5-HT in systemic hypertension: foe, friend or fantasy?

Stephanie W. Watts
Department of Pharmacology and Toxicology, B445 Life Sciences Building, Michigan State University, East Lansing, MI 48824-1317, U.S.A.

ABSTRACT
Since its discovery by Erspamer in the 1930s and identification by Page in the 1950s, 5-HT (5-hydroxytryptamine; serotonin) has been an elusive candidate as a substance that plays a role in the disease of high blood pressure, also known as hypertension. In both animal and human hypertension, arterial contraction to 5-HT is profoundly enhanced. Additionally, 5-HT is a vascular smooth muscle cell mitogen. Because both increased arterial contractility and smooth muscle growth contribute to the disease of hypertension, it is logical to believe that 5-HT is a potential cause of disease, and thus a foe. However, decades of research have produced conflicting results as to the potential role of 5-HT in hypertension. This review will discuss historical findings which both support and refute the involvement of 5-HT in hypertension, and pose some new questions that may reveal novel ways for 5-HT to modify vascular control of blood pressure.

INTRODUCTION
5-HT (5-hydroxytryptamine), originally identified as enteramine and also known as serotonin, is an autacoid synthesized primarily in the enterochromaffin cells of the intestine and in discrete areas of the brain. 5-HT has been identified in a multitude of phyla, including vertebrates, tunicata, mollusca, arthropoda and angiospermae, to name a few [1]. 5-HT can stimulate end responses in virtually every major physiological system, including the central nervous, respiratory, gastrointestinal, genitourinary and even immune systems [2]. Of interest to this review is the effect of 5-HT on the cardiovascular system as it pertains to control of TPR (total peripheral resistance) and BP (blood pressure) in hypertension. Pulmonary hypertension and 5-HT will not be discussed in this review, and the reader can consult cited reviews for more information [3,4]. Prior to detailing the specific effects of 5-HT in the cardiovascular system and the debate as to the involvement of 5-HT in hypertension, it is important to understand the synthesis, metabolism, pharmacology and physiology of 5-HT.

BIOCHEMISTRY OF 5-HT

Synthesis of 5-HT
Tryptophan is an essential amino acid that is the primary building block for 5-HT. Over 95% of the human body load of 5-HT is synthesized in the enterochromaffin cells of the intestine, with the remainder synthesized in the raphe nuclei of the brain and endothelial cells that line the lung [5]. Synthesis depends on the specific action and rate-limiting step of the enzyme TPH.

Key words: blood pressure, cardiovascular system, enteramine, 5-hydroxytryptamine (5-HT), hypertension, serotonin.
Abbreviations: 2K-2C, two kidney-two clip; 5-HIAA, 5-hydroxyindole acetic acid; 5-HT, 5-hydroxytryptamine; Ang II, angiotensin II; BH4, tetrahydrobiopterin; BP, blood pressure; DOCA, deoxycorticosterone acetate; ET-1, endothelin-1; i.c.v., intracerebroventricular; l-5-HTP, L-5-hydroxytryptophan; MAOA, monoamine oxidase A; NET, noradrenaline transporter; NO, nitric oxide; PCPAME, p-chlorophenylalanine methyl ester; PIH, pregnancy-induced hypertension; SERT, serotonin transporter; SHR, spontaneously hypertensive rat; TPH, tryptophan hydroxylase; TPR, total peripheral resistance; WKY, Wistar-Kyoto.
Correspondence: Dr Stephanie W. Watts (email wattss@msu.edu).
Biosynthesis of 5-HT and its metabolites

AANAT, arylalkylamine N-acetyltransferase.

Figure 1  Biosynthesis of 5-HT and its metabolites

AANAT, arylalkylamine N-acetyltransferase.

(tryptophan hydroxylase; EC 1.14.16.4), which transfers a hydroxyl group to the benzyl ring of tryptophan (Figure 1). Consequent decarboxylation by amino acid decarboxylases results in the formation of 5-HT. Sites of 5-HT synthesis in the peripheral non-pulmonary cardiovascular system have not yet been identified, so our current understanding is that the 5-HT exposed to the cardiovascular system is that which is taken up and released by the platelet and/or is naturally freely circulating. An important discovery was made recently with the identification of a second isoform of TPH. Bader and co-workers [6,7], through efforts in knocking out TPH, discovered that a peripheral form of TPH, called TPH1, and a central form, TPH2, are expressed, allowing for distinct sources of peripheral and central 5-HT. In the periphery, platelets possess a high-efficacy SERT (serotonin transporter) which enables the platelet to take up 5-HT from the gut and lung, store and ultimately release 5-HT in a thrombotic event.

Metabolism of 5-HT

Metabolism of 5-HT occurs through actions of MAOA (monoamine oxidase A), an enzyme sensitive to pargyline and isoniazid, to form the metabolite 5-HIAA (5-hydroxyindole acetic acid; Figure 1). Because MAOA is an intracellular enzyme, 5-HT must be taken up inside a cell prior to being acted upon, and both SERT and NET [noradrenaline (norepinephrine) transporter] are capable of this uptake (Figure 2). Body sites that contribute significantly to 5-HT metabolism include lung, intestine and endothelial cells of the arterial system, but any cell that can uptake 5-HT and possesses MAOA has the potential to metabolize 5-HT. SERT is being found increasingly in physiological sites such that 5-HT may be taken up and metabolized by unappreciated sources [8].

PHARMACOLOGY OF 5-HT

The effects of 5-HT are mediated by interaction with 5-HT receptors, integral proteins of the plasma membrane. At present, seven major families of 5-HT receptors exist (5-HT₁–5-HT₇) and subtypes therein exist. Table 1 provides information on these receptors and pharmacological agents used to modulate receptor activation. All receptor families, with the exception of the ion channel 5-HT₃ receptor family, are heptahelical receptors that are largely coupled to G-proteins and effectors as diverse as activation of Ca²⁺ and K⁺ channels, adenylyl cyclase, phospholipase C and the mitogen-activated protein kinases. For a current set of Reviews on 5-HT receptors, see a recent issue of Curr. Drug Targets CNS Neurol. Disorders (volume 3, issue 1, 2004).

Of particular interest to the cardiovascular system are the 5-HT₁B, 5-HT₂ receptor family (5-HT₂A and
5-HT in systemic hypertension: foe, friend or fantasy? 401

Figure 2  Classical neuronal model of the 5-HT synapse
SSRI, selective serotonin re-uptake inhibitors.

Table 1  Pharmacology of relevant cardiovascular 5-HT receptors
DOI, (+)-(4-iodo-2,5-dimethoxyphenyl)-2-aminopropane hydrochloride; 8-OH-DPAT, 8-hydroxy-2-(di-n-propylamino)tетrazin; mCPBG, m-chlorophenylbiguanide; 5-CT, 5-carboxamidotryptamine.

<table>
<thead>
<tr>
<th>5-HT receptor subtype</th>
<th>Agonist</th>
<th>Antagonist</th>
</tr>
</thead>
<tbody>
<tr>
<td>1A</td>
<td>8-OH-DPAT</td>
<td>WAY100635 and N-arom-190.</td>
</tr>
<tr>
<td>1B</td>
<td>Sumatriptan, L-694,247, F11356 and alniditan</td>
<td>GR55562, GR127935 and SB224289.</td>
</tr>
<tr>
<td>1D</td>
<td>Sumatriptan, zolmitriptan, F11356, L-694,247</td>
<td>BRL15572 and GR127935.</td>
</tr>
<tr>
<td>2A</td>
<td>ω-Methyl-5-HT and DOI</td>
<td>Ketanserin, LY33857, MDL100907 and sarapogrelate.</td>
</tr>
<tr>
<td>2B</td>
<td>ω-Methyl-5-HT and BW7232CB6</td>
<td>LY272015, SB204741, SB204553, LY33857 and SD2 SER-082.</td>
</tr>
<tr>
<td>3</td>
<td>2-Methyl-5-HT and mCPBG</td>
<td>Tropisetron, odansetron and LY278504.</td>
</tr>
<tr>
<td>4</td>
<td>BIMUB</td>
<td>GR113808 and SB204070.</td>
</tr>
<tr>
<td>7</td>
<td>5-CT</td>
<td>LY215840, SB205719 and clozapine.</td>
</tr>
</tbody>
</table>

5-HT2B, 5-HT3, 5-HT4 and 5-HT7 receptors. These receptors are found and are active in cardiovascular tissues [9], and are thus those receptors with which 5-HT is most likely to interact in a physiological situation.

PHYSIOLOGY OF 5-HT (Figure 3)

Blood
Blood elements, in particular platelets, are a rich source of 5-HT. 5-HT is avidly taken up by SERT in platelets [10] as platelets do not synthesize 5-HT. The function of 5-HT in the blood is to promote platelet aggregation and blood clotting. Although platelets contain 5-HT, they also possess 5-HT2A receptors which, when activated, promote further platelet activation and aggregation [11]. Thus 5-HT2A receptor antagonists such as ketanserin and sarapogrelate have proven effective anti-platelet/anti-thrombotic agents. Other cells which contain 5-HT include mast cells and macrophages. Interestingly, reports have described platelets in hypertensive subjects as being hyperaggregatable or more fragile [12–16].

Brain/sympathetic nervous system
5-HT acts as a neurotransmitter in the brain, where it is synthesized in distinct nuclei of the raphe, and serves functions as diverse as sleep, satiety and mood. Approx. 1–2% of body 5-HT is found in the brain, and 5-HT must be synthesized in the brain because 5-HT cannot cross the blood–brain barrier. Those areas responsible for synthesizing 5-HT are the clusters of cells in the midline/raphe regions of the pons and upper brainstem. 5-HT has also been localized immunohistochemically to the area postrema, caudal locus coeruleus and interpeduncular nucleus [17]. Collectively, these areas project to the medulla, spinal cord, telencephalon, diencephalon and cerebral cortex.

The central effects of 5-HT on the cardiovascular system are complex. Table 2 summarizes information garnered from work in the rat [18–28]. The effects of 5-HT
Figure 3  Effects of 5-HT on elements of the cardiovascular system

Table 2  Effect of 5-HT administration centrally on rat BP

<table>
<thead>
<tr>
<th>Species</th>
<th>Site (anaesthesia)</th>
<th>Drug/manipulation</th>
<th>Effect on BP</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rat</td>
<td>NTS (urethane)</td>
<td>5-HT</td>
<td>Decrease BP</td>
<td>[18]</td>
</tr>
<tr>
<td>Rat</td>
<td>NTS (chloralose)</td>
<td>5-HT</td>
<td>Increase BP</td>
<td>[19]</td>
</tr>
<tr>
<td>Rat</td>
<td>Dorsal raphe</td>
<td>Electrical lesion</td>
<td>Increase BP</td>
<td>[20]</td>
</tr>
<tr>
<td>Rat</td>
<td>Preoptic area (conscious)</td>
<td>(+)-8-OH-DPAT</td>
<td>Increase BP</td>
<td>[21]</td>
</tr>
<tr>
<td>Rat</td>
<td>Dorsal raphe (urethane)</td>
<td>DOI</td>
<td>Increase BP</td>
<td>[22]</td>
</tr>
<tr>
<td>Rat</td>
<td>i.c.v. (conscious)</td>
<td>5-HT</td>
<td>Increase BP</td>
<td>[23]</td>
</tr>
<tr>
<td>Rat</td>
<td>i.c.v. (conscious)</td>
<td>5-HT</td>
<td>Increase BP</td>
<td>[24]</td>
</tr>
<tr>
<td>Rat</td>
<td>Lateral ventricle (conscious)</td>
<td>5-HT</td>
<td>Increase BP</td>
<td>[25]</td>
</tr>
<tr>
<td>Rat</td>
<td>NTS</td>
<td>5-HT</td>
<td>Decrease BP (SHR fall &gt; WKY)</td>
<td>[24]</td>
</tr>
<tr>
<td>Rat</td>
<td>Cisternally</td>
<td>5,7-DHT</td>
<td>Normal hypertension</td>
<td>[27]</td>
</tr>
<tr>
<td>Rat</td>
<td>NTS</td>
<td>5,7-DHT</td>
<td>Increase BP</td>
<td>[28]</td>
</tr>
<tr>
<td>Rat</td>
<td>NTS (urethane)</td>
<td>5-HT</td>
<td>Decrease BP</td>
<td>[18]</td>
</tr>
</tbody>
</table>

vary according to the site in which 5-HT is administered and the anaesthetic status of the animal. In general, in the rat, central injection of 5-HT increases BP, although there are studies that suggest the opposite. Moreover, effects of central 5-HT in other species, primarily cat and dog, are different from the rat in that 5-HT largely causes a decrease in BP [29]. It is clear that central 5-HT has the capability of altering BP and appears to do so by interacting with both 5-HT$_{1A}$ and 5-HT$_2$ receptors [18–28]. It is unclear whether the central function of 5-HT is altered in hypertension or even whether 5-HT is necessary for development of hypertension. Studies which used toxins of serotonergic neurons, including 5,7- or 5,6-dihydroxytryptamine to destroy serotonergic nerve terminals, demonstrated that removal of central 5-HT did not alter the development or magnitude of hypertension.
in the 2K-2C (two kidney-two clip) rat [27], but did retard development of hypertension in young SHRs (spontaneously hypertensive rats; 6-weeks old) if given i.c.v. (intracerebroventricularly) [30].

Central levels of 5-HT have been reported as altered in hypertension. Specifically, 5-HT was increased at 2 weeks after DOCA (deoxycorticosterone acetate) administration in the rat [31], SHRs had greater levels of brain 5-HT compared with WKY (Wistar–Kyoto) rats [32], brainstem regions of SHRs showed a greater turnover of 5-HT compared with WKY rats [33], 5-HIAA concentration increased in Dahl salt-sensitive rats compared with resistant rats [34], stroke-prone SHRs had a greater concentration of brainstem 5-HT at a time (1.5 months) prior to or just at BP elevation [35], and basal release of 5-HT from the locus coeruleus in SHRs was double that of WKY rats [36]. Alternatively, 5-HT turnover has been reported to be decreased in the telencephalon and hypothalamus of SHRs compared with WKY rats [37]. Thus the central effects of 5-HT are complex, and are not clear cut in terms of the involvement of 5-HT in hypertension.

Heart
5-HT has pluripotent effects in the heart, outside of the well-documented effects in the coronary arteries [38]. Multiple receptors for 5-HT exist in the heart, including those directly on cardiac myocytes and on the vagus and sympathetic nerves. 5-HT stimulation of 5-HT3 receptors on the vagus nerves accounts for the decrease in heart rate through activation of the Bezold–Jarisch reflex. 5-HT can also act as a sympatholytic by activation of 5-HT1 receptors on sympathetic terminals, inhibiting noradrenaline release. However, 5-HT is a positive chronotrope directly in the rat through activation of 5-HT2A receptors, activation of 5-HT2 receptors in the pig and human and 5-HT7 receptors in the cat [39–41]. In isolated cardiomyocytes, 5-HT also stimulates mitogenesis with the 5-HT2B receptor critical for development of the heart in the mouse [42]. Little work has been done in terms of studying the effects of 5-HT in the hypertensive heart or 5-HT as a cause of heart damage in hypertension.

Kidney
Like the brain, the kidney has the ability to synthesize 5-HT from its precursor tryptophan [43–47], with the site of discrete synthesis being the proximal tubule. 5-HT is also a mitogen in mesangial cells [48], but its most profound effect in the kidney is to increase perfusion pressure by increasing renovascular resistance through arterial constriction. This has been demonstrated primarily in isolated perfused kidneys, although 5-HT is vasodilatory in the renal bed of the dog [49]. 5-HT and dopamine appear to play reciprocal effects in the kidney, where 1-5-HTP (1-5-hydroxytryptophan) and 1-DOPA use the same transporter to enter the proximal convoluted tubule, thereby compromising synthesis of the other substance. 5-HT causes sodium retention in the kidney, whereas dopamine promotes natriuresis [50]. Moreover, 5-HT and 5-HT1B receptor agonists stimulate the sodium/phosphate cotransporter to decrease phosphate retention, potentially contributing to renal failure [47].

The ability of 5-HT to increase renal perfusion pressure is enhanced in SHRs [51–54]. Interestingly, it was noted that the pressor response of isolated kidneys from SHRs desensitize to 5-HT at a slower rate compared with WKY rats [55] and that age provides for a similar lesser desensitization [53]. The finding that plasma levels of 5-HT in patients with renal dysfunction were nearly doubled compared with healthy controls has suggested that, in these patients, there is an impairment of 5-HT metabolism [56]. Little or no work has been done investigating the ability of 5-HT receptor antagonists to reduce target organ damage of the kidney in hypertension.

Blood vessels
Blood vessels, in particular arteries, serve an important function in providing a controlled feed of blood to tissues through creation of a resistance to blood flow or TPR. TPR is defined by the reactivity of small arteries and arterioles (\(\leq 200 \mu m\) in humans), where resistance to blood flow is determined by the calibre of the artery. This, in turn, is governed by the size of the lumen based on (i) thickness of arterial wall; (ii) reactivity of the smooth muscle to neuronal and endogenous hormones; and (iii) reactivity of the endothelial cell to endogenous hormones.

The majority of information as to the involvement of 5-HT in hypertension has been derived from in situ or in vitro studies of arteries from hypertensive animals or humans. Table 3 documents results of studies demonstrating, overall, a supersensitivity of isolated blood vessels to 5-HT in hypertension [57–76]. Supersensitivity is observed as a decrease in the lowest concentration of 5-HT necessary to cause contraction (threshold), a decrease in the potency of 5-HT (measured as an EC50 value) and/or an increase in the maximum contraction caused by 5-HT. A majority of these studies have been done in the rat, but a few studies in humans are available [77–82], and variable results are observed. In the majority of blood vessel studies, 5-HT2 receptors have been implicated in contraction to 5-HT. A few studies suggest the presence of 5-HT-stimulated endothelial cell relaxation through 5-HT1 and/or 5-HT1B receptors [83,84].

Whole body
When 5-HT is administered to a whole animal, the pattern of observed BP changes varies based on species. In the rat, intravenous 5-HT elicits a classical triphasic effect: an initial fast depressor (Bezold–Jarisch reflex via 5-HT3 receptor), a pressor response (smooth muscle
Refuting the involvement of 5-HT in hypertension

Several valid findings have been posed as reasons for supporting the lack of involvement of 5-HT in hypertension. First, free circulating plasma levels of 5-HT are relatively low (15–150 nmol/l compared with micromolar levels in whole blood [9]); lower levels have been observed, and there is always concern whether these measures are truly reflective of free 5-HT in the absence of aggregating or activated platelets. This free circulating 5-HT is the 5-HT that would interact with the blood vessels, and thus it has been argued that this is an insufficient level of 5-HT to activate 5-HT receptors normally expressed (primarily 5-HT2A receptors on smooth muscle and 5-HT1 receptors in the endothelial cell). The role of the relatively newly discovered vascular 5-HT7 receptor is less well understood. Secondly, there is little historical evidence that 5-HT is synthesized in arteries and serotonergic nerves in peripheral arteries have not been found.

Thirdly, there are a number of studies examining the effect of 5-HT receptor antagonists in treating high BP which have had negative outcomes. A majority of this work revolves around the use of ketanserin. This 5-HT2A receptor antagonist was touted as an effective antihypertensive strategy over two decades ago. Ketanserin lowered BP of normal and hypertensive subjects, including humans, but BP reduction was largely attributed to α1 adrenergic receptor blockade, not 5-HT2 receptor blockade [89–98]. Presently, ketanserin is used in a population of women with severe PIH (pregancy-induced hypertension) [99]. Additional studies using 5-HT2A receptor antagonists that lacked affinity for the α adrenergic receptors, such as LY53857 and cinanserin, did not lower BP in the rat [100]. Lowering of BP in anaesthetized SHRs by ketanserin or LY53857 seemed to be dissociated from 5-HT2 receptor blockade [101]. In the hypertensive human [102] and in SHRs [103], use of ritanserin did not lower BP (ritanserin lacks affinity for the α1 adrenergic receptor and has a high affinity for the 5-HT2A receptor; see PDSP K database at http://pdsp.cwu.edu/pdsp.asp). These studies suggest that endogenous activation of 5-HT receptors is not important for maintaining elevated BP.

Fourthly, studies using L-tryptophan feeding in SHRs demonstrated a dose-dependent decrease in BP [105], as did studies in DOCA-salt rats [106] and humans [107]. L-Tryptophan is the necessary precursor for 5-HT synthesis and should elevate 5-HT synthesis through law of mass action. Thus, if 5-HT endogenously increases BP, tryptophan should also do so by virtue of increasing endogenous 5-HT. In the latter study, salt intake was reduced by tryptophan, suggesting an activity to decrease in elevated BP.

Finally, depletion of 5-HT by PCPA (p-chlorophenylalanine), an irreversible inhibitor of TPH, did not...
lower the BP of SHRs [108]. Overall, these studies cast doubt as to the involvement of 5-HT in either initiating or maintaining elevated levels of BP.

**Supporting the involvement of 5-HT in hypertension**

For each of the five points made above, opposite outcomes have been observed, providing evidence for the ability of endogenous 5-HT to modulate vascular smooth muscle tone and thereby have the potential to alter TPR and BP.

Firstly, the local and/or circulating level of 5-HT can be considered sufficient to activate endogenous 5-HT receptors. Thrombotic events in which platelets aggregate can result in a high (micromolar) local concentration of 5-HT [10]. 5-HT can also be taken up by adrenergic nerves through the NET and is released upon neuronal stimulation [109]. Moreover, subcontractile concentrations of 5-HT amplify arterial contraction to vasoactive agonists such as Ang II (angiotensin II), ET-1 (endothelin-1) and noradrenaline, to name a few [110–112]. This is important as low concentrations of 5-HT (nanomolar) are able to modify arterial contraction to substances which control TPR (e.g. Ang II, ET-1 and noradrenaline). Thus 5-HT has significant effects on the contractile state of vascular smooth muscle directly and indirectly.

It is argued that, in the absence of a thrombotic event, levels of free (non-platelet bound) circulating levels of 5-HT are insufficient to activate vascular 5-HT receptors. This is true if the receptor considered is the 5-HT$_{2A}$ receptor, for which 5-HT has a $K_A$ of close to 3 µmol/l. However, 5-HT has a significantly higher affinity for the 5-HT$_{2B}$ receptor ($K_A = 10$ nmol/l), and this receptor is expressed in arterial smooth muscle. Importantly, the arterial 5-HT$_{2B}$ receptor is expressed to a significantly greater level in experimental hypertension and 5-HT$_{2B}$ receptor antagonists reduce experimental forms of rodent hypertension [74–76,113,114]. It should be noted, though, that at least one of the antagonists tested previously, LY53857, has equivalent affinity for 5-HT$_{2A}$ and 5-HT$_{2B}$ receptors and yet this compound was ineffective in reducing BP of SHRs [101]. Moreover, there is evidence that 5-HT levels, or at least 5-HT turnover (observed as increased 5-HIAA production and lower 5-HT), are increased in experimental and human forms of hypertension (Table 4); [115–126]. Reduced levels of platelet 5-HT and an increased plasma 5-HT or 5-HIAA, the MAOA metabolite of 5-HT, have been measured in essential hypertensive patients and multiple rat models of hypertension, including cyclosporine A-induced, erythropoietin-induced and NO (nitric oxide) synthase-inhibited models.

We have recently uncovered another potentially important means by which local arterial 5-HT levels may be modified and thereby influence arterial contraction and TPR [127]. The transporter for 5-HT (SERT) exists in endothelial cells and arterial smooth muscle cells of isolated large and resistance arteries (rat and mouse). SERT actively concentrates 5-HT in peripheral arteries, including superior mesenteric arteries and aorta, and also facilitates release of 5-HT. Thus a local arterial system of 5-HT release and uptake may occur, such that sufficient concentrations to at least amplify arterial responses to other hormones are highly possible. The function of this arterial system is supported by findings that, although the SERT inhibitor fluoxetine (Prozac®) is largely safe, there are reports of fluoxetine-induced acute pressor responses in the rat [128], a sustained hypertension during short-term (12-week) fluoxetine treatment in humans [129], frank hypertension in the rat [130] and use of fluoxetine to treat severe refractory orthostatic hypotension [131]. Interestingly, use of fluoxetine or inhibition of reuptake
inhibitors has been associated with a serotonin syndrome [132,133]. Serotonin syndrome is accompanied by an increase in mean arterial BP, suggesting that elevation of extracellular 5-HT is associated with a rise in BP.

Secondly, studies investigating the serotonergic innervation of peripheral arterial blood vessels were performed before the advent of PCR and antibody-based immunohistochemistry and Western analyses. The presence of TPH is considered a hallmark of 5-HT synthesis. It will be important to confirm/refute these studies to determine whether peripheral arteries, or any part of them, truly lack the ability to synthesize 5-HT.

Thirdly, there is a host of studies using 5-HT receptor antagonists as antihypertensive therapy which dissociate the \( \alpha \) adrenergic receptor blockade caused by ketanserin from 5-HT\(_{2A} \) receptor blockade or which demonstrate effectiveness of 5-HT receptor antagonists in lowering BP in the absence of appreciable \( \alpha \) receptor antagonism. This includes use of ketanserin in the DOCA-salt rat [134], use of the 5-HT\(_{2A} \) receptor antagonist sarpyrogrelate [135] and ketanserin [136]. One study [137], published in 1984, demonstrated a reduction in high BP by ketanserin in humans by 22%. This same concentration of ketanserin was not able to reduce a pressor response to the \( \alpha \) receptor agonist phenylephrine, indicating that the reduction in BP caused by ketanserin was independent of \( \alpha \) adrenergic receptor blockade. There are also specific forms of hypertension that have been described as 5-HT dependent. This includes an erythropoietin-driven model of hypertension [125] and cyclosporine-induced hypertension [123].

Fourthly, a number of studies suggest that tryptophan feeding, as a means to increase 5-HT synthesis, may promote higher BP. SHRs given L-tryptophan in their diet had an increased BP within 30 min of administration, and a maximal elevation of BP 60 min post-administration [138]. In a study published in 1991, Ito et al. [139] gave tryptophan to female stroke-prone SHRs prior to mating, and discovered that the offspring of these mothers had a higher BP; they were heavier and had a higher central nervous system load of 5-HT. These findings suggest, but do not prove, that an increased 5-HT level was associated with a higher systemic BP. An interesting point to make is that hypertension has been viewed as a BH\(_{4} \) (tetrahydrobiopterin)-deficient state, allowing for uncoupling of the BH\(_{4} \)-dependent NO synthase from NO to superoxide production [140,141]. It could be surmised that a reduced BH\(_{4} \) may result in a reduced synthesis of 5-HT. Could this, if experienced by a blood vessel, account for arterial supersensitivity? (Table 3). This is somewhat discordant with the findings in Table 4, describing increased turnover and/or plasma concentrations of 5-HT.

Finally, a few studies removing or destroying 5-HT have demonstrated a concomitant decrease of BP in hypertensive animals. In 1976, Buckingham et al. [142] demonstrated that a single injection of 400 mg/kg of body weight (intraperitoneally) of the TPH inhibitor PCPAME (p-chlorophenylalanine methyl ester; Figure 2) produced a fall in BP in DOCA-treated rats ranging from 20–43 mmHg within the first day of injection, and kept BP depressed for 8 days. PCPAME competes directly with TPH and binds irreversibly to the enzyme, hence the long-lasting effects of PCPAME. In the normal rat, a fall of 15–20 mmHg was observed only 8 days after injection of PCPAME; there was no immediate fall in BP. No measurements of 5-HT were made in plasma or arteries of the rats receiving PCPAME, and we propose that part of the fall in BP was because 5-HT was depleted in the periphery. This idea is supported by the findings that central 5-HT depletion by intracisternal injections of the serotonergic neurotoxin 5,6-DHT (5,6-dihydroxytryptamine) did not alter the course of development or magnitude of hypertension achieved in the deoxycorticosterone acetate (DOCA)-salt rat [143] or SHRs [144], nor did 5,6-DHT (i.c.v) alter the ability of PCPA to cause a reduction in BP [142]. Importantly, the study by Buckingham et al. [142] has not been repeated.

**UNANSWERED QUESTIONS**

The above discussions underline the controversy that continues to exist regarding the involvement of 5-HT in hypertension. There are several issues which need to be addressed and argued.

**Ability of 5-HT to activate the \( \alpha \) adrenergic receptor**

Purdy and co-workers [145,146] have demonstrated in the rabbit ear artery that 5-HT directly activates \( \alpha \) adrenergic receptors to cause arterial contraction. Although this occurs at relatively high concentrations of 5-HT (micromolar), this finding raises the possibility that 5-HT may exert effects on BP through not only activation of 5-HT receptors, but \( \alpha \) adrenergic receptors as well. This would thus call into question the interpretation of those studies in which 5-HT receptor antagonists with \( \alpha \) receptor affinity were used (ketanserin and nantenine; [147]). Are these antagonists effective because they block the activation of \( \alpha \) adrenergic receptors by catecholamines, 5-HT or both? It is unknown whether 5-HT activates \( \alpha \) adrenergic receptors in resistance arteries.

**Use of species outside of the rat and appreciation of species differences in the dependence on 5-HT**

A majority of the work presented in this review and available in the literature has used the rat as a model because both experimental and genetic models of hypertension are readily available. However, no mouse models...
have been used to investigate the role of 5-HT in hypertension. The group of Michael Bader has initiated studies using TPH1−/− mice for examination of whether peripheral 5-HT is necessary for BP development in DOCA-salt mice [148]. A number of genetically modified mice, including 5-HT1A, 5-HT1B, 5-HT2A, 5-HT2B, 5-HT2C, 5-HT3A, 5-HT4 and 5-HT7 receptor knockouts, as well as SERT-targeted mutated mice, have been produced and have thus opened up new avenues of research.

Truly definitive human studies and taking advantage of genetics
There is a paucity of human studies investigating the involvement of 5-HT in human hypertension. The advent of genetics in research has recently lent itself to discovery of polymorphism of the 5-HT2A receptor gene promoter in the human such that a sex-specific association in females expressing a T102C polymorphism was found to be an independent risk factor for increased BP [149]. This is, to our knowledge, the only study of its type.

Resolution of the effects of tryptophan feeding
Tryptophan feeding, although certainly providing for a law of mass action-driven increase in 5-HT, also provides for an increase in melatonin (N-acetyl 5-methoxytryptamine) synthesis. Melatonin is a vasodilator in a majority of arteries and is also an antioxidant [150]. Thus the effects of tryptophan feeding that are purely serotonergic are difficult to isolate in the face of increased melatonin synthesis, because results stimulated by 5-HT may be counteracted by those of melatonin.

Test of mixed receptor antagonists
Have we been studying the wrong receptor? Our laboratory has examined the role of the 5-HT2B receptor in hypertension, but these results have yet to be confirmed [113,114].

Genesis of supersensitivity
For decades, reactivity for 5-HT has been used as a marker of arterial disease. Arteries taken from subjects with diseases that include atherosclerosis, hypertension and diabetes are supersensitive to 5-HT [151–153]. Classically, supersensitivity has been observed when a stimulus (in this case 5-HT) has been absent and thus the system up-regulates receptors or other elements so as to be able to detect a small amount of the stimulus. If this is so, then how can it be argued that an increase, not a decrease, in 5-HT is important to hypertension? Why should the 5-HT2B receptor up-regulate and not down-regulate? These remain unsolved dilemmas.

Validation of the lack of ability of arteries to synthesize 5-HT
It is important to define, using current experimental tools, the ability of peripheral arteries to synthesize 5-HT. Regardless of outcome, this knowledge is important.

Understanding the function of intracellular 5-HT
With the discovery that 5-HT is taken up into an artery comes the question as to what happens to this 5-HT once inside the cell. Is it all metabolized to 5-HIAA? Is it stored intracellularly? Does it have a function intracellularly? This last question is particularly important given two recent studies. Firstly, intracellular function of hormones, such as Ang II, is increasingly recognized [154,155]. The entrance of a hormone inside a cell does not necessarily mean that the function of the hormone on that cells is complete. Secondly, intracellular 5-HT itself has been implicated in the function of the platelet. Bader and co-workers [156] demonstrated the ability of 5-HT, being acted upon by the enzyme transglutaminase, to covalently modify the protein RhoA and make this protein constitutively active. We are currently investigating the ability of 5-HT to be acted upon similarly in the arterial smooth muscle cell.

Understanding the function of arterial SERT and its association with cardiovascular disease
In affective disorders, SERT has been discovered as an important predictor of disease. The SERT promoter has been discovered to possess two variants, one long promoter (l) and one short (s) [157]. The long promoter has been associated with an increased expression and thus function of SERT, whereas the short promoter is associated with a decreased expression of SERT. There is a growing body of literature indicating that possession of the ss allele or treatment with 5-HT reuptake inhibitors, such as fluoxetine, may reduce the risk of myocardial infarction [158–160]. The same kinds of associations have not been made with respect to BP, and this therefore remains an open question.

Role of 5-HT and adrenal medullary function
In the 1970s and 1980s, a number of papers described the innervation of the adrenal medulla by serotonergic nerves and the location of 5-HT itself to catecholamine-containing vesicles in the medulla [161–164]. Moreover, 5-HT increases noradrenaline release from the medulla, and causes corticosterone to rise [165–169]. How could 5-HT potentially interplay with these important cardiovascular hormones and modify BP?
SUMMARY

One has to wonder what Erspamer and Page would think as to how far 5-HT has come since its discovery in the gastrointestinal system. More than just a hormone that promotes gut motility, 5-HT has been implicated in diseases as diverse as satiety, depression, pulmonary disease, carcinoid tumour and, as described here, hypertension. The new tools available to us as scientists have afforded us an opportunity to test rigorously the question posed: 5-HT in systemic hypertension: foe, friend or fantasy?

REFERENCES


© 2005 The Biochemical Society

Received 17 December 2004; accepted 31 January 2005
Published on the Internet 22 April 2005, DOI 10.1042/CS20040364

© 2005 The Biochemical Society