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Innervation and vascular pharmacodynamics of the mammalian spleen

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1. Introduction

The principal goal of the following treatise is to consider the effect(s) of potentially vasoactive substances and synthetic agonists, antagonists, or other inhibitors, on the capsule and vasculature of the mammalian spleen. The anatomic distribution of autonomic and sensory nerves as well as the role of neural mechanisms in splenic function also are evaluated in a number of species. Where relevant, an assessment is made of the pathophysiologic significance of elevated levels of neurotransmitters, or other humoral agents, on splenic blood flow in an attempt to elucidate the potential involvement of each in local regulation of blood flow during conditions of optimal circulation and low-flow states. Lastly, the limitations of the experimental per-

turbations used currently to investigate splenic vascular regulation are discussed in order to focus on unresolved questions and avenues for future research.

2. *Autonomic and sensory innervation*

Innervation of the spleen appears to be relatively sparse. While adrenergic (sympathetic) nerves have been demonstrated in the spleens of dogs, cats, rats, mice, and humans using histochemical and electron microscopic techniques, there is limited evidence for cholinergic (parasympathetic) or sensory innervation[5, 6, 10, 26, 44, 48, 59, 60, 68, 69].

There is considerable species variation in the distribution of adrenergic nerves. They are distributed to the smooth muscle of arteries and arterioles of the human and murine spleen. In the rat, cat, and dog, these nerves also innervate the smooth muscle of the capsule and trabeculae. In addition, there is evidence of adrenergic axons innervating reticular cells of the white pulp of the mouse[60] and red pulp of the canine spleen[6]. Some of these axons also have been reported to be contiguous with developing lymphocytes in the white pulp of the mouse[60] and with erythroid elements in the red pulp of the dog[71]. However, a functional relationship between such nerves and the reticulum, or blood elements, has yet to be established. Since the reticular cells of the spleen have been suggested to be contractile[6, 63], they might participate along with the smooth muscle in the capsule and trabeculae in affecting the large decrease in splenic volume observed following adrenergic stimulation[14, 15].

3. *Vascular pharmacodynamics*

a) *Adrenergic mechanisms*

Pharmacologic and neurophysiologic studies of the spleen in the dog, cat, rat, mouse, guinea pig, monkey, and man, have been extensive. In these species, norepinephrine, epinephrine, or isoproterenol, have been demonstrated to be vasoactive, and alpha and beta receptors have been isolated for these chemicals (for a comprehensive review, see Davies and Withrington[19]).

In intact, perfused preparations of the spleen, epinephrine, norepinephrine, or neurostimulation, cause alpha-mediated vasoconstriction and contraction of the capsule in dogs, cats, mice, rats, guinea pigs, rabbits, and man[7, 11–17, 27, 28, 32, 33, 35, 39, 49, 53, 54, 57, 58, 61, 67]. These responses generally are dosage- or frequency-dependant and result in a) a decrease in splenic arterial inflow and volume, and b) a concomitant increase in splenic arterial and venous pressure as well as venous outflow and hematocrit[8, 22, 29, 37]. Epinephrine has been reported to be more potent than norepinephrine; however, the responses evoked by either of the sympathomimetic amines are less than those provoked by electrical stimulation of splenic nerves. In man, capsular contractions are greatly attenuated or even nonexistent[1, 2, 42]. The degree of contraction is more on the order of that seen following adrenergic stimulation of the spleen in mice and rats[49, 57, 58]. The difference in capsular response among these species has been attributed to the sparse distribution of smooth muscle and innervation in the trabeculae and capsule of the spleen[44, 48, 59].

In contrast to the findings in mice, rats, guinea pigs and rabbits[27, 49, 57, 58], lower concentrations of epinephrine and norepinephrine in the dog (less than 0.1 μg/ml) and cat (less than 0.3 μg/ml) produce vasodilation and increased splenic blood flow by activating beta adrenoceptors. At these concentrations, the splenic capsule also contracts and is more sensitive than the vasculature to these chemicals[54, 61]. Isoproterenol (0.1–1.0 μg/ml), a sympathomimetic beta-receptor stimulant, also produces vasodilation and increased splenic blood flow in the dog, cat, and mouse, as well as a slight increase in volume and venous pressure in the spleens of the dog and cat[35, 39, 54, 57, 61]. The magnitude of the response to isoproterenol is directly proportional to vascular tone, which in turn is dependent on the degree of basal sympathetic innervation. Concentrations of isoproterenol in excess of 1 μg/ml have been reported to increase splenic vascular resistance and to decrease splenic weight in dogs and cats but not in the mouse. Both responses are dose-dependent and modified by alpha-adrenergic antagonists.

In vivo microscopic studies by MacKenzie et al.[49], Fleming and Parpart[27], and Reilly and McCuskey[57, 58], indicate that arteries, arterioles, and 'arterial' capillaries of the splenic red and white pulps of mice, rats, guinea pigs and rabbits are the vascular segments responsive to pharmacologic or neural stimulation. While venules and veins in these locations do not respond following alpha- or beta-adrenoceptor stimulation, some vasoactive substances appear to influence the permeability of the venous sinuses to blood cells[52].

b) *Cholinergic mechanisms*

Cholinergic (parasympathetic) innervation from the vagus nerve has been discounted in the spleens of the cat[26, 68, 69], mouse[59, 60], and human[44]. In general, the consensus of opinion is that vascular and capsular responses (i.e., constriction and contraction, respectively) in the spleen after electrical stimulation of the vagus nerve, or the systemic administration of cholinergic substances, are secondary responses caused by a reduction in cardiac output, and a decrease in mean systemic blood pressure[19, 41, 51, 57, 58].

In the isolated spleen of dogs perfused with blood, higher doses of acetylcholine induce contraction of splenic vessels and capsule while lower doses provoke vasodilation[7, 11, 16, 39]. The capsular and vascular responses usually are blocked by an anticholinergic drug, atropine[24, 28, 32, 45, 62]. However, a response to acetylcholine has been reported even after the administration of atropine[23, 31]. These latter responses are antagonized by alpha-receptor blockade[11]. As a result, it is thought that the vasodilation induced by lower doses of acetylcholine is a muscarinic effect, while the vasoconstriction and capsular contractions elicited by higher doses are nicotinic (ganglionic) effects, viz., related to stimulation of sensory nerve endings with the initiation of axonal reflexes, or a direct excitation of, or the release of stores of neurotransmitter from, the postganglionic nerve fibers present in the spleen. Only the latter interpretation

is supported by biochemical and neuroanatomic studies which question the existence of sensory nerves in the feline[69] and murine[59] spleen, but which confirm the presence of adrenergic (sympathetic) nerve fibers in the dog, mouse, cat, and human[5, 6, 10, 26, 44, 48, 59] and of presynaptic muscarinic receptors in the rat[70].

Transillumination studies of the murine spleen in vivo indicate that cholinergic stimulation produces arterial and arteriolar constriction and decreased blood flow[27, 57]. The constriction of arterioles and 'arterial' capillaries is antagonized by atropine which suggests a direct muscarinic action. However, in this species a nicotinic action can not be ruled out, because alpha antagonists and reserpine also block microvascular responses to cholinergic stimulation[57, 58]. In mammalian species other than the mouse, arteries and arterioles are presumed to be the microvascular segments responsive to direct or indirect cholinergic stimulation. However, no in vivo microscopic studies have been reported to support this contention.

c) Vasoactive amines

Serotonin has been reported to produce vasoconstriction and decreased flow in the canine spleen perfused with blood[39, 73], and constriction of venules in the spleens of mice observed by in vivo microscopic methods[57]. This response was blocked by methysergide in the dog[39]. Davies and Withrington[19] have reported that lower doses of serotonin (5 µg) cause slight reduction in volume and slight vasoconstriction in the spleen of dogs, while higher doses of serotonin (up to 25 µg) provoke intense vasoconstriction which completely abolished splenic blood flow.

In the cat, isolated strips of spleen contract when bathed in serotonin; a response which is blocked by phenoxybenzamine, phentolamine, bromolysergic acid dietylamide, cocaine, and dihydroergotamine[46, 56]. Furthermore, serotonin has little effect on the spleen strips of reserpine-treated cats, but splenic contraction is greatly enhanced after higher doses of norephinephrine. This observation suggests that serotonin acted indirectly by releasing norepinephrine in this tissue.

In the conscious dog, histamine causes a decrease in splenic arterial blood flow and a transient increase followed by a prolonged decrease in venous outflow[36]. Fleming and Parpart[27] and Reilly and McCuskey[57, 58] found that topical administration of histamine elicits both capsular contraction and arteriolar constriction in the mouse. Since the responses elicited by histamine are blocked by alpha-adrenergic antagonists and reserpine, Reilly and McCuskey[58] suggest that they were mediated by releasing stored norephinephrine from splenic adrenergic (sympathetic) nerves. However, Reilly and McCuskey[57] noted these responses only in the presence of a H_2-blocker. They found that the predominent effect of histamine in the murine spleen is arteriolar dilation which is mediated via H_2 but not H_1 receptors.

d) Nucleotides and their degradation products, ions, and lactic acid

In the spleen of most species, ATP, ADP, AMP, adenosine, guanosine, inosine, sodium and potassium chloride, inorganic phosphate, and lactic acid, have *not* been implicated in the local regulation of blood flow. Of these constituents, only adenosine and lactic acid have been shown with in vivo microscopic methods to be vasoactive in the murine spleen[57, 58]. Adenosine was found to evoke arteriolar dilation and to increase both the linear velocity of blood flow and the number of channels with flow. These responses were not modified by beta-adrenoceptor antagonism.

In contrast, lactic acid causes alpha-mediated arteriolar constriction and reduced blood flow, and it promotes storage of blood in the red pulp of the mouse spleen[57, 58]. These responses are suggested to be due to the release of norepinephrine from adrenergic nerves, since alpha blockers and reserpine abolished the responses[58]. In higher doses (greater than 10 µg/ml), lactic acid elicits erratic arteriolar contractions which appear to be related to pH, because HCl solutions of equivalent pH (less than 7) elicit similar responses which also are not antagonized by alpha-receptor blockade or reserpine.

e) Prostaglandins (PG), prostacyclin (PGI_2) and thromboxane

In the dog, PGE_1, E_2, $F_{1\text{-alpha}}$, A_1, A_2, or lower doses of $PGF_{2\text{-alpha}}$ or PGI_2 (less than 10 µg/ml blood), cause a reduction in splenic vascular resistance and an increase in splenic volume[16, 18, 21, 64]. The responses to $PGF_{2\text{-alpha}}$ or A_2 are reversed by phenoxybenzamine. At higher doses, $PGF_{2\text{-alpha}}$ causes splenic vasoconstriction in the dog[18]. In all cases, there is little effect of $PGF_{2\text{-alpha}}$ on splenic capsular smooth muscle or on the vasoconstriction elicited by nerve stimulation. However, a slight interaction of PGA_1, A_2, D_2, I_2, E_1, or G_2 with nerve stimulation or injection of epinephrine or norepinephrine has been reported in the dog spleen[17] and the splenic capsule in the rabbit[55]. PGA_1 or D_2 increase, while PGA_2, I_2, E_1, or G_2 decrease, contractions of capsular and/or vascular smooth muscle to adrenergic stimulation.

In the rat, $PGF_{2\text{-alpha}}$ or thromboxane B_2 also produce vasoconstriction; however, in this species they potentiate vascular responses to norepinephrine and neurostimulation[50]. This investigator proposes that PGs other than PGD_2 modulate adrenergic vasoconstrictor responses in the rat spleen.

Administration of PGE_2 in the cat or of arachidonic acid, PGE_1, E_2 or I_2 in the rat, concomitant with adrenergic nerve stimulation or injection of norepinephrine, induced a dose-dependent reduction in the vasoconstriction and capsular contraction evoked by adrenergic stimulation alone[40, 47, 50]. In the cat, lower doses decreased vascular resistance while higher doses potentiate the response induced by nerve stimulation[40]. Based on these observations in the cat and rat, it has been proposed that prostaglandins modulate adrenergic mechanisms within the spleen by inhibiting a) the release of norepinephrine from sympathetic neurons, and b) the response of vascular and capsule smooth muscle to norepinephrine. The fact that indomethacin, which inhibits the synthesis of prostaglandins, potentiates the response of splenic smooth muscle in the cat[25] or rat[50] to injected epinephrine or norepinephrine adds support to this hypothesis.

In the mouse, PGE_2 or $PGF_{2\text{-alpha}}$ administration reduces blood flow and promotes storage of blood in the red pulp of the spleen. Arteriolar constriction also was provoked by each compound; however, this response was observed only at higher doses (100 µg/ml). While the combined effects produced by the PGs are attenuated by alpha-adrenoceptor blockers, they are abolished by reserpine. Such antagonism suggests that PGE_2 or $PGF_{2\text{-alpha}}$ release stored norpinephrine from adrenergic nerves in the murine spleen[57,58].

f) Polypeptides

The neurohypophyseal peptides, vasopressin and oxytocin, have been studied most extensively in the spleens of dogs, cats and man[2,9,20,30,34,35]. These investigations indicate that oxytocin has little or no action in these species while vasopressin induces vasoconstriction. Negligible changes in the splenic capsule or volume are seen in cats and man. However, vasopressin in the dog causes contractions of the vasculature and capsule as well as reduction in volume suggesting that the canine spleen may contribute to the maintenance of peripheral resistance and to circulating blood volume by reduction in splenic capacity[20].

Conversion of angiotensin I to angiotensin II in the lungs produces a highly vasoactive octapeptide[3]. In dogs, cats, and man, angiotensin II provokes vasoconstriction, but negligible changes in splenic volume associated with capsular contraction[1,2,12,34,35]. Most evidence suggests that the constriction due to angiotensin is a direct action on vascular smooth muscle, because this response is unaffected by alpha-adrenoceptor blockers, reserpine, or chronic denervation[2,15,20,43].

In the cat and dog, bradykinin has been demonstrated to release norepinephrine from splenic sympathetic nerve endings[38]. These effects on stored catecholamine result in vasoconstriction. In contrast, a direct action of bradykinin on vascular smooth muscle has been proposed by Reilly and McCuskey[57,58] in the mouse. They indicate using in vivo microsopic methods that bradykinin induces arteriolar constriction, increased storage of blood, and reduced blood flow through the red pulp. These responses are not influenced by alpha-adrenoceptor blocking agents or reserpine.

In the dog, Moerman et al.[53] have reported that close-arterial injection of bradykinin causes vasodilation. The latter response in the dog is unaccompanied by any change in splenic weight, and it can not be antagonized by phenoxybenzamine. Other investigations have shown that the vasodilation elicited after close-arterial injection of bradykinin is not dose dependent, is unaccompanied by any significant change in splenic weight, and unaffected by prior alpha or beta blockade[19]. Therefore, it has been suggested that the vasodilation caused by bradykinin is a direct effect of this substance on vascular smooth muscle.

4. Pathophysiologic significance

Splenic blood flow is principally controlled by splenic arterial resistance. In some mammalian species, but not man, smooth muscle in the capsule and trabeculae contribute to the regulation of flow. Dynamic, moment-to-moment adjustments in muscular tone affect the rate(s) of filtration, synthesis, storage, and release of blood elements in the red and white pulps of the spleen. Vasoconstrictor mechanisms, primarily of an alpha-adrenergic origin, predominate and are relatively strong influences governing blood flow to and from, as well as within and between, the red and white pulps of the spleen. Vasodilator mechanisms, however, are poorly developed in most of these species. These mechanisms are thought to be operative during conditions of optimal circulation (e.g., hyperemia, hematopoiesis) and of low flow (e.g., hemorrhage, shock, polycythemia, anemia, etc.). However, only circulating levels of catecholamines, vasopressin, and angiotensin II have been demonstrated in dogs and cats to accumulate in quantities sufficient to modify splenic blood flow during pathophysiologic conditions such as hemorrhagic, cardiogenic, or traumatic shock[4,35,65,72].

5. Unresolved problems

The principal limitation to vascular studies in the spleen has been the technical difficulties encountered, and although more recent work has made substantial contributions to our knowledge of splenic circulation, many areas remain the subject of relatively few studies. The adaptation of different experimental protocols impose further limitations on the nature and validity of the information derived from them. As a result, caution must be applied to the interpretation of pharmacodynamic and neurophysiologic data on blood flow and other parameters.

Binding of drugs in the blood stream or to vascular receptors peripheral to the spleen must be considered as sources of error when interpreting experimentally-derived data, because the results may not reflect the potential activity of substances when they are released in situ. Conflicting results also may be related to differences in the experimental techniques used and in the type and dosage of anesthetic agents administered or to different concentrations of drugs or vasoactive agents at the effector site(s) within the spleen. Investigations are required to ascertain whether compounds are released in sufficient quantities to modify blood flow during conditions of optimal circulation or low flow. Yet to be elucidated is the role of resident cells in the spleen in secreting such vasoactive compounds. Macrophages produce prostaglandins and hematopoietic elements also may release these or other substances[52] that influence intrasplenic blood flow.

Reported discrepancies in responses to pharmacologic and neural stimulation may reflect real species variations in structure and function[8,37,52,66]. For example, the spleens of mice and cats contain poorly developed sinuses while those of rats, rabbits, and the human have an elaborate sinusoidal network. The extent to which penicillar arterioles and sheathed 'arterial' capillaries are developed also varies considerably in different mammalian species as does the distribution of adrenergic (sympathetic) innervation. This raises the as yet unresolved question as to whether or not a direct relationship exists between these morphologic differences and

the patterns of intrasplenic circulation reported during experimentally-induced conditions of optimal circulation and low flow.

Even more ambiguous are the mechanisms regulating blood flow through the splenic microvasculature. Most information on microvascular regulation has been extrapolated from investigations of humoral effects on innervated vessels larger than 300 μm or from measurements of flow, resistance, and clearance following arterial or venous injections of vasoactive substances or tracers. These studies fail to elucidate directly the sensitivity of the microvascular system to these substances in situ, the discrete site(s) of chemical interaction within the 'resistance' vessels of the spleen, or the role of hormones, the nervous system, or other stimuli, in direct and indirect microvascular responses. Indirect effects are induced by these chemicals modifying the responses of vascular and capsular smooth muscle to vasoactive agents or causing the release of stores of neurotransmitter(s) from adrenergic (sympathetic) nerves. Since microvascular innervation appears to be sparse, a strong theoretical case can be made for the role of humoral agents other than neurotransmitters in the local regulatory mechanisms that maintain homeokinesis.

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The regulation of hemopoiesis in the spleen

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1. Introduction

Bone marrow in humans and in adult rodents is recognized as the primary site for hemopoiesis. This prominence, however, has not always been enjoyed phylogenetically or even ontogenetically. This paper reviews: 1) the relative contribution and regulation of splenic hemopoiesis in health and specific experimental and disease states where reversion to its embryonic hemopoietic capacity occurs, 2) the role of the stromal