Can Metarteriolar Vessels Occlude Their Lumens in Response to Vasoactive Substances?[1] (36656)

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Vasomotion, or the cyclical opening and narrowing (or closing) of metarterioles and precapillary sphincters, is thought to be the major mechanism, in the microcirculation, by which blood flow is ultimately regulated into the true endothelial capillary exchange vessels (1–3). Although the precapillary sphincters are the vessel types thought to primarily determine the distribution of blood to the capillaries, as well as dictate the surface area available for transcapillary exchange (1–7), in some tissue areas (as well as in certain organs) the tissues are completely devoid of precapillary sphincters (8). In these latter tissue areas other muscular microvessels such as precapillary arterioles, metarterioles and/or endothelial cells are thought to finely regulate flow into the true capillaries (8). Thus, the metarterioles might in many cases control transcapillary exchange function. In most mammalian regional vascular beds the terminal arterioles become thin as they extend distally and exhibit walls which have a discontinuous investment of smooth muscle (1, 3, 9). Such vessels which give rise to the true capillaries, at approximately right angles, have been termed metarterioles or thoroughfare channels (1, 10).

It is generally assumed, *in vivo*, that the metarterioles (composed of endothelial cells and scattered, discontinuous elements of smooth muscle [1, 3, 9]) always exhibit some active blood flow and hence do not close down, even in conditions of curtailed blood flow (1, 3, 10–12) while the endothelial capillaries exhibit an intermittent flow (1–3, 10–12) and do, at times, completely shut down via effects on the precapillary sphincters (1–3, 13). Thus, it would appear that the metarteriolar channels cannot completely occlude their lumen diameters in response to intrinsic physiologic stimuli. Furthermore, although the metarterioles have been shown by many investigators to respond to vasoactive substances and to changes in intravascular pressures (1, 10, 13–17), it is not known whether these microscopic vessels do so in a graded or in an all-or-none fashion. Up until now, however, these fundamental contractile properties of metarterioles have not received adequate quantitative, investigative attention. Recently, a preliminary report has appeared (18), using a combination of direct *in vivo* microscopy and an image-splitting television microscope recording system (19), which suggests that the rat mesenteric metarterioles can not only exhibit graded, dose-dependent contractile responses to at least one vasoactive material, namely dopamine, but can contract to the point of complete lumen occlusion. The present experiments were undertaken with additional vasoactive substances in order to extend these observations. The development of the image-splitting television microscope recording system allows one to rapidly make, *in vivo*, accurate quantitative measurements up to magnifications of 6500× on a television screen of the different components of the mammalian capillary bed (13, 16, 18, 20). (The use of this image-splitting device allows one to make measurements with an accuracy 10 times that of the resolving power of the light microscope [21].)

The present report, employing this quanti-

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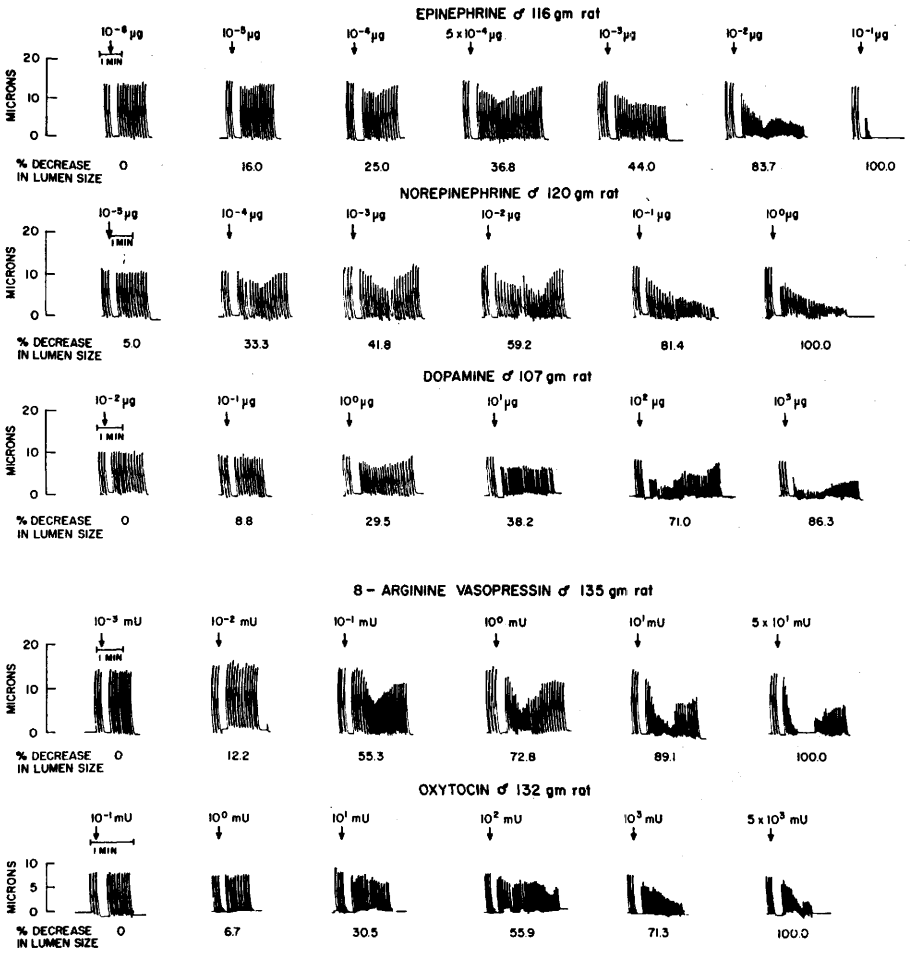

FIG. 1. Electrographic recordings of the effects of topically applied graded doses of catecholamines and neurohypophyseal hormones (vasopressin and oxytocin) on lumen sizes of metarterioles in rat mesocecum. Each electrographic deflection is a value of the vessel lumen diameter (in microns) at the instant of measurement. The space in the electrogram (immediately after arrows) represents the time necessary for the observer to initiate image-splitting following local application of the amines and peptides. The doses refer to the salts (in case of catecholamines) and bases (peptides) in this figure as well as Figs. 2 and 3.

tative technique of Baez (19), unequivocally demonstrates that metarterioles not only contract to complete lumen closure in a graded, dose-dependent manner to a variety of vasoactive substances (amines, peptides) but respond to extremely low (physiologic?) doses of these materials.

Methods. The experiments were carried out *in vivo* by direct microscopic observation of metarterioles (at a few microns [*e.g.*, 5–8 μm] in distance from a precapillary sphincter) in mesentery of the anesthetized rat. The rat mesentery was prepared and kept under physiologic conditions according to procedures described previously (22). Measurements for changes in metarteriolar lumen size were made before (control) and after topical application of graded doses (6 to 12 in number) of the catecholamines epinephrine (Adrenaline hydrochloride, Parke, Davis, and Co.), norepinephrine (noradrenaline bitartrate, Levophed, Winthrop Labs.),

and dopamine hydrochloride (Sigma Chemical Co.) as well as the pure, synthetic (preservative-free) neurohypophyseal peptide hormones—8-arginine vasopressin (approx. 400 IU/mg, Sandoz Ltd.) and oxytocin (400 IU/ml, Sandoz Ltd.). *In vivo* microscopic observations for discrete drug effects were made at magnifications up to 4000× using the image-splitting television microscope recording system (19). The Ringer gelatin irrigation of the mesentery was temporarily interrupted during topical drug applications. The effects of vasoactive agents on lumen diameter were recorded for at least 2–3 min after topical application either to the point where the contractile response stabilized or until complete lumen occlusion. Complete lumen occlusion is defined here as a touching of both internal walls of a metarteriolar vessel and was simultaneously observed visually on the TV monitor and recorded on the polygraph. Leitz–Ultrapak water immersion objectives, 32× and 55×, were used in conjunction with 10× Bausch and Lomb oculars on a Bausch and Lomb Dyna-Zoom microscope equipped with a trinocular head.

Results and Discussion. Figure 1 shows the electrographic recordings of typical changes in metarteriolar lumen sizes seen in five different rats, in response to graded constrictor doses of the catecholamines and neurohypophyseal hormone peptides. Topical application (0.1 ml volumes) of all three catecholamines as well as both peptide hormones, in increasing doses, resulted in dose-dependent contractile responses (to complete lumen occlusion) which were clearly not an all-or-none phenomenon. Figures 2 and 3 show the relative sensitivity of the metarterioles to the three naturally occurring catecholamines, as well as to the two naturally occurring neurohypophyseal hormones, and thus indicate that these vessels are quantitatively more sensitive to epinephrine, norepinephrine and vasopressin than heretofore believed, responding to threshold doses of 10^{-5} and 10^{-4} μg/ml for epinephrine and norepinephrine, respectively, and 5×10^{-2} mU/ml for vasopressin.[3] Other quantitative data (obtained with the image-splitter TV system) indicates that these vessels are on the average 500–1,000 times more sensitive to the constrictor actions of epinephrine and norepinephrine

[3] These approximate threshold concentrations were extrapolated from the 0.1 ml volumes of drug solutions that were routinely applied to the rat mesocecum. Since 0.1 ml volumes of Ringer gelatin were superfused over the entire rat mesocecum, the actual threshold drug concentrations must be appreciably less.

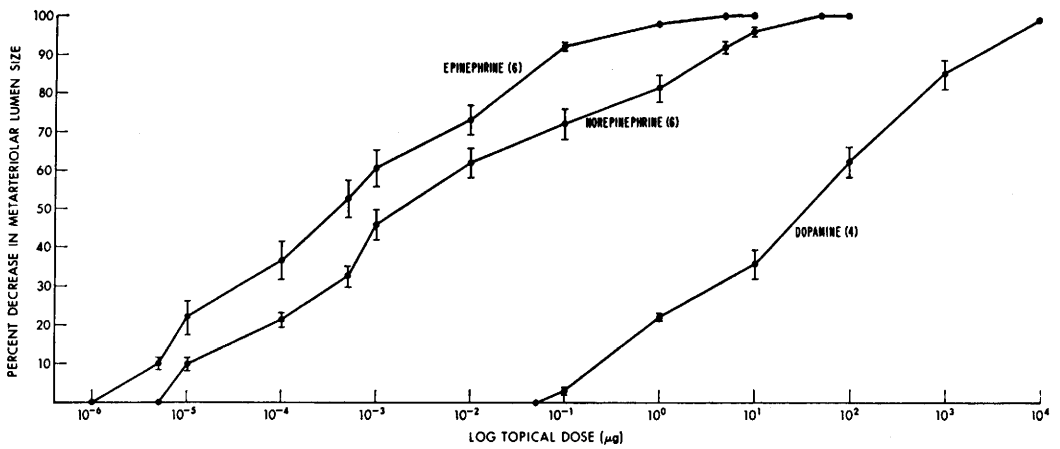

FIG. 2. Graded contractile responses of metarterioles to topically applied catecholamines. Each point represents the mean value obtained from measurements on vessels from different male rats (indicated by numbers in parentheses). Only one type of catecholamine was tested on each rat mesentery. The bars represent the SEM. The mean control lumen sizes for the metarterioles (μm ± SEM) were: epinephrine, 11.5 ± 0.8; norepinephrine, 10.7 ± 0.8; and dopamine, 10.5 ± 1.0.

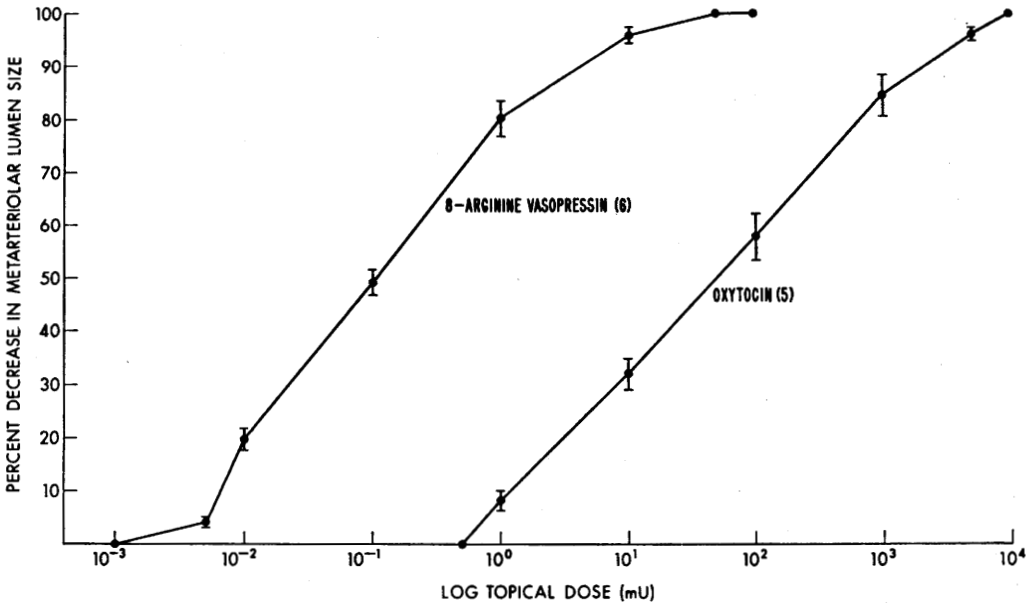

FIG. 3. Graded contractile responses of metarterioles to topically applied 8-arginine vasopressin and oxytocin. Note that the abscissa is expressed in milliunits of peptide. Other symbols and conventions are similar to those in Fig. 2. The mean control lumen sizes for the metarterioles were: vasopressin, 11.6 ± 1.0; and oxytocin, 10.9 ± 1.0.

than are the rat mesenteric arterioles (18) and 2–10 times less sensitive to the constrictor actions of 8-arginine vasopressin than are the arterioles (unpublished data). Although it is generally believed that oxytocin is a vasodilator (23), the present experiments strongly oppose such a tenet and in fact demonstrate that this pituitary hormone is a potent constrictor, at least in male rats; the present findings with pure synthetic oxytocin (preservative-free) thus serve to extend and confirm our previous work in regard to a constrictor action for this peptide in the microcirculation (15, 18). Rat plasma epinephrine and norepinephrine levels are on the average 6–9 $\times$ 10^{-3} μg/ml (25), while rat plasma vasopressin levels vary between 0.008–6.0 mU/ml (26). Thus, these metarteriolar vessels not only respond to circulating plasma catecholamine and vasopressin levels, but could decrease their lumens up to 40–70% in response to these circulating vasoactive substances under normal conditions. Since vasopressin is known to potentiate the constrictor actions of catecholamines (27, 28), the magnitudes of these lumen changes are probably much greater. Experiments are currently in progress to test this tenet.

The present *in vivo* quantitative data suggest that complete closure of metarteriolar lumens can only be attained with catecholamine plasma levels equivalent to 10–50 μg/ml and vasopressin plasma levels equivalent to 100–500 mU/ml, concentrations which are approximately 10,000 times greater than those present in rat plasma (25, 26). Thus, although the present study unequivocally demonstrates that metarterioles can respond to circulating vasoactive substances in a dose-dependent graded fashion to complete lumen occlusion, it is highly unlikely that these microvessels can narrow, in response to these substances, to the point of complete closure *in vivo* under normal physiologic conditions. However, in pathophysiologic conditions such as shock, where: (i) catecholamine and vasopressin levels are known to become markedly elevated (26, 29); and (ii) metarterioles are known to become hypersensitive to both catecholamines (1, 22, 29, 30) and vasopressin (31), it is distinctly possible that these substances could effect closure of these

microscopic blood vessels, especially in view of the synergistic actions of these vasoactive agents (27, 28).

In view of the quantitative data presented in this communication, one must consider the strong probability that metarterioles functionally and anatomically perform as *sphincters* in regulating flow into and out of the endothelial capillaries in the absence of true precapillary sphincters (8). In addition, the present observations strongly suggest that even in the presence of true precapillary sphincters, metarterioles probably act to *very finely regulate* transcapillary exchange by more actively changing precapillary pressure and distribution of blood flow than is currently believed.

Summary. Metarterioles are not currently thought by most investigators to either (i) be able to narrow their vascular walls to the point of occlusion in response to circulating vasoactive substances; or (ii) contract in a dose-dependent manner to vasoactive agents. *In vivo* experiments, using the rat mesentery and a high magnification (up to 6500×) image-splitting television microscope recording system, were designed to test these tenets. The results clearly and quantitatively demonstrate that metarterioles, at least in rat mesentery, not only contract to complete lumen occulsion in a graded, dose-dependent manner to catecholamines and neurohypophyseal hormone peptides but respond to extremely low (physiologic ?) doses of these substances.

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