cultured pulmonary artery smooth muscle cells (PASMC; Ref. 26). At first glance, this appears to scupper any idea of an EDCF-mediated increase in Rho kinase activation during sustained HPV. However, the increase in Rho kinase activity in cultured PASMC was a rather meager ~20% after 40 min of hypoxia, rising to a rather disappointing ~40% after 1 h. This is in stark contrast to the rather impressive ~260% increase in Rho kinase activity recently reported in isolated pulmonary arteries of the rat during sustained HPV (16). Moreover, this activation of Rho kinase in rat pulmonary arteries was endothelium dependent and in endothelium-denuded arteries the increase in Rho kinase activity was similar (~40%) to that reported for cultured PASMC. Furthermore, the translocation of RhoA to the plasma membrane of the pulmonary smooth muscle, a causal event in the activation of Rho kinase, was similarly dependent on the presence on an intact endothelium.

In closing, the case for the EDCF-RhoA/Rho kinase-Ca2+-sensitisation axis being responsible for the maintenance of sustained HPV is solid. My most worthy opponent will probably try to convince the reader that the endothelium merely “modulates” the hypoxic constrictor response. However, when disruption of said axis results in removal of the hypoxic constrictor response, then “modulate” must surely be changed to “mediate”!

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COUNTERPOINT: RELEASE OF AN ENDOTHELUM-DERIVED VASOCONSTRICTOR AND RHOA/RHO KINASE-MEDIATED CALCIUM SENSITIZATION OF SMOOTH MUSCLE CELL

CONTRACTION ARE NOT THE MAIN EFFECTORS FOR FULL AND SUSTAINED HPV

The great tragedy of Science—the slaying of a beautiful hypothesis by an ugly fact

Thomas H. Huxley (1825–1895)
With a simple experiment 15 years ago, Jane Madden et al. (9) slayed the beautiful (then unborn) hypothesis that a (still mysterious) vasoconstrictor secreted from the endothelium in response to hypoxia causes hypoxic pulmonary vasoconstric-
tion (HPV) via the Rho-kinase pathway (1, 21). Freshly isolated (and nondedifferentiated) pulmonary artery smooth muscle cells (PASMC), but not systemic arterial SMC, were able to contract to hypoxia in vitro (9); no endothelium, no mysterious factors needed (Fig. 1). There is nothing ugly to this experiment, although it might appear ugly in the eyes of our opponent. We could rest our case right here, but let’s continue.

Our job becomes even easier because of the way the debatable statement is worded: “...the main effectors...” being the key words. Many mechanisms contribute to full HPV, from the nervous to the endocrine system and multiple local mediators. But the main effector, the cornerstone of an HPV theory, has to support all the fundamental features of HPV. To win the argument, we simply need to prove that our opponent’s theory is in conflict with only some of these.

HPV is intrinsic to the pulmonary circulation. While the resistance PAs constrict to hypoxia (to maintain ventilation-perfusion matching), the systemic arteries do not, and often dilate, increasing blood delivery to the hypoxic/ischemic tissues. This means that a mechanism for HPV has to explain its selectivity to the resistance PAs. Similarly, it needs to be specific to hypoxia and ideally not shared by the many pathways involved in nonspecific PA and systemic artery constrictions. For example, HPV cannot be explained by a mechanism involved in the vasoconstriction to prostanoids or phenylephrine, which constrict proximal, resistance PAs and systemic arteries in a similar manner. Based on these, here are three points that support our case.

**Point 1:** An argument that our opponent will use is that the well-documented increase in [Ca$^{2+}$]i caused by hypoxia in PASMC is small and transient, not enough to cause full and sustained contraction (1). Therefore, he will claim, a “boost” from an endothelium-derived vasoconstrictor and an increase in Ca$^{2+}$ sensitivity, mediated by Rho kinase, is necessary. This argument is often used to justify pretone strategies, i.e., exposure to a nonspecific vasoconstrictor prior to and during hypoxia. The breakdown of HPV to phase I and II [phase II is the endothelium- and Rho kinase-dependent phase (1)] is not a universal feature of HPV. For example, it is seen in the isolated PA model, but not in the perfused lung or in vivo models. It also appears that pretone is required in some but not all laboratories. For example, in our hands, rat resistance PAs can have full HPV without pretreatment (3). Although species differences might exist, it is ironic that in the cat Pas [the model in which HPV was first described (24)], no preconstriction is required for HPV (7, 10). Furthermore, in the cat, rat, lamb, dog, and other species (3, 6, 8, 10, 25), full HPV can be observed in isolated PAs effectively denuded from endothelium (Fig. 1B). While methodological differences can be debated in the isolated PA model, our strongest argument comes from the freshly isolated PASMC model (9). These cells can effectively contract within a few minutes of exposure to physiological hypoxia and without the need of pretreatment with vasoconstrictors (Fig. 1A); obviously the increase in [Ca$^{2+}$]i, in this case is by itself enough for effective contraction.

Our opponent’s theory has a conflict with this observation. This is not the case with competing theories. Take for example the theory describing an oxygen sensor within the PASMC (the mitochondria, or the balance of redox couples), which in response to changes in P$O_2$, alters the production of a mediator (for example ROS, whether increased or decreased), which regulates the function of an effector (for example, voltage-gated K+ channels, Kv) (15, 26). No need for endothelium, mysterious factors, pretone requirements, etc. In its simplicity, it is also shared by many other oxygen-sensing systems in the body, including neurons or neuroendocrine cells (26). The Kv channel inhibition in isolated PASMC occurs within minutes from exposure to physiological hypoxia (19). The resulting depolarization leads to increased [Ca$^{2+}$], and contraction. If one keeps these cells in hypoxia for days (chronic hypoxia), he will observe a sustained Kv channel inhibition and a sustained increase in [Ca$^{2+}$]i (18). Interestingly this does not occur in systemic arterial SMC treated identically (17, 27). This sustained mechanism, supported by molecular events that take place in chronic hypoxia, has been proposed as the basis of hypoxic pulmonary hypertension (11, 15, 26).

A proposed mechanism for HPV has to explain PA constriction but also lack of effects or (ideally) dilatation of systemic...
arteries. Furthermore, it should not be a part of any nonspecific vasoconstrictor response. The vasoconstrictor-Rho kinase theory suffers badly here.

Point 2: The inhibition of HPV by Rho-kinase inhibitors (in a manner similar to the inhibition caused by lack of endothelium) is used by our opponent to defend his point (1). But Rho-kinase inhibitors inhibit angiotensin II and KCl-induced constriction in a manner similar to HPV (5). In contrast, blocking Kv channels pharmacologically (3) or molecularly (2, 14) is used by our opponent to defend his point (1). But our opponent suffers badly here.

Now look at just one example of a drug (rotenone) that blocks the mitochondrial complex I, thus proximally inhibiting the mitochondria-Kv channel oxygen-sensing system that we discussed above. In a system where a rat lung and kidney are perfused in series, rotenone mimics hypoxia and constricts the pulmonary while it dilates the renal circulation; this is confirmed in endothelium-denuded vascular rings and in isolated SMC where rotenone inhibits Kv current in PA but activates Kv current in renal artery SMC (13). This is explained by the fact that mitochondria appear to be different in the PA vs. systemic arterial SMC (13). Furthermore, dichloroacetate (DCA), a drug that inhibits a mitochondrial enzyme (pyruvate dehydrogenase kinase), when given systemically in several models of PHT, significantly decreases pulmonary vascular resistance, without affecting systemic arterial pressure (4, 12, 14). While Rho-kinase inhibitors appear to be nonspecific for the pulmonary circulation, targeting the mitochondrial-Kv channel axis selectively inhibits HPV and reverses PHT, sparing systemic vessels.

HPV remains mysterious and elegant. In a future debate, we could defend the mitochondria-ROS-Kv channel theory. For this one, we only had to reject our opponent’s theory; a much easier job. Now let’s clarify something. Our opponent’s work is brilliant. We have been his and his team’s students over the years. Their most recent review (1) is one of the best, most balanced, and comprehensive reviews we have seen in this field. Their original description of this pathway remains one of the most influential in the field (21). The Rho-kinase pathway is an important one for pulmonary vascular biology; but it is not THE one for HPV.

If you are out to determine what it is that makes a Ferrari accelerate so differently than a Fiat, you have to first accept that there is probably more than one reason. However, if you deflate the tires and the Ferrari does not run, you can’t conclude that you discovered what makes the Ferrari a Ferrari; tires are equally important for all cars, like Rho kinase is important in all blood vessels.

To reveal the elegance and uniqueness of a Ferrari, you have to dig further, deep inside its engine, deep into the mitochondria.

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REBUTTAL FROM DR. ROBERTSON

“Two words came to mind as I read my opponent’s side of the argument: “smoke” and “mirrors.” To quote my esteemed opponents; “our strongest argument comes from the freshly isolated PASMC model.” While I do not regard these experiments as “ugly,” they may have more than a few unsightly blemishes! The observation that enzymatically dispersed PASMCs contract to hypoxia in no way negates a primary role for an endothelium-dependent Ca^2+ sensitization in the intact artery or in the whole animal. To infer that the PASMC is the sole repository for the mechanism(s) of HPV as it can constrict locally, with complete disregard to the myriad of cellular changes that likely occur during its rather traumatic isolation, is one inference too far!

I must also take issue with my opponent’s argument that HPV “cannot be explained by a mechanism involved in the vasoconstriction to prostanoids or phenylephrine.” I must admit to finding this argument rather baffling. Could not the specificity, with respect to HPV, be attributed to an endothelium-derived constrictor factor released locally within the lung, and/or one that acts solely within the pulmonary circulation rather than in the signal transduction pathway it activates?

It is interesting that my opponents state that “methodological differences can be debated in the isolated PA model.” Indeed they can and since my opponents state that “in our hands, rat resistance PAs can have full HPV” (1), this seems like a suitable point to debate some methodological differences! In their work, the PAs were ~74 μm in diameter and had a resting tension of 700 mg (~6.9 mN). With a generous allowance of between 1 and 2 mm vessel length, for an artery of this diameter, the equivalent transmural pressure would probably lie between ~500 and ~1,000 mmHg compared with 15–30 mmHg reported from other laboratories (2–9). Moreover, the resultant constrictor responses in this report were, perhaps unsurprisingly, given the “resting” conditions, rather small (~100 mg or ~1 mN) compared with those reported by other investigators using small PAs (~30 mN; Refs. 2, 4, 6, 7).

In the laboratories in which I have had the privilege to work, a pulmonary artery with a resting tension of 7 mN and a subsequent constrictor response of 1 mN would have been a candidate for life support measures!

Finally, HPV (like a Ferrari) is multifactorial. It requires a spark (the mitochondria and increases in cytosolic Ca^{2+}), fuel (glucose for glycolysis), and an accelerator (Rho kinase). But one thing is missing in this Ferrari...oh yes, the driver—something to put the pedal to the metal when it’s really needed. . .now I wonder what that could be?

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REBUTTAL FROM DRS. ROCHEFORT AND MICHELAKIS

Opening his argument, our opponent calls you, the readers, to a gambling exercise; admittedly a safe bet. Because in one of his reviews on the subject, only four papers found the endothelium to be nonessential for HPV, compared to the 15 that found it to be essential (1), he asks you to bet your money on his side of the argument. However, even in Las Vegas you get in trouble if you play safe bets, but with weighted dice! To support their side of the story in that review, the authors listed (in Table 1 of that review) apparently all the papers that were studying sustained HPV (more than 20 min of hypoxia) (1). We do not understand the basis on which 20 min was used as the cutoff between acute and “sustained HPV.” But, even using this rule, several papers were omitted from that table and argument, which, interestingly, strongly suggest a nonessential role of the endothelium. For example, Wang et al. (3) studied...