B. Observations in Living Animals

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Use of Pharmacologic Antagonists to Evaluate Humoral Regulation of the Microcirculation*

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Current concepts of local regulation of blood flow postulate a balance between vasodilator and vasoconstrictor factors. Studies on the terminal vascular bed of the rat mesocecum were designed to evaluate the role of some of these neuro-humoral materials by differential blockade with known pharmacologic antagonists or tissue depletion with agents known to interfere with synaptic transmitter mediation; both systemic and local effects were studied and evaluated by noting direct vasomotor actions and changes in reactivity.

Methods

The mesentery of female rats, anesthetized with pentobarbital, was exposed for microscopic observation (Altura and Zweifach, 1965a) either before or after subjecting the animals to various pharmacologic antagonists, amine depletors, etc. The response of the small blood vessels to different vasoactive test stimuli (epinephrine [E], norepinephrine [NE], angiotensin [A], serotonin [5-HT], vasopressins [Vp], histamine, acetylcholine, bradykinin and isoproterenol) was

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examined and analyzed according to previously defined criteria (ALTURA and ZWEIFACH, 1965a). As a precaution all specific pharmacologic antagonists were tested for cross-blocking effects against other topically applied vasoactive agents which may also possibly be inhibited in order to rule out any non-specific blocking effects.

Results

Local autonomic blockade. Two structurally unrelated alpha-adrenergic blocking agents, phenoxybenzamine (150 μg) and chlorpromazine (50 μg), when applied topically, to the surface of the mesentery, produce a widespread vasodilation of all muscular components in the terminal bed *thereby suppressing vasomotion.* Despite the fact that both drugs clearly prevented the constriction induced by topical threshold doses of catecholamines, vascular reactivity to other constrictors such as 5-HT, A and Vp and their analogues remained unaffected or, in some instances, were potentiated. Topical local anesthetics, such as lidocaine and procaine (200–300 μg) rapidly induced relaxation of small arteries and arterioles which was followed with 1–2 min by metarteriolar and precapillary relaxation. Both lidocaine and procaine initially potentiated the constrictor responses to threshold doses of topical NE. Locally applied yohimbine (50 μg), another alpha-adrenergic blocking drug, produced almost immediately a partial constriction of arterioles followed by metarteriolar and precapillary vasoconstriction. These excitatory responses usually disappeared within 3–4 min giving way to arteriolar, metarteriolar and precapillary relaxation. These data with locally applied specific alpha-adrenergic blockers and local anesthetics seem to indicate, in each case, an interference with the constrictor action of endogenous catecholamines on the effector smooth muscle cells. Locally applied *atropine* (a selective cholinergic blocker) led to constriction of arterioles, metarterioles and precapillaries. The dose regimes of atropine (30–300 μg) specifically antagonized the vasodilator responses induced by topical acetylcholine. Most of the constrictor actions, of atropine, were potentiated after selective adrenergic blockade with either phenoxybenzamine or chlorpromazine. Such observations may implicate other humors such as angiotensin or vasopressin-like peptides or other unknown factors involved in the regulation of basal vascular tonus.

Systemic autonomic blockers. Systemic administration of phenoxybenzamine or chlorpromazine produced widespread microvascular

dilation and specific adrenergic blockade (Table I) similar to that seen with topical application. I.v. administration of the ganglionic blocking agent hexamethonium, in low concentrations, produced relaxation of arteries and arterioles (Table I). Higher doses, after 20–30 min, failed to induce metarteriolar and precapillary relaxation in addition to artery and arteriolar effects. These experiments serve to buttress the thesis implicating endogenous constrictor catecholamines in maintenance of vascular tone. Administration of dichloroisoproterenol (DCI), a beta adrenergic blocker, produced a transient venular constriction and increased vascular reactivity to locally applied constrictor catecholamines while specifically blocking the local vasodilator action of topical isoproterenol (Table I). Data of this nature are strongly suggestive of β-receptors in the venules and may indicate that these specific microvessels are normally being dilated by circulating E (ALTURA and ZWEIFACH, 1965b). When *atropine* is administered i.v. in a dose sufficient to selectively block locally applied acetylcholine, some of the precapillary sphincters, metarterioles and arterioles become moderately narrowed (Table I) producing results very similar to those seen on local application. These results with i.v. atropine serve to reinforce those obtained with local administration and seem to indicate a role for endogenous circulating acetylcholine in the local regulation of blood flow.

Catecholamine depletors. Chronic administration of reserpine and guanethidine (two known tissue catecholamine depletors) or alpha-methyl dopa, an agent which interferes with norepinephrine sythesis producing a false sympathetic transmitter substance, viz. alpha-methyl norepinephrine, produced widespread dilation in most cases *thereby suppressing vasomotion* (Table II).

Antiserotonin compounds. Experiments with topical or i.v. administration of the antiserotonin compounds 2-bromo-lysergic acid (BOL) or LSD, in specific serotonin blocking doses, clearly failed to produce any noticeable effect on the tonal appearance or vasomotion of the rat mesenteric microvessels.

Comment

These data with local or systemic adrenergic blockers, catecholamine depletors, local anesthetics or agents such as alpha-methyl-dopa which interfere with the synthesis of NE indicate, in each case, a selective relaxation of arteries, arterioles, metarterioles and precapillaries

Table I. Effect of i.v. Autonomic Blocking Agents on Rat Mesenteric Microvessels

Drug	Dose (mg/kg)	Arteriole (25 μ)	Metarteriole (18 μ)	Precapillary (12 μ)	Venule (40 μ)	Vasomotion
Phenoxybenzamine	1.0	dilated*	dilated	dilated	dilated	absent
Chlorpromazine	5.0	dilated	dilated	dilated	dilated	absent
Hexamethonium	1.0	dilated	no effect	no effect	no effect	no effect
Dichloroisoproterenol (DCI)	10.0	no effect	no effect	no effect	constricted	no effect††
Atropine	1.5	constricted†	constricted	constricted	no effect	increased**

* Represents at least a 20–30 % increase in vessel diameter.
† Represents at least a 20–30 % decrease in vessel diameter.
†† Vasodilator response to topical isoproterenol (1. Oμg) blocked.
** Vasodilator response to topical acetylcholine (1. Oμg) blocked.

Table II. Effects of Catecholamine Depleting Agents on Rat Mesenteric Microvessels

Drug	Dose-Duration (mg/kg; days)	Arteriole (25 μ)	Metarteriole (18 μ)	Precapillary (12 μ)	Venule (40 μ)	Vasomotion
Reserpine*	1.0; 6	dilated†	dilated	dilated	dilated	absent
Guanethidine**	10.0; 15	dilated	no effect	no effect	dilated	decreased
Alpha-Methyl Dopa**	100; 15	dilated	dilated	dilated	dilated	absent

All three catecholamine depletors were administered I.M.
* Mesenteric observation made 48 hours after the last dose.
† Represents at least a 20–30 % increase in vessel diameter.
** Mesenteric observations made 24 hours after the last dose.

and lends firm support to a role for endogenous catecholamines in the regulation of vascular tone and vasomotion in the rat mesentery. In view of the data presented here it is unlikely that endogenous serotonin, at least under normal circumstances, is a regulator of local blood flow in the rat mesentery. Experiments utilizing an agent which produces selective cholinergic blockade indicates that acetylcholine may play a role as an endogenous vasodilator. Further experiments utilizing a combination of cholinergic and adrenergic blockade indicate that some vascular tone is still maintained in the terminal vascular bed and may be due to a constrictor peptide like angiotensin and/or vasopressin-like substances (ALTURA and HERSHEY, 1966). Experiments utilizing *antihistamines* were not undertaken as previous work has shown that these antagonists may not be a valid test for the presence of microcirculatory histamine (ALTURA and ZWEIFACH, 1965a, c; ALTURA and ZWEIFACH, 1966). Overall the present data serve to support the concept of local regulation of blood flow by neuro-humoral substances.

Summary

Studies were designed to evaluate the role of neuro-humoral materials in microcirculatory regulation. This was accomplished by differential blockade with known pharmacologic antagonists and with agents known to interfere with autonomic transmission. Evidence was presented for a role of endogenous catecholamines and acetylcholine in the local regulation of blood flow. Further experiments suggested that peptides such as angiotensin and vasopressin may also play a role in microcirculatory regulation.

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