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Serotonin-induced Hypotension is Mediated by a Decrease in Intestinal Vascular Resistance

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ABSTRACT

Background: Long-term serotonin (5-hydroxytryptamine, 5-HT) infusion produces a sustained reduction in mean arterial pressure (MAP) and a decrease in total peripheral resistance (TPR) through a mechanism dependent on Nitric Oxide Synthase (NOS) in rats. This study sought to determine if the reduction in resistance induced by 5-HT is caused by NOS-dependent relaxation of vessels to all major organs of the body or in a specific vascular bed. **Materials and Methods:** Alzet mini-pumps containing vehicle (saline) or 5-HT (25 μg kg^{-1} min^{-1}) were implanted in male Sprague Dawley rats. A separate group of rats was treated with the NOS inhibitor Nω-nitro-L-arginine (LNNA; 0.5 g L^{-1}) prior to 5-HT administration. **Results:** 5-HT reduced MAP after 24 h (veh = 87±3, 5-HT = 75±2 mm Hg; $p<0.05$), a fall prevented by LNNA (LNNA/5-HT= 115±10 mm Hg). Yellow-green fluorescent microspheres, used to measure blood flow (300, 000 spheres; size 15 μm), were introduced into the left ventricle of the heart. Microspheres were recovered in arterial reference blood sample and organs using sedimentation through centrifugation. The intensity of dye extracted from the microspheres was measured spectrophotometrically. The 5-HT significantly decreased resistance to the spleen (48%) and small intestine (35%) compared to vehicle-treated animals; this decrease was abolished by LNNA. By contrast, resistance to the ear (175%) was increased by 5-HT. Resistance in brain, liver, lungs, kidneys and muscle did not show a statistically significant reduction by 5-HT. **Conclusion:** These findings underscore the importance of the splanchnic (particularly intestinal) circulation and NOS in 5-HT-induced reduction in blood pressure.

Key words: 5-HT, intestinal blood flow, blood pressure

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INTRODUCTION

While serotonin (5-hydroxytryptamine, 5-HT) has long been known as a vasoconstrictor, peripheral administration of 5-HT (25 μg kg^{-1} min^{-1}) to conscious rats over the course of one week and even one month causes a sustained decrease in blood pressure (Diaz *et al*., 2008; Davis *et al*., 2012, 2013). This finding could prove markedly important given that the precursor of 5-HT, 5-hydroxytryptophan, diminishes the development of deoxycorticosterone (DOCA)-salt hypertension (Fregly *et al*., 1987). The question is how this fall in blood pressure occurs.

The Nitric Oxide Synathase (NOS) inhibitor Nω-nitro-L-arginine (LNNA) abolished the ability of 5-HT to lower blood pressure (Diaz *et al*., 2008; Tan *et al*., 2011). This suggests that arterial relaxation to 5-HT, likely endothelium and NOS-dependent, mediates the 5-HT-induced fall in blood pressure. However, studies support that 5-HT does not cause a direct relaxation or endothelium-dependent relaxation when tested in rat aorta, superior mesenteric artery or resistance arteries (Davis *et al*., 2012). It is possible the arterial bed that mediates the hypotension has not been studied. The present study addresses the identity of the vascular site(s) contributing to the fall in blood pressure; could it be shown that 5-HT increased flow to particular organs, underscoring a fall in blood pressure? Use of fluorescent microspheres to track blood flow allowed for testing, in many different tissues, the ability of 5-HT to change blood flow (Deveci and Egginton, 1999; Glenny *et al*., 1993). The present study tests the working hypothesis that chronic 5-HT would increase blood flow (decrease resistance) in multiple vascular beds, with the hope of identifying a site of action of 5-HT to decrease blood pressure. Because LNNA abolished 5-HT-induced chronic hypotension, a group of animals treated with 5-HT and LNNA are included.

MATERIALS AND METHODS

Materials and animals: Unless otherwise noted, all chemicals were obtained from Sigma Chemical Company (St. Louis, MO USA). Yellow-Green

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microspheres (15 μ diameter, 10^6 beads mL^{-1}) were obtained from Life Technologies (Grand Island, NY USA). All protocols were approved by the MSU Institutional Animal Care and Use Committee and follow the "Guide for the Care and Use of Laboratory Animals", 8th edition, 2011. Male Sprague Dawley rats (Charles River Laboratories, 225-250 g) were used. Some rats received LNNA (0.5 g L^{-1}; protected from light) in their drinking water one week prior to implantation of pumps.

Surgery: Under isoflurane anesthesia, radiotelemeter transmitters (PA-C40; Data Sciences International, MN) were also implanted subcutaneously through a 1-1.5 cm incision in the left inguinal area as previously described (Diaz *et al*., 2008). Rats were allowed 3-4 days to recover post-operatively and then 3-4 days of baseline measurements were made. At this time, osmotic pumps (Model 2ML1, Alzet Osmotic Pumps) were implanted subcutaneously between the scapulae, also under anesthesia. Mean arterial pressure, pulse pressure and heart rate were recorded for 10 sec every 10 min using a commercially available data acquisition system (Dataquest, DSI). Pumps contained either vehicle (sterile saline with 1% ascorbate, pH balanced to between 6-7) or 5-HT (25 μg kg^{-1} min^{-1}, s.c.).

Microsphere delivery and quantitation: Yellow-green fluorescent microspheres (sonicated; 300,000 total in 0.3 mL volume; 15 μ diameter) were delivered in a glass-glass Hamilton syringe over a few seconds, followed by a 0.6 mL flush of saline, into the right carotid artery of the anesthetized rat. This microsphere load does not affect cardiovascular parameters itself (Kobayashi *et al*., 1994). The left femoral arterial catheter was used for blood pressure determination (Powerlab; ADInstruments, CO USA) and for reference blood withdrawal. A perfusion pump run in withdrawal mode (New Era Pump Systems NE-4000) was begun 30 sec prior to ventricular injection of the microspheres at a rate of 0.5 mL min^{-1}. Two and 1/2 min after microsphere injection, tissues were removed and weighed. These included adrenals, heart, spleen, liver, lungs, right kidney, left kidney, small intestine (duodenum), leg muscle, leg skin, visceral fat, brain and ears. Yellow-green spheres were reclaimed through the sedimentation method after 1 M KOH digestion (60°C, overnight) following Triton Technologies Protocols (www.physiology.com; sedimentation protocol). Acidified cellosolve acetate [10 μL HCl/100 mL Cellosolve (2-ethoxyethyl acetate); 250 μL] was added to each dried sample, samples vortexed and sat for 15 min to dissolve the fluorescent coating. Fluorescent supernatant was recovered through centrifugation. Standard curves were generated for yellow-green beads (0 to 10,000 beads mL^{-1}). Fluorescence of samples was read on a white 96 well plate (Packard Optiplate, 6005290, Perkin Elmer, Walther MA, USA) for use in a Labscan Fluoroskan Ascent FL plate reader using an excitation wavelength of 485 nm and emission wavelength of 510 nm.

Data analyses: All points represent Mean±SEM for the number of animals (N) reported. For blood pressure data analysis over time, within group differences were assessed by a one-way repeated measures ANOVA with post-hoc multiple comparisons using Dunnett's procedure (GraphPad Instat 3). Between group differences were assessed by a two-way mixed design ANOVA and post-hoc testing at each time point was performed using Bonferroni's procedure to correct for multiple comparisons (GraphPad Prism 5):

$$\text{Blood flow to organ of interest (i)} = \text{Reference blood flow } (0.5 \text{ mL min}^{-1}) \times \frac{\text{Fluorescence organ of interest (i)}}{\text{Fluorescence of reference blood}}$$

$$\text{Organ vascular resistance} = \frac{\text{Mean arterial pressure}}{\text{Organ blood flow [mm Hg/(mL min g)]}}$$

In all cases, a p-value of <0.05 was considered significant.

RESULTS

5-HT caused a chronic fall in mean arterial blood pressure in conscious, unrestrained rats (Fig. 1a). The nadir of blood pressure fall occured between days 1 and 2 (24-48 h). It was at this time (24 h) that microspheres were infused to anesthetized rats. Under anesthesia, baseline mean arterial blood pressure was lowered significantly by 24 h of 5-HT infusion when compared to vehicle-infused rats (75.4±2.0 mm Hg vs. 87.3±3.5 mm Hg, $p<0.05$). LNNA given one week prior to infusion with 5-HT abolished the fall in blood pressure (115±10 mm Hg, $p<0.05$). Animals were euthanized for tissue procurement after microsphere administration.

The mass of the tissue samples taken between vehicle and 5-HT-infused groups was statistically similar (not shown) and the standard curves for yellow-green microspheres were robust with an r value of 0.996 (N=10 separate experiments). The microsphere burden in blood samples was not different between the vehicle, 5-HT or 5-HT/LNNA rats (~500-600 microspheres/0.5 mL). Similar basal flow to the left (2.48±0.42 mL min^{-1} g^{-1}) and right kidneys (2.31±0.40 mL min^{-1} g^{-1}; $p>0.05$) validated the even circulation of microspheres through the body.

Blood flow was significantly elevated in the spleen and in the small intestine of animals infused with 5-HT

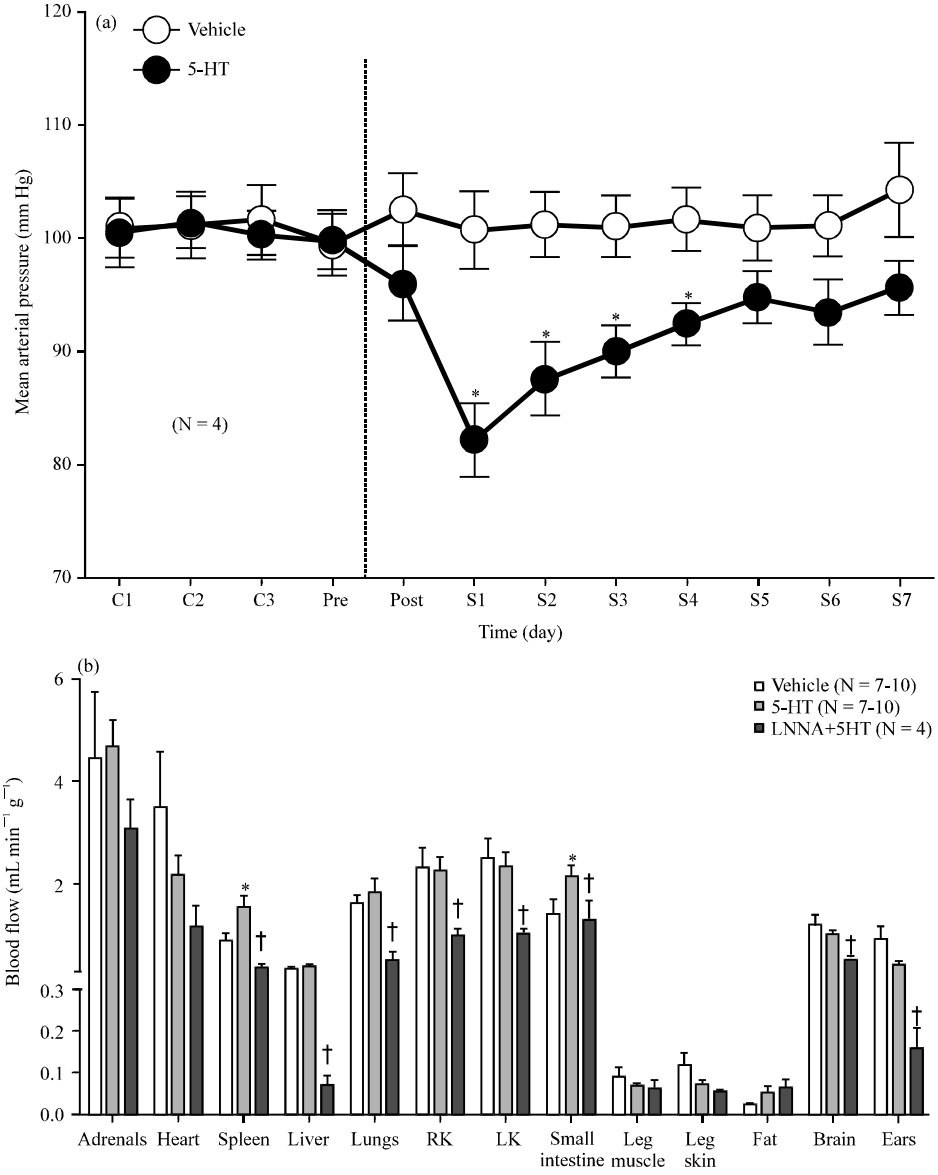

Fig. 1(a-b): (a) Effect of vehicle or 5-HT (25 μg kg^{-1} min^{-1}) in normal male Sprague dawley rats over the course of 10 days. Points represent Mean±SEM for the number of animals in parentheses. *indicate statistically significant differences from control values prior to pump implantation, $p<0.05$) and (b) Effect of a one week administration of LNNA on collective blood flows in animals receiving vehicle (open bars) or 5-HT (black or gray bars). Points and bars represent Mean±SEM for the number of animals in parentheses. *statistically different from vehicle, † different from 5-HT-infused

compared to vehicle (Fig. 1b). A group of LNNA treated animals in which vehicle was placed in the pumps was created but a majority of these animals died during placement of catheters for microspheres due to the fragility of the blood vessels; we do not have these data to share. The increase in flow to 5-HT observed in both the intestine and spleen was abolished by LNNA. By knowing individual animal blood pressures, resistances [mm Hg/(mL min^{-1} g^{-1}] from measured blood flow could be calculated. For the small intestine, vehicle resistance was 61.89±10.64, 5-HT-infused 39.83±4.93 and LNNA+5-HT-infused

103.31+25.79 ($p<0.05$). Similarly, in the spleen vehicle resistance was 113.19±18.39, 5-HT-infused 58.26±7.71 and LNNA+5-HT infused 323.4+72.71 ($p<0.05$).

DISCUSSION

This study is important because 5-HT caused the mean arterial blood pressure of experimentally hypertensive rats to drop over 50 mm Hg, compared to approximately 20 in the sham group (Diaz *et al*., 2008). 5-HT virtually normalized blood pressure. Moreover, the administration of 5-HT for a longer, 30-day period of time continued to depress blood pressure in hypertensive rats (Davis *et al*., 2013). These findings support the potential use of 5-HT, precursors of 5-HT such as 5-hydroxytryptophan, or drugs that modify 5-HT concentration in treatment of elevated blood pressure. 5-HT did not cause direct arterial relaxation in large and small resistance mesenteric arteries (Davis *et al*., 2012). This suggests the *in vivo* mechanism for 5-HT to cause blood pressure to fall is: (1) In a bed different than those previously measured and (2) Involves mechanisms not previously considered. Use of microspheres *in vivo* allowed us to encompass both possibilities.

Qualitatively and quantitatively, the blood flows observed in this study are consistent with published results (Gervais *et al*., 1999; Mishra *et al*., 2010). In the rats receiving 5-HT chronically, an increase in splenic and small intestinal blood flow was consistently observed. Liver blood flow, also a part of splanchnic circulation, was not modified by 5-HT. LNNA abolished the elevated flow and reduced blood pressure caused by 5-HT, suggesting that the fall in resistance in the intestinal/splenic beds may be a mechanism by which 5-HT lowers blood pressure. LNNA did not lower the flow in all tissues (e.g., adrenal, leg muscle) such that one could argue a global and insurmountable vasoconstriction was not established (Takahashi *et al*., 1995).

How can the 5-HT-induced changes in splenic and intestinal blood flow be understood relative to changes in blood pressure? It would be surprising for the spleen to have large control over blood pressure as a significant but small (5%) of cardiac output is received by the spleen (Mendell and Hollenberg, 1971). By contrast, the activities of 5-HT in the intestine, outside of changing gut motility, are better understood. Biber *et al*. (1973) demonstrated that 5-HT given acutely increased intestinal blood flow in the cat in a tetrodotoxin (TTX)-sensitive manner and use of TTX converted a typically vasodilatory response into a vasoconstrictor response. Splanchnic blood flow is recognized to have the ability to modify blood pressure (Mishra *et al*., 2010). 5-HT has been suggested to sensitize β adrenergic receptors (Fozard and Leach, 1968) and to antagonize α adrenergic receptors to promote vascular relaxation. However, interference with either the α or β adrenergic receptors would be observed as a more global (not tissue specific) increase in flow/decrease in resistance and this was not observed. 5-HT must work in through other mechanisms.

There are two new ideas that emanate from this study. First, 5-HT could inhibit sympathetic neuroeffector junctions to decrease sympathetic tone and vascular resistance, an effect observed in a number of different vascular beds (Engel *et al*., 1983; Gothert *et al*., 1986; Molderings *et al*., 1987). Garcia-Pedraza *et al*. (2013) recently described that activation of the 5-HT_{1D} and 5-HT_{7} receptor inhibited sympathetic neurotransmission in rats treated with the 5-HT_{2} receptor antagonist sarpogrelate (2013). Importantly, the $5\text{-HT}_{1B/1D}$ receptor has been linked to NOS (Fujita *et al*., 2004). Second, the contributions of the venous circulation to the increase in flow caused by 5-HT should be considered. The simplest difference to point to between the present observation of a 5-HT-induced increase in intestinal flow but lack of direct 5-HT-induced arterial relaxation is 5-HT-induced venous relaxation. If veins increase their capacitance, then more flow can occur. Studies of isolated veins support the ability of 5-HT to cause relaxation and to involve NO (Datte and Offoumou, 2004; Ellis *et al*., 1995). This does not mean that 5-HT does not also have the ability to contract veins but that 5-HT possess both contractile and relaxant activity in these vessels. This is an exciting possibility given new attention paid to the importance of venous capacitance to blood pressure determination (Fink *et al*., 2000).

CONCLUSION

There is now substantial evidence that *in vivo* 5-HT reduces blood pressure. The present findings suggest that 5-HT interacts to increase intestinal blood flow by reducing resistance in these tissues. It is a goal to understand what is presumably a receptor-initiated mechanism set into motion by 5-HT to change intestinal flow and blood pressure.

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