Receptor mechanisms of serotonin-induced prenodal lymphatic constriction in the canine forelimb

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Dobbins, David E. Receptor mechanisms of serotonin-induced prenodal lymphatic constriction in the canine forelimb. Am. J. Physiol. 274 (Heart Circ. Physiol. 43): H650–H654, 1998.—Numerous endogenous vasoactive agents have been shown to cause lymphatic smooth muscle contraction. In this study, we assessed the ability of serotonin (5-HT) to alter lymphatic smooth muscle activity and elucidated the receptor mechanisms of 5-HT’s actions. Both intralympathic and intra-arterial administration of 5-HT significantly increased lymphatic smooth muscle activity in lymphatics perfused at constant flow, as indicated by an increase in lymphatic perfusion pressure. The 5-HT-induced increase in lymphatic perfusion pressure is attenuated but not blocked by the intra-arterial infusion of phentolamine, suggesting the involvement of α-adrenoreceptors and 5-HT receptors. Intralymphatic infusion of the 5-HT2-receptor-agonist α-methylserotonin significantly increased lymphatic perfusion pressure, either alone or when administered into an α-receptor blocked preparation, whereas the 5-HT1-receptor-agonist carboxamidotryptamine maleate did not effect the prenodal lymphatics. These data indicate that the lymphatic smooth muscle contractile response produced by 5-HT is mediated both by lymphatic α-adrenoreceptors and 5-HT2 receptors.

lymphatic contractility; lymphatic smooth muscle; transvascular fluid flux; prenodal lymphatics; lymphatic function

THE LYMPHATIC SYSTEM is crucial to the maintenance of circulatory homeostasis in that it returns critical amounts of fluid and protein to the circulation daily. Several studies have suggested that alterations in lymphatic smooth muscle activity play a pivotal role in determining lymph flow, hence impacting lymphatic function under both normal and pathophysiological conditions (13, 14, 16, 18). We have previously shown that lymphatic smooth muscle contracts in response to the intralymphatic administration of numerous endogenous vasoactive agents, including epinephrine, norepinephrine, acetylcholine, histamine, bradykinin, prostaglandins, endothelin-1, platelet-activating factor, and neurokinin A (4, 5, 7–10). Additionally, intra-arterial infusion of many of these agents also results in significant lymphatic smooth muscle contraction.

To date, the receptor mechanisms by which endogenous vasoactive agents produce prenodal lymphatic smooth muscle contraction and relaxation in vivo have not been adequately addressed. Ascertain the specific receptor mechanisms by which endogenous agents contract or relax lymphatic smooth muscle will help to identify pharmacological targets (receptors) through which the drainage function of the lymphatic system could be enhanced to clinically manage edema. Previous studies have shown that the receptor mechanisms involved in the mediation of lymphatic constriction can be studied by administering vasoactive agents intralymphatically before and during intra-arterial administration of appropriate receptor antagonists. By this approach, we have shown that constriction of prenodal lymphatics by either epinephrine or norepinephrine can be blocked by the intra-arterial infusion of the equipotent α1- and α2-receptor-antagonist phentolamine (6). These data indicated that epinephrine- and norepinephrine-induced lymphatic constriction is mediated through stimulation of prenodal lymphatic α-receptors. Additionally, since the specific α1-receptor-antagonist prazosin attenuates but does not completely block epinephrine and norepinephrine-induced lymphatic smooth muscle activation, it appears that both α1- and α2-receptors are involved. This interpretation of the antagonist data was confirmed by the fact that both the α1-receptor-agonist phenylephrine and the α2-receptor-agonist α-methyl norepinephrine also significantly constrict prenodal lymphatics.

Serotonin (5-HT) has been proposed to play a significant role in both normal and pathophysiological circulatory conditions, such as ischemic heart disease (where loss of 5-HT-induced vasodilation is subsequently to down-regulation of the endothelial nitric oxide system may contribute to pathogenesis), control of cerebral blood flow, and changes in the permeability of the blood-brain barrier, microcirculatory adjustments in pancreatitis, migraine (where chronically low systemic 5-HT levels predispose patients to migrainous headache), and altered permeability of the hepatic sinusoids (2, 3, 11, 21). The potential impact of 5-HT on lymphatic function under either normal or pathophysiological conditions has not yet been adequately examined.

The receptor mechanisms through which 5-HT interacts with the vasculature are complex. 5-HT has been shown to interact with α- and β-adrenergic receptors as well as with 5-HT1A, 5-HT1B, 5-HT1C, and 5-HT2 receptors. The vasodilatation seen in blood vessels in many beds is mediated through the interaction of 5-HT with the 5-HT2 receptor. However, controversy exists in the literature. The vasorelaxation seen in such preparations as the human or canine basilar artery has been attributed to 5-HT2 receptors by some investigators and 5-HT1 receptors by others. The receptor actions of 5-HT in the prenodal lymph vessels have not yet been determined. In this study, we report both the actions of 5-HT on prenodal lymphatic smooth muscle and the receptor mechanisms operant in 5-HT-mediated prenodal lymphatic constriction.

METHODS

Adult mongrel dogs of either sex were anesthetized with pentobarbital sodium (35 mg/kg iv and supplemented as
needed) and were intubated and ventilated with a positive-pressure ventilator. A femoral vein and artery were cannulated for the administration of supplemental anesthetic and for drawing arterial blood from which the lymphatic perfusate was made. Blood from this artery was then delivered through polyethylene tubing to the brachial artery of the right forelimb at a constant flow with a pressure-independent roller pump (Cole-Parmer model 7520–20). In the experiments involving the intra-arterial infusion of 5-HT, the blood flow from the pump was measured at the end of the experiments to allow for the calculation of plasma 5-HT concentrations attained at each concentration infused. A small ventral metacarpal artery and a superficial dorsal metacarpal vein in the paw were cannulated for the measurement of small artery and vein pressures. Forelimb perfusion pressure was measured from a cannulated side branch of the brachial artery. Systemic arterial pressure was measured through a catheter inserted retrograde into the brachial artery upstream to the cannula used to perfuse the forelimb. An external jugular vein was cannulated with a cardiac catheter, the tip of which was positioned in or just upstream to the right atrium, for the measurement of central venous pressure.

A small lymph vessel on the dorsal surface of the paw was cannulated in the direction of normal lymph flow (4) with a section of polyethylene tubing (PE-10). The other end of this catheter was attached to a 30-gauge hypodermic needle and connected through two three-way stopcocks to 5-ml syringes. The first stopcock was altered such that all three ports were confluent. The 30-gauge needle was attached to the first port. The middle port was connected to a transducer for the measurement of pressure, and the third port was attached to the second stopcock. This second stopcock allowed flow to be delivered from one or the other of two syringes by a double-channel (Harvard Apparatus model 940) infusion pump. The lymph vessels were perfused at constant flow at a volume flow rate of 0.034 ml/min. The perfusate used to perfuse the lymphatic was made by mixing freshly drawn autologous arterial blood with heparinized tricarboxylic acid solution (1:1) and centrifuging it to obtain a diluted supernatant plasma.

Because the lymphatics are connected to the venous system, changes in central venous pressure could effect lymphatic volume and hence lymphatic pressure. Thus we monitored central venous pressure from an indwelling cardiac catheter. All pressures were measured continuously with low-volume displacement transducers (Statham P-23 GB) and recorded on a direct-writing oscillograph (Hewlett-Packard model 7758B).

All drugs were prepared fresh daily, immediately before use. 5-HT, the 5-HT1 agonist 5-carboxyamidotryptamine maleate, and the 5-HT2 agonist α-methylserotonin were prepared in normal saline in a stock solution of 1.5 mg/ml. The appropriate final dilutions of these drugs for intralymphatic administration were made by adding the artificial lymph solution immediately before infusion of the drug. Final dilutions for the intra-arterial infusion of 5-HT were made by adding the appropriate amount of saline to an aliquot of the stock solution. In the α-adrenoceptor studies, phentolamine was diluted in normal saline and infused into the arterial blood supply to the forelimb via a needle-tipped catheter.

The experimental protocol was as follows. After the completion of all cannulations, the lymphatic vessel was perfused with control perfusate for a minimum of 15 min to ensure that all measured pressures had achieved steady-state values. In the 5-HT intralymphatic experiments (n = 7), the lymph vessel was then perfused with perfusate containing 5-HT in a concentration of $9.32 \times 10^{-9}$, $9.32 \times 10^{-8}$, $9.32 \times 10^{-7}$, and $9.32 \times 10^{-6}$ M for a minimum of 15 min at each concentration or until the peak pressure had been obtained. After the infusion of each concentration of 5-HT, the lymph vessel was perfused with control perfusate until the lymphatic perfusion pressure returned to control values before proceeding to the next highest concentration of 5-HT. In the 5-HT intra-arterial experiments (n = 7), the lymphatic was perfused continuously with control perfusate. 5-HT solutions were infused intra-arterially to obtain plasma concentrations of $6.22 \times 10^{-10}$, $6.22 \times 10^{-9}$, $6.22 \times 10^{-8}$, and $6.22 \times 10^{-7}$ M for a minimum of 15 min or until the peak change in lymphatic pressure had been obtained. Between each infusion of 5-HT, sufficient time was allowed for all measured pressures to return to control values. In the 5-HT and phentolamine experiments (n = 7), 5-HT was first given intralymphatically at a concentration of $9.32 \times 10^{-7}$ M to establish a control 5-HT response in these animals. The lymphatic was then infused with control perfusate until all pressures returned to control values. Phentolamine was then infused at 400 µg/min intra-arterially. Five minutes after beginning the phentolamine infusion, the intralymphatic infusion of 5-HT was repeated during the continued infusion of phentolamine. In the 5-HT receptor agonist studies, the 5-HT1 agonist 5-carboxyamidotryptamine maleate was infused intralymphatically (n = 7) in a concentration of $7.65 \times 10^{-7}$, $7.65 \times 10^{-6}$, and $7.65 \times 10^{-5}$ M. The 5-HT2 receptor agonist α-methylserotonin was infused intralymphatically (n = 7) in a concentration of $7.34 \times 10^{-8}$, $7.34 \times 10^{-7}$, $7.34 \times 10^{-6}$, and $7.34 \times 10^{-5}$ M. In a final series of experiments, α-methylserotonin was infused intralymphatically (n = 9) in a concentration of $7.34 \times 10^{-5}$ M before and during the intra-arterial infusion of phentolamine at 400 µg/min to determine if the lymphatic constriction seen with the 5-HT2 agonist was mediated in part by lymphatic α-adrenoceptors.

All data were analyzed using Student's t-test, as modified for paired replicates. The pressures obtained during the peak of the response were compared with those obtained immediately before the administration of any drug.

**RESULTS**

The intralymphatic infusion of 5-HT (Fig. 1) significantly increased lymphatic perfusion pressure at the three highest concentrations infused. These data indi-
cate that the threshold concentration necessary to produce lymphatic constriction lies between $9.32 \times 10^{-2}$ and $9.32 \times 10^{-1} \text{M}$. At the two highest concentrations infused, the lymphatic perfusion pressure more than doubled. The intra-lymphatic infusion of 5-HT had no significant effect on mean systemic, forelimb perfusion, skin small artery, skin small vein, or central venous pressures.

The intra-arterial infusion of 5-HT (Fig. 2) likewise significantly increased lymphatic perfusion pressure at the three highest concentrations infused, indicating that the threshold concentration of 5-HT necessary to produce significant lymphatic constriction when 5-HT is administered into the bloodstream was between $6.22 \times 10^{-10}$ and $6.22 \times 10^{-9} \text{M}$.

The infusion of 5-HT intralymphatically at a concentration of $9.32 \times 10^{-7} \text{M}$ before phentolamine (Fig. 3) significantly increased lymphatic perfusion pressure from a control value of 5 mmHg to a peak value of 11.5 mmHg. The infusion of phentolamine intra-arterially did not significantly affect lymphatic perfusion pressure (data not shown), and the repetition of the same dose of 5-HT intralymphatically in the presence of $\alpha$-adrenoreceptor blockade still significantly increased lymphatic perfusion pressure; however, the increase seen was significantly less (Student’s t-test as modified for paired replicates) than that seen before $\alpha$-receptor blockade.

The intralymphatic infusion of the 5-HT$_1$-receptor-agonist carboxyamidotryptamine maleate at three concentrations failed to significantly alter lymphatic perfusion pressure (Fig. 4).

Intralymphatic infusion of the 5-HT$_2$-receptor-agonists 5-methylserotonin at four concentrations (Fig. 5) resulted in significant increases in lymphatic perfusion pressure at the three highest concentrations infused, indicating that the threshold concentration needed to produce significant lymphatic constriction lies between $7.34 \times 10^{-8}$ and $7.34 \times 10^{-7} \text{M}$. At the highest concent-

![Fig. 2. Intra-arterial infusion of 5-HT. *P < 0.05, paired t, comparing the peak response (hatched bars) with the corresponding control (open bars).](image)

![Fig. 3. Intralymphatic infusion of 5-HT before and during an intra-arterial infusion of phentolamine. *P < 0.05, paired t, comparing the peak response (hatched bars) with the corresponding control (open bars).](image)

![Fig. 4. Intralymphatic infusion of the 5-HT$_1$-receptor-agonist carboxyamidotryptamine maleate. Hatched bars, peak response; open bars, corresponding control.](image)

![Fig. 5. Intralymphatic infusion of the 5-HT$_2$-receptor-agonist $\alpha$-methylserotonin. *P < 0.05, paired t, comparing the peak response (hatched bars) with the corresponding control (open bars).](image)
Fig. 6. Intralymphatic infusion of the 5-HT2-receptor-agonist \( \alpha \)-methylserotonin before and during intra-arterial infusion of phenotolamine. \( * P < 0.05 \), paired \( t \), comparing the peak response (hatched bars) with the corresponding control (open bars).

**DISCUSSION**

It is well established that numerous vasoactive agents are capable of altering lymphatic smooth muscle tone in a variety of either in vitro or in vivo experimental preparations. Ohhashi et al. (18–20) have made an extensive study of the contractile responses of the longitudinal smooth muscle of isolated bovine mesenteric lymphatics in vitro. They reported that contraction of lymphatic smooth muscle could be induced by 5-HT, prostaglandin \( \mathrm{F}_2 \), norepinephrine, histamine, dopamine, and acetylcholine. These authors concluded (18), in agreement with a number of previous authors, that contraction of lymphatic smooth muscle likely plays a major role in the propulsion of lymph under physiological conditions. A number of investigators have reported the effects of catecholamines or nerve stimulation on lymphatic vessels. Browse (1) reported static increases in pressure in ligated efferent vessels of the popliteal lymph node in greyhounds during stimulation of the lumbar sympathetics. McHale and Roddie (16) have shown that catecholamines in nanogram quantities increased the frequency and decreased the amplitude of the spontaneous contractions observed in isolated bovine mesenteric lymphatics in vitro. Hayashi et al. (12) reported that hemorrhage resulted in a significant increase in lymphatic pumping in mesenteric lymphatics of anesthetized sheep. McHale and Roddie (17) reported that the intravenous infusion of norepinephrine in conscious sheep increased the frequency of lymphatic contractions and increased lymph flow in popliteal, prefemoral, and mesenteric efferent lymphatic vessels. We have previously reported that a wide array of endogenous vasoactive agents are capable of constricting prenodal lymphatic vessels in the canine forelimb in vivo. These agents include epinephrine, norepinephrine, endothelin-1, prostanoids, acetylcholine, bradykinin, histamine, and neurokinin A (4, 5, 7–10). The results of the current study clearly demonstrate that 5-HT is likewise a member of the pool of endogenous vasoactive agents that can cause activation of prenodal lymphatic smooth muscle. Both the intralymphatic (Fig. 1) and intra-arterial (Fig. 2) infusion of 5-HT result in significant lymphatic constriction. Previous work has revealed that the threshold concentrations of intralymphatically administered vasoactive agents required to produce significant lymphatic constriction varies from the extremely potent endothelin-1 (10\(^{-10}\) to 10\(^{-12}\) M) to markedly less potent agents, such as prostaglandin \( \mathrm{E}_2 \) and arachidonic acid (10\(^{-8}\) to 10\(^{-3}\) M). The results of the current experiments indicate that the threshold concentration of intralymphpatic 5-HT (Fig. 1) lies in the range of 10\(^{-9}\) to 10\(^{-8}\) M. Thus it would appear that 5-HT, when given intralymphatically, is one of the more potent activators of lymphatic smooth muscle. The threshold concentration of intra-arterial 5-HT (Fig. 2) lies in the 10\(^{-10}\) to 10\(^{-9}\) M range.

The current study demonstrates that the lymphatic constriction produced by intralymphatic 5-HT is significantly attenuated by the intra-arterial infusion of phentolamine (Fig. 3), suggesting a role for lymphatic \( \alpha \)-receptors in 5-HT-mediated lymphatic constriction. The lymphatic constriction seen to remain in the \( \alpha \)-receptor-blocked preparations subsequent to 5-HT infusion could indeed be the result of lymphatic 5-HT receptors, and the experiments with the 5-HT receptor agonists confirm this notion. Although the intralymphatic infusion of the 5-HT1-receptor-agonist 5-carboxyamidotryptamine maleate is without effect (Fig. 4), infusion of the 5-HT2-receptor-agonist \( \alpha \)-methylserotonin (Fig. 5) does produce significant lymphatic constriction. These data indicate the presence of 5-HT2 receptors in the prenodal lymphatic vessels. However, because it was already shown that 5-HT itself interacts with the \( \alpha \)-receptors contained within the lymphatics, it was necessary to establish that the 5-HT2-receptor-agonist would also produce constriction in an \( \alpha \)-blocked preparation. As can be seen in Fig. 6, \( \alpha \)-methylserotonin does indeed still constrict lymphatics after the lymphatic \( \alpha \)-receptors have been blocked by phentolamine. These data then serve to corroborate that the prenodal lymphatics do contain 5-HT2 receptors and that the constriction seen with intralymphatic infusion of 5-HT is indeed likely mediated through a combination of \( \alpha \)-adrenergic receptors and 5-HT 5-HT2 receptors.

The results of this study, previous studies in the canine forelimb, and another in vivo and in vitro experiments clearly demonstrate that many endogenous vaso-
active substances can alter lymphatic smooth muscle tone and hence impact lymphatic vessel function. Previous reports indicate that, while many similarities exist between the manner in which lymphatic smooth muscle and vascular smooth muscle respond to vasoactive agents, differences also exist. For example, the potent vasodilators histamine, bradykinin, and acetylcholine all constrict lymphatic vessels in the canine forelimb (9). Whether these differences are a reflection of differing receptor profiles between the two smooth muscle populations or differences in the endothelial cell/ smooth muscle cell axis remains to be determined. Accumulated data on lymph vessel function and lymphatic vessel receptor populations suggest that it may be possible to develop a class of drugs that interact with the lymphatic system to enhance its function in disease states. Thus alterations in transvascular fluid flux in the numerous disease states in which it is manifest could be treated with a double-pronged therapeutic approach, both by anti-inflammatory drugs that restore microvascular permeability to macromolecules and by drugs that enhance the ability of the lymphatic system to transport fluid. Clearly, perturbations in the lymphatic system can cause or exacerbate edema formation in numerous disease states. It has been noted, for example, that lymphedema is associated with loss of vascular smooth muscle from the lymphatic collecting vessels of the affected organ (22). Further understanding of the mechanisms that cause lymph vessels to constrict and dilate are critical in evaluating the potential clinical manipulation of the system in the many disease states where increased transvascular fluid flux and edema formation are an instigating or exacerbating circumstance of the disease process.

I acknowledge the generous gifts of SKF 82526-f from Smith Kline & Beecham Laboratories (King of Prussia, PA) and prazosin from Pfizer Central Research (Groton, CT).

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Received 17 July 1997; accepted in final form 27 October 1997.

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