The two faces of serotonin

Working in collaboration with Dr Susan Barman, Dr Gregory D Fink and Bridget M Seitz, Professor Stephanie Watts is investigating vascular dysfunction. She describes the surprising finding that 5-HT, or serotonin, can lower blood pressure and outlines their groundbreaking concept of a future treatment for hypertension via selectively targeting the venous circulation.

Why is hypertension such a severe problem worldwide? What are the main factors that need to be addressed in tackling this issue?

Hypertension is a serious condition that, when combined with other diseases such as diabetes or high cholesterol, becomes even more detrimental to health. We know that weight, cigarette smoking, alcohol intake, unhealthy eating and inactivity all contribute to elevated blood pressure. The solutions to these misbehaviours are available but are hard to enact, human behaviour is enormously difficult to change! Drug treatment for hypertension may be a more realistic course, and there are truly excellent drugs available for reducing blood pressure. However, even with medications, a significant proportion of individuals with hypertension can’t get their blood pressure to acceptably lower levels. We still don’t understand all the ways in which blood pressure is established or elevated, meaning that there is much room for discovery!

What was the significance of your finding that 5-hydroxytryptamine (5-HT) could reduce blood pressure in animals with hypertension?

We found a higher level of free 5-HT (not in platelets, where a lot of 5-HT is stored) in plasma from hypertensive animals. 5-HT constricts arteries when they are isolated from the body, and vasoconstriction elevates blood pressure. This is also true for veins. We hypothesised that we would observe an elevation of blood pressure when 5-HT was infused over days. We were wholly surprised when just the opposite occurred, and 5-HT nearly normalised the blood pressure of hypertensive animals. With that first experiment, we realised that there was much we did not understand about how 5-HT functions.

How can this finding be reconciled with observations such as the elevated 5-HT plasma levels in cardiovascular disease?

Excellent point. It can be reconciled with the hypothesis that plasma 5-HT levels elevate for the purpose of reducing raised blood pressure, though this isn’t something we have been able to prove. It may be an adaptive mechanism – although it is clearly not perfect, since hypertension still exists where there are higher 5-HT levels.

How is your team working to deduce the mechanism of this action?

We are utterly dedicated to the science, as well as to slow and meaningful experimentation, constant discussion of the data in front of us and exploration of any disagreements. What’s brilliant about our team is that each member’s expertise contributes strongly to our end goals; we complement one another in what we do and how we think.

Can you outline your successes so far and explain how you have overcome experimental challenges?

Our primary experimental challenge has been to figure out how blood pressure falls if the arterial system does not directly relax to 5-HT and the sympathetic nervous system is not inhibited by 5-HT. This has forced us to think broadly and in new directions. Successes have included being able to determine what 5-HT does not do to determine blood pressure. This then led to a series of studies which ultimately arrived at the venous circulation as a target for 5-HT.
Hormonal therapy for hypertension?

Integrating multiscale pharmacological, biochemical and neurophysiological techniques, Michigan State University researchers have proved that the vasoconstrictor 5-HT can reduce blood pressure, and are now investigating its positive action against hypertension.

Can you briefly explain how the venous system may be responsible for the reduction of blood pressure in response to 5-HT?

By increasing venous capacitance (the amount of blood the veins can hold) through relaxing venous smooth muscle, ‘effective blood volume’ is removed from the arterial circulation and thus blood pressure falls. With our 5-HT dosing in vivo, we must be hitting relaxant receptors, but none or a minority of contractile receptors, so that the total body outcome of infusing 5-HT is a fall in blood pressure.

How do you see your work developing over the next few years?

5-HT is involved in so many physiological systems that simply dosing a human with it or one of its precursors would likely cause more problems than solve them. Developing an appropriate drug for the venous side of the circulation will take careful consideration and there is much we must first learn. A very high level goal would be to understand how one could selectively deliver a drug to the venous system. At present, we simply don’t know if this is possible – but that is the beauty of science, and there are many scientists who can help us answer these questions.

SEROTONIN, OR 5-HYDOXYTRYPTAMINE

(5-HT), is a neurotransmitter synthesised primarily in certain cells in the gastrointestinal tract and in the central nervous system (CNS). Known as a ‘happy hormone’, it is mainly stored in the platelets of the blood. Its activity is mediated by 5-HT receptors, of which there are at least seven types in different areas of the body, with each type prompting a different response. In the CNS, serotonin helps to regulate body temperature and mood; it also plays a role in sexuality, sleep and appetite. Disturbances in the serotonin system are thus implicated in mental disorders such as depression, anxiety and bipolar disorder, as well as in physical conditions such as migraine, obesity, irritable bowel syndrome, tinnitus and fibromyalgia.

In instances where blood pressure is severely reduced, such as during anaphylactic shock and in some cancers, levels of 5-HT have been found to increase by approximately six-fold. Whether there is in fact a causal relationship between lowered blood pressure and raised 5-HT is thus the focus of the work conducted in the laboratory of Professor Stephanie Watts in the Department of Pharmacology and Toxicology at Michigan State University (MSU), USA. Together with her team, Watts is seeking to identify the mechanisms by which 5-HT becomes elevated and acts on the vasculature, with the ultimate goal of improving treatment for hypertension.

EXPERIMENTAL APPROACH

Watts’ laboratory is unusual in that it integrates analyses of serotonergic systems from the molecular scale to that of the whole living animal, using a wide array of techniques: western blot and protein analyses, real-time polymerase chain reaction and super arrays, gene array-based pathway mapping, high-performance liquid chromatography, immunohistochemistry, immunocytochemistry, cell culture, kinase assays, animal surgery, blood pressure measurement and pharmacology. These are supplemented with methods for measuring smooth muscle contraction via isometric tension recordings in isolated tissue baths, and small artery contraction via myography. In addition, Watts’ team tracks responses to precisely timed and measured doses of compounds in the form of blood pressure changes in experimental animals while they are conscious. This is done by combining a microscale drug infusion pump device, specifically designed for the automated

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INTELLIGENCE

TWO FACES OF 5-HT: A VASOCONSTRICTOR THAT LOWERS BLOOD PRESSURE

OBJECTIVES
To determine the mechanism(s) by which 5-HT causes a reduction in blood pressure in vivo.

KEY COLLABORATORS
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PROFESSOR STEPHANIE W WATTS earned her PhD in 1992 from Indiana University/Purdue University in Indianapolis, performed a postdoctoral fellowship at the University of Michigan and has been a faculty member at MSU since 1995. Watts is currently Full Professor and Assistant Dean in the Graduate School.

DR SUSAN BARMAN earned her PhD at Loyola University in 1976, performed a postdoctoral fellowship at Michigan State and remained to become Full Professor in the Department of Pharmacology and Toxicology. Her expertise is in measurement of sympathetic nerve activity measurement and whole animal physiology.

BRIDGET M SEITZ earned her MS in Pharmacology and Toxicology in 2010 after working in Pfizer laboratories for six years as a whole animal surgeon.

DR GREGORY D FINK earned his PhD at Tulane University in 1975, performed a postdoctoral fellowship at the University of Iowa and has been Professor of Pharmacology and Toxicology at MSU since 1977.

administration of experimental compounds to small animals, with radiotelemetry – and delivers results to a time resolution of a second.

Interestingly, 5-HT was originally identified as a substance that changed the tone of blood vessels via constriction, thus raising blood pressure and potentially leading to hypertension. Watts therefore hypothesized that the infusion of 5-HT would increase blood pressure in both normal and hypertensive animals, as she had found in tests of 5-HT on excited rat arteries in vitro.

SURPRISING FINDINGS
Contrary to predictions, in 2008, along with MSU hypertension specialist Professor Gregory Fink, Watts found that infusing 5-HT into a conscious hypertensive animal resulted in the reduction of elevated blood pressure down to nearly normal levels. Further exploration of the reasons for this, however, hit a dead end. Watts theorised, but could not show, that 5-HT relaxed arteries, particularly those in the site of the visceral organs, the splanchnic bed. Also, in collaboration with her colleague Dr Susan Barman, she found that 5-HT did not interfere with the sympathetic nervous system responsible for the light- or flight response in animals, which plays a role in controlling blood pressure and blood flow and supplying nerves to the splanchnic bed.

Together with pharmacology researcher Bridget Seitz, Watts’ team accordingly decided to use small fluorescent microspheres to identify where in the body an increase in blood flow or a decrease in resistance might occur. This showed that microsphere accumulation grew in the intestinal/splanchnic region when 5-HT was present. On further analysis, it was found that veins relax in response to low levels of 5-HT.

This led Watts and her team to conclude that, because veins can pool blood when relaxed, this could be the key to why 5-HT infusion reduces blood pressure. “The finding emphasised to us that you cannot take the results of any one experiment in isolation,” observes Watts. “In vivo experiments are incredibly useful at so many levels, but if you are going to investigate a parameter like blood pressure, at some point you have to apply your question and experimentation to the whole animal. Just think what we would have missed if we hadn’t done that!”

CURRENT INVESTIGATIONS
Watts’ current 5-HT project now follows on from the premise that 5-HT elevates blood flow to the splanchnic region, including the flow in the superior mesenteric vessels. Her hypothesis is that serotonin relaxes the superior mesenteric vein to varying degrees, according to the dose when administered over an extended period – for, say, 30 days. Relaxation is primarily achieved via the 5-HT receptors.

Because of their widespread usage, many antidepressants based on suppression of serotonin reuptake or its metabolism, are known to have a side effect of reducing human blood pressure. However, some are also known to have no effect, such as citalopram, while others, such as fluoxetine, have variable effects on the blood pressure of rodents. Whether these exceptions are brought about by elements in these compounds other than 5-HT is unclear. For this reason, Watts’ is excluding any possible skewing of study results by directly increasing 5-HT concentrations in experiments.

Importantly, Watts’ new study will also seek to demonstrate that serotonergic agonists can cause a long-term fall in blood pressure, that certain 5-HT receptor antagonists could block a fall in blood pressure induced by 5-HT – and, finally, to establish the physiological relevance of such a fall.

VENOUS THERAPY FOR HYPERTENSION
Once Watts understands the mechanisms of vein dilation by 5-HT, she is hopeful that this will help in the development of new antihypertensive therapies using serotonergic drugs to target the veins. This is ambitious as drugs that act on the veins rather than the arteries are highly unusual in pharmacological strategies.

Yet, going forwards, if such an approach turns out to be unfeasible, Watts envisions that her research will nevertheless set the foundation for an important shift from the arterial to the venous circulation in pharmacology. “I am inspired by my work. It is exciting to think that our collaborative research will enable us to arrive at a better understanding of just what hypertension is and how it can be treated,” she concludes.