td>
<td>14</td>
<td>131</td>
</tr>
<tr>
<td>ATP</td>
<td>5</td>
<td>5</td>
<td>—</td>
</tr>
<tr>
<td>Gp</td>
<td>15</td>
<td>15</td>
<td>—</td>
</tr>
<tr>
<td>HEPES</td>
<td>25</td>
<td>25</td>
<td>5</td>
</tr>
<tr>
<td>P₁</td>
<td>1</td>
<td>1</td>
<td>—</td>
</tr>
</tbody>
</table>

The pH of Solutions A and B was 7.2; free Mg²⁺ was 2.3 mM; Solution C had a pH of 7.4. Experiments were carried out at room temperature (20–22°C). P₁ = inorganic phosphate.

Table 1: [Ca²⁺] 100 µM containing crude α-toxin from Staphylococcus aureus (final concentration 2 mg/ml) [19]. Tension rose slowly over a 10–15 min period as the Ca²⁺ gained access to the myofilaments. When tension had plateaued, the α-toxin was removed and the [Ca²⁺] lowered to 1 nM (Solution B, Table 1). Lowering the [Ca²⁺] caused the muscle to relax. Experiments were carried out at room temperature to limit deterioration of maximum Ca²⁺-activated force and at ambient oxygen tension.

2.3. Solution composition

Solution composition is given in Table 1. The Ca²⁺ buffer ethylene glycol-bis(β-aminoethyl ether)-N,N,N',N'-tetraacetic acid (EGTA) was used to control [Ca²⁺]. The solutions contained adenosine triphosphate (ATP) and phosphocreatine (PCr) to support contraction of the permeabilised muscle. A range of [Ca²⁺] was obtained by mixing Solutions A and B in differing proportions. The equilibrium concentrations of metal ions were calculated using a computer programme with affinity constants for H⁺, Ca²⁺, and Mg²⁺ binding to EGTA taken from Smith and Miller [20]. The affinity constants used for ATP and PCr are those quoted by Fabiato and Fabiato [21]. Corrections for ionic strength, EGTA purity and the principles of the calculation are detailed elsewhere [20]. All chemicals were purchased from Sigma, UK, except α-toxin (Glascow University) and PGE₂ (Upjohn, Kalamazoo, MI, USA). The stock solution of PGE₂ [(5Z,11º,13º,15º)-11,15-di-hydroxy-9-oxoprosta-5,13-dien-1-oic acid] was dissolved in ethanol. All experiments were performed in the presence of 1 µM indomethacin to inhibit intrinsic prostaglandin production by the smooth muscle.

2.4. Statistics

Experiments were carried out on tissue from at least 4 separate animals unless otherwise stated. The fitted curves drawn through the data in Figs. 2 and 3 were the best fit
curves using the Hill equation (see legend, Fig. 2) using the computer programme Fig-P (Biosoft, UK). The values for the calculated variables within the Hill equation (\(T_{\max}, K_m\) and \(N\)) are quoted with the 95% confidence interval (CI) in the legends of Figs. 2 and 3. Statistically significant differences between the curves in Figs. 2 and 3 were calculated by comparing the 95% confidence limits on the variables. All \(EC_{50}\) values quoted are the reciprocal of the calculated \(K_m\) in the Hill equation. Statistically significant differences in the data shown in Figs. 4 and 5 were tested using the appropriate Student \(t\)-test and the data are expressed as mean values ± standard error of the mean (s.e.m.).

3. Results

3.1. Comparison of \(Ca^{2+}\) sensitivity of force production in \(\alpha\)-toxin-permeabilised ductus arteriosus, aorta and main pulmonary artery

The \(Ca^{2+}\) sensitivity of force production was measured by cumulatively raising the [\(Ca^{2+}\)] from 1 nM to 100 \(\mu\)M for 10 min at each [\(Ca^{2+}\)] in \(\alpha\)-toxin-permeabilised smooth muscle from ductus arteriosus, aorta and main pulmonary artery. Typical examples of normalised \(Ca^{2+}\)-activated force are shown for each of the tissues in Fig. 1. Preparations derived from ductus arteriosus achieved on average a maximum force of 0.4 ± 0.05 mN, \(n = 25\); aorta, 0.1 ± 0.02 mN, \(n = 4\); main pulmonary artery, 0.3 ± 0.02 mN, \(n = 4\) (mean ± s.e.m.). The threshold [\(Ca^{2+}\)] for activated force production was 0.1 \(\mu\)M in the three permeabilised muscles. However, maximum \(Ca^{2+}\)-activated force was achieved at 10 \(\mu\)M for smooth muscle from ductus arteriosus, but not until 100 \(\mu\)M in aorta or pulmonary artery. Fig. 1 suggests that the rate of development of tension development is more rapid in the preparations from ductus arteriosus than from aorta and main pulmonary artery; however, this was not a consistent observation and there appeared to be no significant difference in the rate of rise of tension at any of the [\(Ca^{2+}\)]s used in the study.

The relationship between \(Ca^{2+}\) and tension for the three permeabilised smooth muscles is shown in more detail in Fig. 2. The curves drawn through the data (see legend, Fig. 2) are expressed as a percentage of the response at 100 \(\mu\)M \(Ca^{2+}\) than either aorta (RA) or main pulmonary artery (RPA) [RDA, \(EC_{50}\) 2.00 ± 0.18 \(\times\) \(10^{-7}\) M; RA, \(EC_{50}\) 5.18 ± 1.38 \(\times\) \(10^{-7}\) M; RMPA, \(EC_{50}\) 7.19 ± 1.54 \(\times\) \(10^{-7}\) M (mean ± 95% CI, \(n = 4\)), \(P < 0.05\)], whereas there is no significant difference between the curves drawn through the data for aorta and main pulmonary artery [RA, \(EC_{50}\) 5.18 ± 1.38 \(\times\) \(10^{-7}\) M; RMPA, \(EC_{50}\) 7.19 ± 1.54 \(\times\) \(10^{-7}\) M (mean ± 95% CI, \(n = 4\)), \(P > 0.05\)].

3.2. The effect of 1 \(\mu\)M indomethacin on \(Ca^{2+}\) sensitivity of permeabilised ductus arteriosus

Fig. 3 shows the relationship between \(Ca^{2+}\) and tension production by the contractile proteins in \(\alpha\)-toxin-permeabilised ductus arteriosus in the presence and absence of 1 \(\mu\)M indomethacin. Indomethacin was present in all solutions to inhibit intrinsic PGE2 production by the smooth muscle [6,2]. The results suggest that indomethacin (1 \(\mu\)M) caused a small increase in the \(Ca^{2+}\) sensitivity of the contractile proteins of \(\alpha\)-toxin-permeabilised ductus arteriosus, however, the effect was not statistically significant [1 \(\mu\)M indomethacin, \(EC_{50}\) 2.00 ± 0.18 \(\times\) \(10^{-7}\) M; 0 indomethacin, \(EC_{50}\) 2.48 ± 0.44 \(\times\) \(10^{-7}\) M (mean ± 95% CI, \(n = 4\)), \(P > 0.05\)].

3.3. The effect of PGE2 on submaximal \(Ca^{2+}\)-activated force in permeabilised ductus arteriosus

Increasing [\(Ca^{2+}\)] from 1 nM to 0.3 \(\mu\)M produced a sustained increase in force on average 67 ± 4% (mean ± s.e.m., \(n = 4\)) of maximum \(Ca^{2+}\)-activated force. Fig. 4 shows the effect of 1 nM PGE2 on submaximal \(Ca^{2+}\)-activated force (0.3 \(\mu\)M). PGE2 was added to the bathing
Fig. 2. Cumulated data examining the relationship between Ca\(^{2+}\) and tension production by the contractile proteins in α-toxin-permeabilised rabbit ductus arteriosus, rabbit aorta, and rabbit main pulmonary artery. Data plotted are the steady-state tensions (mean ± s.e.m.) from 4 experiments from different blood vessels. Continuous line represents the best-fit curve of the following form of the Hill equation:

\[
\frac{T}{T_{\text{max}}} = \frac{[\text{Ca}^{2+}]}{K_m + [\text{Ca}^{2+}]}^N,
\]

where \(T/T_{\text{max}}\) is a fraction of maximal Ca\(^{2+}\)-activated force \((T_{\text{max}})\) and \(K_m\) is the apparent affinity constant of the myofilaments for Ca\(^{2+}\).

<table>
<thead>
<tr>
<th>Tissue</th>
<th>(T_{\text{max}}) \text{ (x10}(^6) M)</th>
<th>(K_m) \text{ (x10}(^{-7}) M)</th>
<th>(E_{50}) \text{ (x10}(^{-7}) M)</th>
<th>(N)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ductus arteriosus</td>
<td>0.99 ± 0.03</td>
<td>5.01 ± 0.46</td>
<td>2.00 ± 0.18</td>
<td>1.81 ± 0.25</td>
</tr>
<tr>
<td>Aorta</td>
<td>0.97 ± 0.07</td>
<td>1.93 ± 0.53</td>
<td>5.18 ± 1.38</td>
<td>1.27 ± 0.39</td>
</tr>
<tr>
<td>Pulmonary artery</td>
<td>0.97 ± 0.05</td>
<td>1.39 ± 0.25</td>
<td>7.19 ± 1.54</td>
<td>1.44 ± 0.34</td>
</tr>
</tbody>
</table>

Values quoted are the means ± 95% CI.

Fig. 3. The relationship between Ca\(^{2+}\) and tension in the presence and absence of 1 µM indomethacin in α-toxin-permeabilised rabbit ductus arteriosus. Data plotted are the steady-state tensions (mean ± s.e.m.) from 4 experiments from different blood vessels. Continuous line represents the best fit curve of the Hill equation given in Fig. 2.

<table>
<thead>
<tr>
<th>Tissue</th>
<th>(T_{\text{max}}) \text{ (x10}(^6) M)</th>
<th>(K_m) \text{ (x10}(^{-7}) M)</th>
<th>(E_{50}) \text{ (x10}(^{-7}) M)</th>
<th>(N)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Indomethacin 1 µM</td>
<td>0.99 ± 0.03</td>
<td>5.01 ± 0.46</td>
<td>2.00 ± 0.18</td>
<td>1.81 ± 0.25</td>
</tr>
<tr>
<td>Indomethacin 0</td>
<td>0.98 ± 0.05</td>
<td>4.03 ± 0.69</td>
<td>2.48 ± 0.44</td>
<td>1.54 ± 0.35</td>
</tr>
</tbody>
</table>

Values quoted are the means ± 95% CI.

Fig. 4. The effect of IBMX (1 and 10 µM) and PGE\(_2\) (1 nM) on submaximal Ca\(^{2+}\)-activated force (0.3 µM Ca\(^{2+}\)) in α-toxin-permeabilised rabbit ductus arteriosus. IBMX and PGE\(_2\) were added to the bathing medium as indicated above the trace. Bathing [Ca\(^{2+}\)] was changed as indicated below the tension trace.

Fig. 5. Mean effect of IBMX (10 µM) and PGE\(_2\) (1 nM) on submaximal Ca\(^{2+}\)-activated force (0.3 µM Ca\(^{2+}\)) in α-toxin-permeabilised rabbit ductus arteriosus. Tension has been expressed as a percentage of the force achieved at 0.3 µM Ca\(^{2+}\) alone. In combination, IBMX and PGE\(_2\) depressed submaximal Ca\(^{2+}\)-activated force to 60 ± 1% (mean ± s.e.m., \(n = 4, P < 0.01\)).
4. Discussion

The results presented in this paper show that tension production by contractile proteins of α-toxin-permeabilised smooth muscle from rabbit ductus arteriosus has a higher Ca\(^{2+}\) sensitivity than that observed in smooth muscle from its two neighbouring vessels, namely the aorta and main pulmonary artery. We have also shown that PGE\(_2\) (1 nM) depresses submaximal Ca\(^{2+}\)-activated force and further results suggest that PGE\(_2\) acts via the stimulation of adenylate cyclase and the production of cAMP.

### 4.1. Comparing the Ca\(^{2+}\) sensitivity of rabbit ductus arteriosus, aorta and main pulmonary artery

One other study has examined the Ca\(^{2+}\) sensitivity of the contractile proteins of ductus arteriosus smooth muscle [24] isolated from fetal lambs. The Ca\(^{2+}\) sensitivity reported was significantly lower than that observed in this study. The reason for this disparity is unknown—certainly comparing results across species may not be valid, or the difference may arise from the use of saponin to permeabilise the smooth muscle cells [24] rather than α-toxin from Staphylococcus aureus (present study). It is known that essential contractile proteins are lost from saponin-permeabilised smooth muscle [25,26], yet the selective permeabilisation produced by α-toxin will prevent the loss of cytosolic proteins.

Tension production by the smooth muscle from rabbit ductus arteriosus was significantly more sensitive to Ca\(^{2+}\) than the muscle from two neighbouring vessels (i.e., aorta and main pulmonary artery). Indeed, of the smooth muscles investigated in this laboratory rabbit ductus arteriosus is the most sensitive to Ca\(^{2+}\). The [Ca\(^{2+}\)] required to achieve half the maximal Ca\(^{2+}\)-activated force in a variety of smooth muscle preparations is as follows (µM): 0.20, rabbit ductus arteriosus; 0.25, rat myometrium; 0.33, rat portal vein; 0.37, rat anococcygeus; 0.43, human umbilical artery; 0.52 rabbit aorta (this study); 0.72 rabbit main pulmonary artery (this study); and 1.04, rabbit portal vein. One point to note is that the experiments described in this study were carried out at ambient oxygen tension, but that much lower oxygen tensions are normally experienced by the ductus arteriosus in utero [1,2]. It is conceivable that oxygen tensions may affect the Ca\(^{2+}\) sensitivity of the contractile proteins and further investigations are required to examine this point.

### 4.2. Effect of IBMX, PGE\(_2\), cAMP and forskolin on submaximal Ca\(^{2+}\)-activated force

Previous studies have shown that the intact ductus arteriosus relaxes in response to PGE\(_2\) when precontracted by oxygen or an agonist such as noradrenaline [7,27]. These studies also demonstrated that indomethacin, a cyclo-oxygenase inhibitor, will contract the isolated ductus arteriosus, suggesting that the intact vessel synthesises and releases prostaglandins that act on the smooth muscle to produce a chronic vasodilation. In this study indomethacin
had no effect on the developed tension of permeabilised smooth muscle from rabbit ductus arteriosus, indicating that there was no significant endogenous prostaglandin production by the smooth muscle under these experimental conditions.

Reports in the literature suggest that cAMP mediates the intracellular effects of PGE$_2$ [10,28]. Increased intracellular [cAMP] is known to lower intracellular [Ca$^{2+}$] (via the stimulation of the sarcoplasmic reticulum Ca$^{2+}$ pump) and reduce the responsiveness of the contractile proteins to Ca$^{2+}$ and in these two ways reduce tone in a range of smooth muscle types [29]; however, work on the smooth muscle from the ductus arteriosus is rare.

This study reports that addition of cAMP to permeabilised smooth muscle from rabbit ductus arteriosus markedly reduces Ca$^{2+}$-activated force. As with previous studies [22,23], the effective concentrations of cAMP were much higher than the expected intracellular levels due to the activity of phosphodiesterase within the preparation; much higher sensitivities are observed in the presence of phosphodiesterase inhibitors [30]. Further evidence for the role of cAMP in this tissue is the observation that forskolin, a specific stimulator of the enzyme adenylate cyclase, markedly reduces Ca$^{2+}$-activated force in permeabilised smooth muscle from ductus arteriosus. The concentrations of forskolin needed to produce effects in the permeabilised preparation are greater than in the intact muscle [27]. Possible explanations for this are: (i) diffusion of endogenously produced cAMP out of a permeabilised preparation may prevent the development of high localised concentrations; (ii) the further relaxation caused by the cAMP-mediated decrease of intracellular [Ca$^{2+}$] in intact cells will not apply in permeabilised preparations since the [Ca$^{2+}$] next to the contractile proteins is fixed by the external bathing solution.

Ca$^{2+}$-activated force was depressed in the permeabilised smooth muscle from the ductus arteriosus by PGE$_2$, but only in the presence of a phosphodiesterase inhibitor (IBMX). These results suggest that cAMP mediates the effects of PGE$_2$ on the contractile proteins. Comparable levels of PGE$_2$ markedly decrease tone in intact ductus arteriosus without the addition of IBMX [7]. This disparity between intact and permeabilised preparations may reflect the absence of a barrier to the loss of cAMP generated by the stimulation of adenylate cyclase from permeabilised preparations.

In summary, the experimental work presented in this paper suggests that circulating PGE$_2$ can maintain ductus arteriosus patency in the fetus in part by depressing the Ca$^{2+}$ responsiveness of contractile proteins of the smooth muscle within the wall of the ductus. This effect appears to be mediated via cAMP. The higher Ca$^{2+}$ sensitivity of the contractile proteins of the ductus arteriosus over the neighbouring vessels may be beneficial to the function of the vessel in utero. Normal cytosolic [Ca$^{2+}$] (100–200 nM) within the smooth muscle of the ductus arteriosus will generate a greater tone than in other vascular smooth muscle; thus the properties of the contractile proteins may contribute to the closure of the ductus when the PGE$_2$ levels fall after birth.

Acknowledgements

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References


