

# Electrical Properties of Pulmonary Lymphatic Smooth Muscle Cells

**Mustafa Ahmadzai**

Honours Biology & Pharmacology, Class of 2012

The Laboratory of Dr. Luke Janssen, McMaster University

*Mustafa Ahmadzai is a fourth-year student in the Honours Biology and Pharmacology (Co-op) Program. Mustafa spent this previous summer working at the Firestone Institute for Respiratory Health at St. Joseph's Hospital in downtown Hamilton. His research focused on the electrophysiological properties of lymphatic smooth muscle cells in the lungs and how they participate in the etiology of asthma and other related pulmonary diseases. The work involved using a technique known as voltage clamping, wherein electrical probes are used to stimulate isolated cells and measure the resulting currents. Like pieces to a puzzle, these electrical responses may give us an insight into how the tissue behaves under complex disease states like asthma and chronic obstructive pulmonary disorder (COPD).*

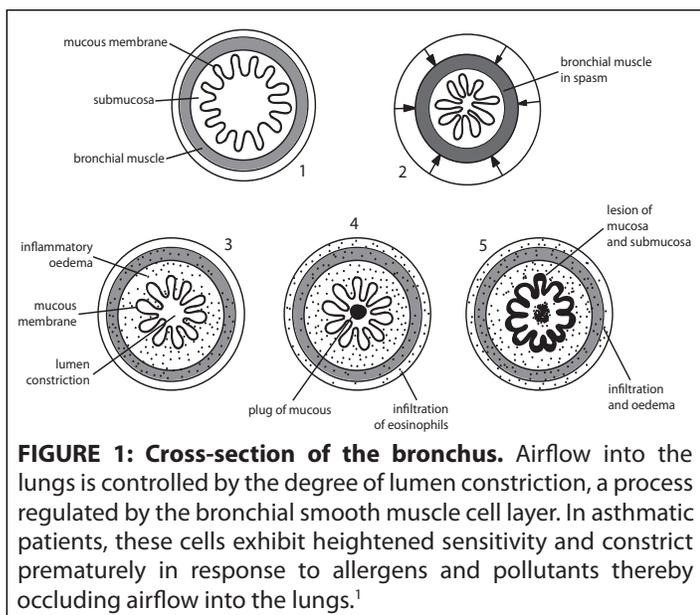
Pathology arises when a physiological function is compromised; treatment entails restoring this dysfunction to within homeostatic ranges. When concerned with illnesses like asthma or chronic obstructive pulmonary disorder (COPD), treatment depends exclusively on pharmacological interventions that target precise signalling pathways that underlie the outward symptoms associated with the disease. To understand the scope of these interactions, the researcher is confined to the level of the cell along with its many complexities. It is through these pursuits that the most meaningful and far-impacting results are obtained: data extracted at this microscopic level facilitate the production of life-saving drugs that attempt to target the right proteins and pathways, at the right time.

When we consider asthma, the difficulty in breathing arises from the hyperresponsiveness of the smooth muscle cells that circumscribe the upper airway tract (see Figure 1).<sup>1</sup> Ultimately, their constriction is mediated by calcium influx into the cytosol through selective ion channels embedded within the cell membrane. In light of this, it is important to note that an ion channel is a large, membrane-bound protein that facilitates the influx or efflux of ions. Since proteins are often specific in function, some are selective for only one type of ion. A channel that permits sodium influx is referred to as a sodium channel; one that is selective for potassium is a potassium channel, and so on. The main physiological ions of concern are sodium, potassium, calcium and chloride. As such, the overstimulation of smooth muscle cells, mediated by these integral channel proteins impedes

airflow into the pulmonary alveoli, where gas-exchange is known to occur.<sup>2,3</sup> With this serving as a starting point, two crucial questions then arise: which signalling molecules are of greatest relevance to the activation of this pathway and secondly, how are they important to the overall etiology of the disease?

## ISOPROSTANES AND THEIR NOVEL ROLE IN ASTHMA

It is well known that environmental allergens, pollutants and other irritants are strongly linked to the incidence of asthma.<sup>4,6</sup> Whether or not these factors are direct or indirect contributors to this process is critical to understanding asthma. Isoprostanes comprise one such family of molecules that are seen in situations of oxidative stress, which are characterized by the uncontrolled production of free radicals. Consequently, these compounds are now being implicated in airway hyperresponsiveness.<sup>7</sup> As structural isomers to the broad family of prostaglandin signalling molecules, their plasma levels have been classically interpreted as indicators of the extent of oxidative damage that has occurred in a tissue.<sup>8,9</sup> A key difference between the prostaglandins and isoprostanes, however, derives from the source of their synthesis. Prostaglandins are produced as downstream products of tissue damage in a reaction catalyzed by the enzyme phospholipase A2. Alternatively, isoprostanes are by-products of lipid peroxidation, a process mediated by free radicals (see Figure 2).<sup>9,10</sup>



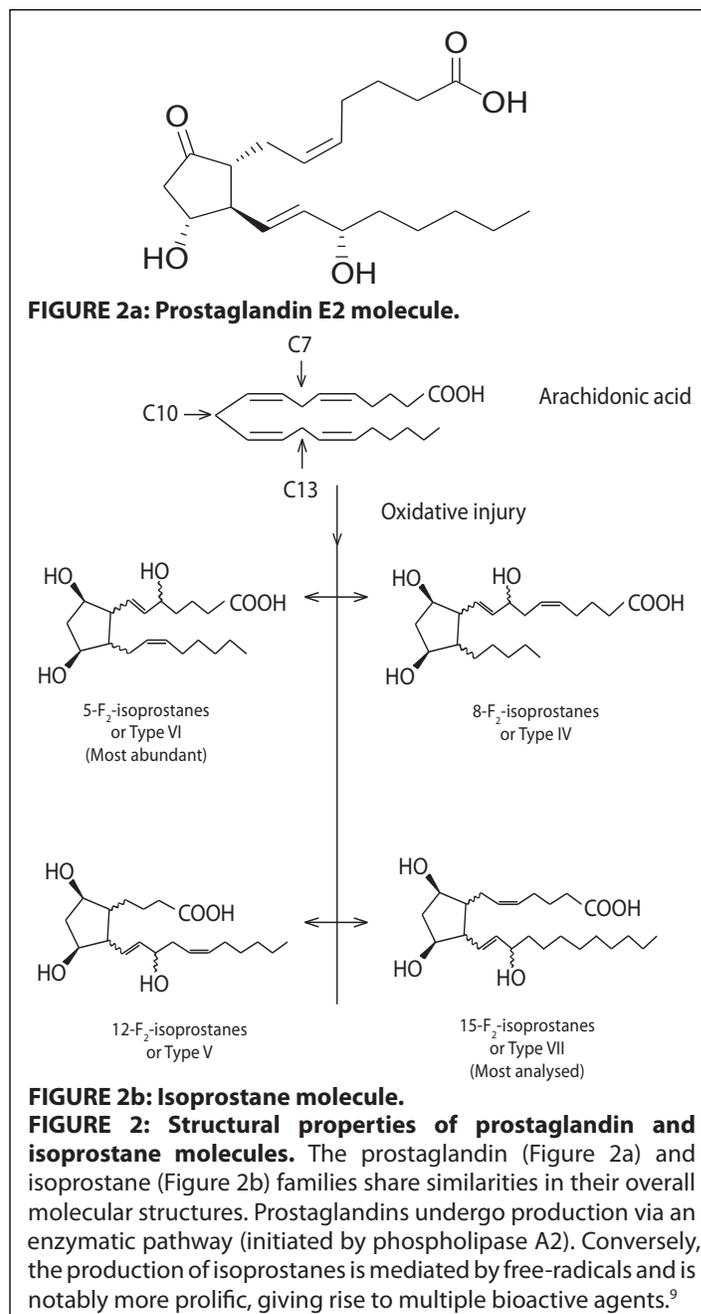
The newfound bioactivity of isoprostanes is believed to arise from their molecular similarities to prostaglandins. When applied to segments of the canine trachea, for instance, the agonistic properties of isoprostanes induce contractions of the smooth muscle layer, thereby mimicking the symptoms of an asthma attack.<sup>11</sup> In order to explain these results, it has been hypothesized that the prostaglandin receptor, which normally mediates this response, cannot detect the minute differences between the prostaglandin and isoprostane ligands.<sup>12</sup> An additional explanation put forth is the potential existence of an isoprostane-selective receptor that only responds to this ligand.<sup>12</sup> On a large scale, free-radical-mediated production of isoprostanes, like that seen from inhaling toxic pollutants, could exacerbate the symptoms of asthma and perhaps even trigger a full-scale asthma attack.<sup>11</sup> Conversely, pharmacological antagonism of these receptors would suppress this cycle and presents one possible direction for asthma drug development.

### SHIFTING THE FOCUS FOR GREATER INSIGHT

As we seek to develop new treatment options for asthma, our understanding remains incomplete without a thorough knowledge of how other tissue systems of the lungs interact with isoprostanes. How these adjoined tissue layers contribute to the onset of asthma may ultimately be far more profound than what was previously known, which is very little considering the lack of research within these areas. Pulmonary edema, which is characterized by a build-up of fluid in the lungs, is an often-cited symptom of numerous cardiothoracic pathologies in addition to asthma. In light of

this, it is necessary to investigate the impact of isoprostanes on the lymphatic system, which returns excess fluid to the heart for recirculation. The structure of the lymphatic vessel system is very diverse, region-specific and not as extensively studied to date.<sup>13</sup> As a result, this inherent heterogeneity provides strong rationale for investigating how isoprostanes and lymphatic smooth muscle interact and contribute to symptoms of asthma – if at all.

Like in blood vessels, the smooth muscle cells of lymphatic vessels encompass a layer of endothelial cells, which line the vessel wall (Figure 3). In veins and arteries, the communication between these two distinct layers gives rise to the body's blood pressure control mechanisms; this process is regulated by the balance between vasodilation (mediated by nitric

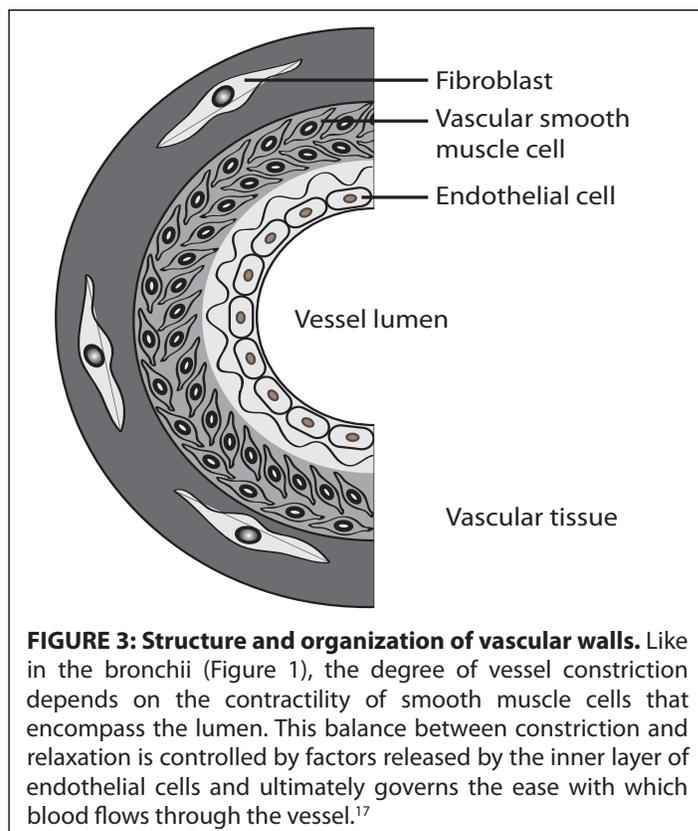


oxide) and vasoconstriction (mediated by endothelin). Consequently, if isoprostanes are found affecting lymphatic smooth muscle in the same manner that they affect tracheal smooth muscle, then this could represent a novel inlet by which to treat edema and related pulmonary disorders. Moreover, as seen in airway hyperresponsiveness, lymphatic smooth muscle contraction is calcium-mediated. As such, this contractile response can be readily manipulated and measured through various advanced *in vitro* experiments, like those outlined below.

### THE “CHARGE” BEHIND DRUG DEVELOPMENT

Electrophysiological experiments measure ionic fluxes through selective channels such as the calcium channels that trigger bronchoconstriction, which were discussed above.<sup>14</sup> Ultimately, the movement of an ion depends upon three basic factors. The first of these is the local electric field of the cell. The second factor is the chemical concentration gradient in which the ions are distributed across the membrane.<sup>14</sup> Overall, equilibrium is established between these two forces, thereby giving rise to the so-called electrochemical gradient. The final element that determines ionic movement is the gating of the ion channel; that is, whether or not the channel’s pore is open to permit the flux of ions.<sup>15</sup>

Basic physics dictates that charge separation, which in this case is the differential distribution of ions across the cell membrane, confers a voltage (also known as potential) upon the cell. When voltage changes occur, they serve as intrinsic signals that further propagate this initial depolarization: one channel’s opening leads to the opening of many others.<sup>14,15</sup> As this continues, opposing ionic fluxes serve to “reset” the cell to the initial voltage that prevailed before the whole process began.<sup>16</sup> The cycle repeats itself in this manner and is the basis of cell physiology. Where asthma is concerned, a novel frontier involves studying how, and if, isoprostanes trigger a “turning on” of the excitatory pathway – such that contraction of the lymphatic smooth muscle occurs – or whether it inherently triggers the “reset” mechanism. In this latter circumstance, cell contraction and the tissue’s propulsion of lymph would decrease drastically since the calcium influx would be inhibited. Due to the role of the lymphatic system in draining excess fluid back to the bloodstream, this inhibition would amount to a build-up of fluid, which would clinically manifest as edema. Whether the “on” or “off” signal is preferentially stimulated not only determines how isoprostanes manifest their influence on the lymphatic system in the thoracic region, but also whether



**FIGURE 3: Structure and organization of vascular walls.** Like in the bronchii (Figure 1), the degree of vessel constriction depends on the contractility of smooth muscle cells that encompass the lumen. This balance between constriction and relaxation is controlled by factors released by the inner layer of endothelial cells and ultimately governs the ease with which blood flows through the vessel.<sup>17</sup>

they contribute to the occurrence of edema, asthma and related lung pathologies.

Emerging experiments indicate that certain isoprostanes do in fact interact with lymphatic smooth muscle cells. The exact nature of this interaction, however, is unclear. Accordingly, much of this evidence has been derived using a technique known as patch clamping. This technique involves the use of microscopic electrodes that are carefully annealed to a segment (a “patch”) of the cell membrane, while a computer amplifier varies (by “clamping”) the voltage imposed upon the patch. As this occurs, the resulting electric current undergoes measurable fluctuations. The change in these fluctuations in the presence of a drug – in this case, isoprostanes – confirms a drug-cell interaction.

Gaining a more profound understanding of this interaction would potentially amount to the development of new drugs. Intense research, however, would have to be pursued within the industry before human trials can be conducted to these ends, a process that may take years. Since the data obtained from patch clamping studies are to some extent absolute, this approach affords the researcher a direct means by which to assess the effect of a compound on a cell. Such electrophysiological studies were pivotal in characterizing how airway smooth muscle and isoprostanes interacted. Whether or not this is the case with lymphatic smooth muscle cells, however, is only a matter of time. ■

## Reviewed by Dr. Luke Janssen, Ph.D.

Dr. Luke Janssen is a Professor of Medicine (Division of Respiriology) at McMaster University. He obtained his PhD in Physiology and Pharmacology from McMaster University. Currently, his research interests span various topics, including smooth muscle physiology, ion channels, and isoprotane biology.

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