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The love of a lifetime: 5-HT in the cardiovascular system

Stephanie W. Watts
Department of Pharmacology and Toxicology, Michigan State University, East Lansing, Michigan

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Watts SW. The love of a lifetime: 5-HT in the cardiovascular system. Am J Physiol Regul Integr Comp Physiol 296: R252–R256, 2009. First published August 27, 2008; doi:10.1152/ajpregu.90676.2008.—Serotonin [5-hydroxytryptamine (5-HT)] is an amine made from the essential amino acid tryptophan. 5-HT serves numerous functions in the body, including mood, satiety, and gastrointestinal function. Less understood is the role 5-HT plays in the cardiovascular system, although 5-HT receptors have been localized to every important cardiovascular organ and 5-HT-induced changes in physiological function attributed to activation of these receptors. This manuscript relates a few scientific stories that test the idea that 5-HT is important to the control of normal vascular tone, more so in the hypertensive condition. Currently, our laboratory is faced with two different lines of experimentation from which one could draw vastly different conclusions as to the ability of 5-HT to modify endogenous vascular tone and blood pressure. Studies point to 5-HT being important in maintaining high blood pressure, but other studies solidly support the ability of 5-HT to reduce elevated blood pressure. This work underscores that our knowledge of the functions of 5-HT in the cardiovascular system is significantly incomplete. As such, this field is an exciting one in which to be, because there are superb questions to be asked.

serotonin

HENRY PICKERING BOWDITCH was a man of many talents. A physiologist, photographer, kite builder, and singer (though tone deaf, according to his son Manfred). His dedication to physiology and scholarly activity is famous, and thus it was a true honor to give a lecture in his name. This paper will tell a story that is ongoing and has taken unexpected, exciting, and confusing turns. The focus of this story is a small molecule, serotonin [5-hydroxytryptamine (5-HT)].

5-HT has a long history in cardiovascular physiology. In 1868, scientists knew that the blood contained a substance(s) that caused blood vessels to contract. In the late 1930s, Vittorio Erspamer isolated a substance, enteramine, from gastric mucosa that also contracted blood vessels, and demonstrated this substance to be 5-HT (12). At the same time, Page and colleagues (26, 27, 29) also identified 5-HT in the blood. 5-HT is synthesized from the essential amino acid tryptophan in the enterochromaffin cells of the intestine, raphé nuclei of the brain, and other discrete sites. In these sites, 5-HT is best known as a neurotransmitter that can modify gastrointestinal motility and mood. 5-HT is released from the cells and acts on postsynaptic receptors. 5-HT can be taken back up into the neuron by the serotonin transporter (SERT), where it can be stored again, or metabolized into 5-hydroxyindole acetic acid (Fig. 1). The key piece of information here is that 5-HT exists both intracellularly and extracellularly, and thus has the potential of modifying function through both extracellular and intracellular mechanisms.

Since the discovery of 5-HT, researchers have been dedicated toward understanding its role in the cardiovascular system. This lecture is dedicated to the scientists who have invested time and energy into this complex area. There are so many to thank, but they include David Bohr, Don McGregor, Sir Horace Smirk, Paul Vanhoutte, and the special people, discussed below, who have helped me to become a scientist. Because this paper will not be comprehensive in its discussion of 5-HT in general or in the cardiovascular system, I direct you to some excellent resources here (2, 9, 11 15, 18, 20, 25, 34, 36).

Falling

I was introduced to 5-HT by my thesis mentor, Dr. Marlene Cohen. Dr. Cohen was an adjunct Professor of Pharmacology and Toxicology at Indiana University-Purdue University at Indianapolis, and a prominent Senior Research Fellow at Lilly Research Laboratories. She graciously opened her laboratory at Eli Lilly to me, and it felt like coming home. I discovered the powerful pharmacological/physiological technique of the isolated tissue bath. Smooth muscular tissue (and even atrial!) from multiple species can be placed in this bath for measurement of isometric contractile force. I was impressed with the power that this system afforded the researcher and the versatility it allowed. I also discovered 5-HT, which had been a focus of Dr. Cohen’s research for some time. Her laboratory had been dedicated to identifying the 5-HT receptor that...
mediated contraction of the isolated rat stomach fundus. Sir John Vane had introduced this tissue as a bioassay for 5-HT, as the stomach fundus is exquisitely sensitive to 5-HT. Dr. Cohen knew that a receptor mediated contraction to 5-HT in the stomach fundus, but the pharmacology and amino acid sequence of this receptor differed from anything that was known. Over time, she and her colleagues identified the fundal receptor as a new 5-HT receptor that was most closely related to the 5-HT2 receptors, and this receptor was called the 5-HT2B receptor (14, 19). This receptor is significant because of all vascular contractile 5-HT receptors (7 major subtypes exist, 15 subdivisions) (17), 5-HT has among the highest affinity for this receptor. One of the hallmarks of the 5-HT2B receptor is the significantly low affinity the 5-HT2 receptor antagonist ketanserin possesses for this receptor (4, 5). Thus, the field was back to square one as to whether 5-HT was involved in blood pressure. During this time, other 5-HT2 receptor antagonists that lacked α1-adrenergic receptor affinity (ritanserin, LY53857) were examined in hypertensive models, and a majority of studies suggested that 5-HT2 receptor blockade did not reduce blood pressure (36). What then does hyperresponsiveness to 5-HT mean?

The 5-HT2B Receptor

I have looked through a number of Bowditch lecture proceedings, and virtually every one contains the word “serendipity.” How true it is that science and a scientific career can be blessed with serendipity. I had the good fortune to be in two different places that allowed me to put together two pieces of information that led to one of the first major hypotheses in my laboratory. While a graduate student with Dr. Cohen, I learned that the rat stomach fundus contracted to the metabolite kynuramine, defining kynuramine as a 5-HT2B receptor agonist. While I was a postdoctoral fellow, the Webb laboratory was using kynuramine and similar derivatives, demonstrating that arteries from hypertensive animals were hyperresponsive to these compounds (38). Could it be that the 5-HT2B receptor, which can be activated by kynuramine, is more active in arteries from hypertensive vs. normotensive models?

Thus began a major push in my laboratory. In two different models of hypertension, we were able to demonstrate a dependence of the elevated blood pressure on 5-HT2B receptor activation (3, 31, 35, 37). In arteries, we showed upregulated expression of the 5-HT2B receptor mRNA and protein in hypertension. Importantly, this translated into increased function of the 5-HT2B receptor, supported by significant amounts of pharmacological studies in isolated arteries. Moreover, administration of the 5-HT2B receptor antagonist LY272015 reduced blood pressure of the hypertensive rat (7, 31, 37). One reason this hypothesis was and is so attractive is that 5-HT has such a high affinity for the 5-HT2B receptor compared with the normally expressed vascular 5-HT receptors (5-HT2A, 5-HT1B) and the 5-HT2B receptor is relatively insensitive to ketanserin.
It could be argued that the circulating level of 5-HT was sufficient to activate expressed 5-HT2B receptors and, in fact, the free circulating levels of 5-HT (nonplatelet) are elevated in one of the models used, the DOCA salt hypertensive rat (10). This must occur, to some degree, to explain the ability of LY272015 to reduce blood pressure of hypertensive animals. Figure 2 depicts this hypothesis pictorially.

Where Does the 5-HT Come From?

While working on the 5-HT2B receptor hypothesis, we started thinking about from where the 5-HT that could activate the 5-HT2B receptor might come. Classical teachings suggest that the platelet is the primary source of circulating 5-HT, having taken up 5-HT from the enterochromaffin cells of the gastrointestinal system by means of a membrane SERT. Sympathetic neurons can also take up and store 5-HT, largely through actions of the norepinephrine transporter. Could arteries take up 5-HT and release 5-HT? The ability of 5-HT to be released from peripheral tissues was discovered with use of the 5-HT-releasing substances fenfluramine/norfenfluramine (30). Used in the medical management of obesity, fenfluramine functions to release 5-HT in the central neuron to promote feelings of satiety. Unfortunately, it was discovered that the fluramines do this not only in the intended target neurons but also in pulmonary arterial cells and aortic valves. The end result of this stimulation is pulmonary hypertension and valvulopathy (1, 30). Thus, we embarked on a hypothesis that SERT existed in peripheral arteries.

Not only did we find that SERT was present and functional in systemic arteries but that a full serotonergic system existed (Fig. 3). Peripheral arteries, which included the aorta and superior mesenteric artery, can synthesize 5-HT, take up 5-HT, metabolize 5-HT, release 5-HT, and bind 5-HT intracellularly (22–24). Perhaps the most intriguing finding was that arteries could make their own 5-HT. While the quantity of this 5-HT paled compared with the 5-HT made by the intestine, it is an important finding because it draws attention to two points. First, the blood/blood platelet is not the only source of 5-HT for the artery. Second, the intra-arterial synthesis of 5-HT and retention of 5-HT intracellularly begs the question of exactly what 5-HT is doing inside the cell. Could it be released to activate the upregulated 5-HT2B receptor in hypertension? It seemed at this point that we had identified two key lines of evidence that suggested 5-HT was pathogenic in hypertension. A local circuit of 5-HT release and activation of the 5-HT2B receptor would support endogenous activation of the receptor in support of elevated blood pressure.

Fig. 2. Depiction of hypothesis of upregulated 5-HT2B receptors in hypertension. 2A, 5-HT2A receptor; 2B, 5-HT2B receptor.

Fig. 3. Depiction of the serotonergic system within the arterial wall. TPH, tryptophan hydroxylase; AADC, amino acid decarboxylase. [From Ni W, et al. (22).]
Part of the reason for pursuing 5-HT as a pathogenic factor in hypertension is that plasma levels of 5-HT are elevated in hypertension subjects compared with subjects with normal blood pressure (5, 13). One could envision this higher level of 5-HT activating vascular receptors to promote increases in total peripheral resistance and blood pressure. To test this idea, we performed a series of experiments that radically changed how we think about 5-HT and blood pressure control.

**What Does Elevated Plasma 5-HT Do to Systemic Blood Pressure?**

We implanted miniosmotic pumps, filled with 5-HT or vehicle, subcutaneously in normal and mineralocorticoid hypertensive rats (DOCA-salt). The concentration of 5-HT chosen was based on studies by Gustafsson et al. (16) in which 5-HT, given over 90 days, caused valvulopathy. These studies suggested that rats could tolerate the constant exposure to elevated 5-HT. The pumps were implanted in animals with both normal and high blood pressure. Our hypothesis was that blood pressure may go up in the normal animal but would be elevated 5-HT, given over 90 days, caused valvulopathy. These studies suggested that rats could tolerate the constant exposure to elevated 5-HT. The pumps were implanted in animals with both normal and high blood pressure. Our hypothesis was that blood pressure may go up in the normal animal but would certainly become significantly elevated in the hypertensive rats. This hypothesis was based on the idea that the exogenous 5-HT would activate the more highly expressed 5-HT2B receptor in the hypertensive animal, thereby elevating total peripheral resistance and blood pressure. What we discovered was completely surprising. 5-HT, determined through HPLC to be elevated in the plasma, did not elevate blood pressure in the normal rat. Within 48 h of pump initiation, blood pressure had fallen ~20 mmHg in the normal rat. Moreover, 5-HT did not elevate blood pressure in the hypertensive rats but nearly normalized elevated blood pressure by dropping pressure by >50 mmHg (10). Clearly, we did not (and still don’t) understand the totality of actions of 5-HT.

There is certainly a great deal of evidence that suggests 5-HT modifies the function of other organs/systems that modify blood pressure. 5-HT can change neuronal function, heart rate and strength, kidney perfusion pressure, and blood coagulation (36). Further studies suggested that 5-HT reduces sympathetic activity through ganglionic transmission (10), and this is consistent with the knowledge that sympathetic activity is elevated in many forms of hypertension. Moreover, blockade of nitric oxide synthase (NOS) abolished the hypotensive effect of 5-HT, indicating that 5-HT depended on NOS activity for its function. We are currently in the midst of understanding the multiplicity of actions of 5-HT in the cardiovascular system, and how 5-HT interacts with NOS.

**Where Do We Go from Here?**

The past 15 years of research have shown me that as much as I think I know about 5-HT, there is so much more to learn. The following are a few of the questions with which we wrestle.

- Where is the primary site of action of 5-HT that results in decreased blood pressure?
- Are 5-HT receptors involved in the hypotension stimulated by 5-HT? We tested the ability of methiothepin as a general 5-HT receptor antagonist to block the effects of 5-HT, but the sedation of the animals upon administration of methiothepin that ensued made it difficult to interpret any effects on blood pressure.
- How does 5-HT act inside the cell? Is 5-HT stored?
- Is the elevated plasma 5-HT observed in hypertension a cause of disease or an adaptation to try to lower blood pressure?
- How do we put together the seemingly disparate findings of the 5-HT2B receptor supporting hypertension but yet 5-HT stimulating hypotension?

These are the primary questions we are pursuing, and it is thrilling to have a chance to do so.

I can’t express the privilege and gratefulness I feel for having the opportunity to work in science. How many people can say that they actually get paid for getting to think, create, and pass on a tradition? My world of science has been enormously influenced by many. Some of these individuals you met earlier in this paper. In particular, I owe a great deal of thanks to the students and fellows that have been a part of my laboratory. Their energy and ideas, willingness to try new things, and work as a team is one of the greatest things I will ever witness. These people include: Jennifer Florian, Amy Banes, Carolyn McKune, Zachary Hickner, Carrie Northcott, Amber Russell, Keshari Thakali, Wei Ni, Kevin Ogden, Theo Szasz, Jessica Diaz, Jessie Priestley, Nate Tykocki, Patrick Davis, and Elizabeth Linder. My colleagues at Michigan State University have also given me license to dream and have been true colleagues through the years. So, thanks to Sue Barman, Greg Fink, Jim Galligan, and JR Haywood. Thanks, too, to Ken Moore for giving me the chance to be a faculty member. Janice Thompson has been my right hand through the years, ever witness. These people include: Jennifer Florian, Amy Banes, Carolyn McKune, Zachary Hickner, Carrie Northcott, Amber Russell, Keshari Thakali, Wei Ni, Kevin Ogden, Theo Szasz, Jessica Diaz, Jessie Priestley, Nate Tykocki, Patrick Davis, and Elizabeth Linder. My colleagues at Michigan State University have also given me license to dream and have been true colleagues through the years. So, thanks to Sue Barman, Greg Fink, Jim Galligan, and JR Haywood. Thanks, too, to Ken Moore for giving me the chance to be a faculty member. Janice Thompson has been my right hand through the years.

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We acknowledge the British Journal of Pharmacology for allowing publication of Fig. 3 (23).

**REFERENCES**


