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Discussion

DR. MARK A. WILSON (Louisville, Kentucky): President McDonald, Secretary Copeland, Fellows, and Guests. As many of us are aware, nitric oxide has been recognized as a central mediator of many physiologic and pathophysiologic processes as evidenced by the myriad publications about this molecule. However, there is little data and only a handful of studies that have examined intracellular availability of arginine and its transport across cell membranes.

Dr. Souba and his coauthors are to be congratulated for this current elegant study which, I believe, adds significantly to our understanding of endothelial cell biology as related to the second messenger and mechanisms of arginine transport. I have three questions for the authors.

First, can you tell us whether these responses of the arginine transporter also occur in other cell types or with other mediators and, if so, whether the mechanisms are similar? Secondly, the importance of extracellular arginine in the function of nitric oxide synthase is controversial, particularly in endothelial cells and particularly in the case of the constitutive nitric oxide synthase. Several studies by Bogle have demonstrated that hormones such as bradykinin increase the y+ transporter affinity for arginine and increase the rapidity of arginine within just seconds after stimulation.

Yet the significance of this finding has been questioned because intracellular levels of arginine in endothelial cells are several-fold higher than those levels that are necessary for optimal function of the constitutive enzyme. In light of these studies, would you please elaborate on the presumed importance of extracellular arginine for function of both the constitutive and inducible nitric oxide synthases, both in endothelial cells and other cell types.

Next, much of your previous research has been focused on the decrease that occurs in glutamine transport under similar conditions. Because glutamine can serve as an intracellular source of arginine, please speculate about the interrelationships of these two systems.

Finally, are there any prospects for selective antagonists of arginine transport which might be useful in differentiating the potentially beneficial effects of constitutive nitric oxide synthase from the potentially detrimental effects of larger amounts of nitric oxide produced by the inducible system?

I very much enjoyed the opportunity to review the manuscript and thank the Association for the privilege of the floor.

DR. R. NEAL GARRISON (Louisville, Kentucky): President McDonald, Secretary Copeland. Dr. Souba asked me to discuss this paper, and I feel honored to do so, but find it difficult to follow Dr. Wilson's in-depth discussion.

One aspect that I would like to focus on relates to the endothelial cell and its overall function. The endothelial cell is very complex. At times, it is an immunologically reactive type of cell, and the transplant surgeons worry about it. At other times, it is an antithrombogenic type of cell, which brings comfort to the hearts of vascular surgeons. And, thirdly, it redistributes blood flow or regulates—autoregulates blood flow, a process the trauma surgeons worry about quite often.

These studies utilize umbilical vein endothelial cells; yet most, if not all, inflammation occurs under a different set of circumstances. In our *in vivo* microscopy studies, the most reactive vessels are the small precapillary microvessels where au-

toregulation of blood appears to be under endothelial cell control.

I could expect that the same findings in endothelial cells from this microcirculation of the splanchnic circulation, specifically, the small intestinal tract, would be different. Would you care to comment on whether there is a difference in these cells from the microcirculation?

This work is very important, and it is a tremendous addition to our understanding of vascular control of blood flow distribution during stress. I thank the Association for the privilege of the floor.

DR. FRANK G. MOODY (Houston, Texas): Thank you, John, and Colleagues. This is such a marvelous paper that I just want to broaden its context because, as Chip knows, we've been working with the inducible nitric oxide in the gut as it relates to ileus.

For this purpose, we pursued the availability of arginine from the gut side, seeing what brings it into the cell to antagonize alanine. We found we could manipulate this system very simply by changing the concentrations of sodium and glucose in the gut.

But, more importantly, we've been able to develop a very specific probe for iNOS, an antibody that's very clean. And it's interesting that the inducible iNOS we thought was going to be in the muscle; actually was in the epithelium just above the crypts.

I'd like to ask you if you have used LPS because we're thinking that LPS is getting at this iNOS to generate the nitric oxide to cause the ileus. Have you exposed your cells to LPS to see if you get the same response as with TNF?

DR. WILEY W. SOUBA, JR. (Closing Discussion): I thank Drs. Wilson, Garrison, and Moody for their comments. I will attempt to answer the questions in some sort of sensible fashion.

Do these responses or similar responses occur in other cell types? The answer appears to be yes. We've looked at arginine transport in the liver, specifically, rat and human hepatocytes. These cytokines will induce the System y+ carrier in those cells as well, and the response does not appear solely confined to TNF. It can be observed in the presence of interleukin-1 and IL-6 as well.

Dr. Moody asked along with those lines, do we observe these responses in the presence of LPS? It appears to be cell dependent, Dr. Moody. In the human endothelial vein cells, LPS does not appear to alter arginine transport. In pulmonary artery endothelial cells, LPS stimulates transport of arginine, and that can be blocked about 60% by adding antibody to TNF and IL-1, making us think that in response to inflammatory agents such as LPS, the production of TNF and IL-1 by the endothelial cells increases. Those mediators secreted into the medium and act in an autocrine fashion to upregulate arginine transport.

Dr. Moody, iNOS has been well known to increase 20- to 30-fold in response to LPS. That, too, is in most systems a cytokine mediated event.

Dr. Wilson asked an important question about the role of intracellular *versus* extracellular arginine levels. This is important question because in endothelial cells, the concentration of the enzyme arginase, which is very abundant in hepatocytes because of its role in the urea cycle, is not so abundant in endothelial cells. Therefore, intracellular arginine levels can accumulate in endothelial cells.

Is there enough arginine within the cell so that you really don't need arginine from the blood? I don't have the answer to that question. I can speculate that early on after exposure to the inflammatory stimulus the intracellular of arginine may be adequate to support the nitric oxide pathway. But as that level becomes depleted, it may be that a continuing update from the bloodstream is necessary.

The glutamine question is also probably germane in that, unlike arginine, stimulation of PKC actually inhibits glutamine transport rather than stimulating it. We think that may be important because in these endothelial cells glutamine has been shown to block the reproduction of arginine from citrulline.

So that the cell might say, "I certainly don't want to have a lot of glutamine around at a time when I need to fuel these arginine pathways."

We should point out that nitric oxide is not necessarily a bad molecule; in fact, it is a very important molecule. When it's produced in excess amounts, it may have more detrimental effects than is desirable. But, normally, it plays a very important role in modulating cell metabolism.

Dr. Garrison asked have we seen these responses in microvascular cells. We have not looked at the small intestine, Neal, but we have looked at the pulmonary artery microendothelium. Dr. Wilson asked about a selective blockade. As many of you know, nitric oxide synthase inhibitors are now being clinically evaluated. Patients with profound pulmonary artery hypertension can be treated successfully by adding nitric oxide to the inhaled gases. Its role systemically is less well defined.

In general, during inflammation, arginine requirements are increased. So it may be that a substantial portion of this arginine is used to supply a substrate for the other cells in the tissue in the particular organ. And that that occurs by first passing through the microendothelium.

I thank you for your time and your attention and for the privilege of the floor.