Vascular Leakage in the Kidney and Lower Urinary Tract: Effects of Histamine, Serotonin and Bradykinin (36497)

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Functional and ultrastructural studies over the past 10 years have clarified the morphologic mechanisms that lead to increased vascular permeability in acute inflammation. It is now accepted that chemical mediators of inflammation such as histamine, serotonin and bradykinin induce the formation of intercellular gaps in the vascular endothelium (1, 2), probably due to contraction of endothelial cells (3); that such leakage is restricted to small and medium-sized venules; and that direct injury to the endothelium causes leakage in vessels of all calibers due to the formation of discontinuities in the endothelium (4, 5). However, the vast majority of these observations have been made on skin, skeletal muscle, and mesentery, and the course of events in other organs is still incompletely understood. The ultrastructural characteristics of blood vessels vary considerably among different organs, and even among different parts of the same organ (2, 6), and there is evidence that the response of vessels to histamine-like mediators is also organ-dependent. In a recent study Gabbiani, Badonnel and Majno (7) showed that the blood vessels of the testis, Gasserian ganglion and brain are not responsive to histamine, serotonin or bradykinin. Preliminary observations suggested that vascular leakage in the blood vessels of renal parenchyma does not occur in response to histamine injections (8). In the present communication we report the results of experiments designed to study the response of blood vessels in the kidney and lower urinary tract to chemical mediators. Vascular leakage was assessed by the method of vascular labeling with carbon (8) and by electron microscopy. These studies confirm that histamine, serotonin and bradykinin delivered to the kidney by a variety of routes fail to cause vascular leakage in renal parenchymal vessels; in contrast, the vessels in the renal capsule, pelvis, ureter and urinary bladder respond to these mediators in a manner similar to that seen in skin, muscle and mesentery.

Materials and Methods. 1. Animals. All experiments were performed on Sprague–Dawley female rats obtained from the Charles River Laboratories, Worthington, MA. For intra-arterial injections larger animals (350–450 g) were used but all other experiments were done on rats that weighed 175–225 g.

2. Vascular leakage. Increased vascular permeability was assessed and localized by the method of vascular labeling with carbon (8). Colloidal carbon ("Biological ink," Pelikan Co., Hanover, Germany, batch No. C11/1431a) was injected intravenously in a dose of 0.1 ml/100 g body weight, and the animals were sacrificed 1–2 hr later when they had cleared the circulating carbon. The carbon was injected immediately before or after the test injections (see below). Tissues were fixed in buffered formalin and examined for carbon labeling both grossly and in histological sections stained with hematoxylin and eosin. The presence of intramural deposition of carbon, indicating vascular leakage (8), was confirmed by examination of thick Epon sections of tissues processed for electron microscopy and in some experiments by ultrastructural studies. Tissues were fixed for 3–4 hr in 2.5% glutaraldehyde and 2% formaldehyde in 0.1 *M* cacodylate buffer (pH 7.2), rinsed, washed overnight in buffer, postfixed in 2.0% osmium tetroxide, dehydrated in graded alcohols and embedded in Epon 812. One-micron thick sections were stained with

toluidine blue and examined with a light microscope. Thin sections were stained with uranyl acetate and lead citrate.

3. Test solutions. (a) Histamine phosphate (Eli Lilly Co., CalBiochem, or Sigma Chemical Co., Lot No. 71C-1760); (b) 5-hydroxytryptamine (Sigma Chemical Co., Lot No. 16-B1530; (c) bradykinin (Sandoz Pharmaceutical Co., Hanover, NJ).

4. Routes of injection of test substances. (a) Intra-arterial injections. The animals were anesthetized with ether. Under sterile conditions the abdomen was opened, and the viscera were retracted with a saline-soaked sponge to expose the aorta. The aorta was separated from the inferior vena cava, and ligatures were passed above the left renal artery so that traction could effectively occlude the aortic blood flow. The aorta was then ligated distal to the renal artery and a fine glass cannula was inserted into the aortic lumen in a retrograde manner, following a glass leader. While the cannula was being inserted, traction on the proximal aortic ligature for about 30 sec prevented all but the slightest bleeding. Strong pulsations of aortic blood could be observed within the glass cannula. The test solutions were then administered slowly in a volume of 1 ml 0.85% sodium chloride while the aorta was being occluded proximally by traction on the ligature. The effectiveness of this intra-arterial injection technique was confirmed in several experiments by direct observation of staining of renal parenchyma and ureteral urine after injection of methylene blue. Carbon was injected into the tail vein either immediately before or immediately after the injection of the test solutions. The abdomen was then closed and the animal was killed 1–2 hr later for determination of vascular leakage.

(b) Intravenous injections were made into the tail vein under light anesthesia.

(c) Injections into the urinary bladder. The urethra was cannulated with a blunt-tip 19-gauge needle as described previously (9). Isotonic saline was then introduced until it flowed out around the needle (approx 2.0 ml). The saline was then withdrawn and measured and the test substance was given in an equal volume of saline. The urethra was clamped and in some instances the bladder was gently massaged; the clamp was then released. Under these circumstances reflux of fluid from the bladder into the ureter and renal pelvis occurs regularly in the rat.

(d) Intraparenchymal. The left kidney was exposed and the test substance was administered via a 29-gauge needle in a volume of 0.05, 0.1 and 0.2 ml. With the larger volumes some of the fluid oozed out of the injection site. A control injection of similar volumes of saline was injected into the right kidney. Injections were made both in the cortex and medulla.

Results. Table I summarizes the results of experiments with vasoactive substances. None of these caused vascular labeling with carbon in the renal parenchymal vessels, even when they were injected directly into the kidney. Occasionally there were a few carbon plugs in the capillaries of the papilla and in some peritubular capillaries; these were most numerous after intrarenal injection of bradykinin. In contrast, in most experiments there was either focal or widespread labeling of small and medium-sized venules in the adventitia, and often within the muscular wall of the renal pelvis, ureter and urinary bladder. The intracystic injections caused labeling in the pelvic vessels, almost certainly due to the vesicoureteral reflux that occurs regularly in rats after injection of 2 ml of solution into the urinary bladder (9). After injections of the mediators directly into the lower urinary tract the majority of leaking vessels were the venules of the adventitia and outer muscularis, there being only spotty labeling of the *submucosal* vessels and no labeling of small capillaries. Examination of thick Epon sections and selected specimens by electron microscopy confirmed that the vascular labeling was due to intramural deposition of carbon in the medium-sized venules. The endothelium appeared relatively well-preserved, and the ultrastructural characteristics of the leaking venules were essentially identical with those reported in other tissues (2).

It has been shown that the enzyme horseradish peroxidase induces mast cell damage,

TABLE I. Summary of Results.[a]

Test substance	Dose	Route of administration	Carbon labeling			
			Kidney	Pelvis	Ureter	Bladder
Histamine[b]	4500 μg, 1500 μg, 150 μg, 15 μg/100 g	Intravenous	0	+	+	+
	150 μg/100 g	Intra-arterial	0	+	—	—
	0.5–20 mg	Intracystic	—	+	+	+
	2 μg, 20 μg, 200 μg	Intrarenal	0	—	—	—
Serotonin	1500 μg, 150 μg, 15 μg, 1.5 μg/100 g	Intravenous	0	+	+	+
	15 μg/100 g	Intra-arterial	0	+	—	—
	1.5 mg	Intracystic	—	+	+	+
	30 μg	Intrarenal	0	—	—	—
Bradykinin	100 μg/100 g	Intravenous	0	+	+	+
	100 μg/100 g	Intra-arterial	0	0	—	—
	200 μg	Intracystic	—	+	+	+
	20 μg	Intrarenal	0	—	—	—
Horseradish peroxidase	5–20 mg/100 g	Intravenous	0	+	+	+

[a] 0 = no labeling; + = positive labeling; — = not applicable, since with these routes of administration the tissues in question are not exposed to test substance and/or tracer.

[b] Calculated as histamine diphosphate.

histamine liberation and increased vascular permeability when injected intravenously in the Sprague-Dawley rat (10). Examination of the kidney and urinary tract after intravenous injection of peroxidase (Table I) showed widespread labeling of the vessels in the peripelvic fat, the wall and adventitia of the ureter, the adventitia of the urinary bladder and the renal capsule. No leakage whatsoever was seen in renal parenchymal vessels. The labeled vessels corresponded in location to the distribution of mast cells in the kidney: toluidine blue stain confirmed (11) that mast cells were present in the pelvis and capsule of the kidney but not within the renal parenchyma, except occasionally around a larger blood vessel at the hilus.

Discussion. Our experiments indicate that three vasoactive substances (histamine, serotonin and bradykinin) are unable to induce vascular leakage within the renal parenchyma, as determined by the method of vascular labeling with carbon (8).

Successful interpretation of the latter method requires the presence of circulating carbon in the susceptible vessel at the time when the mediator is active. In both intra-arterial and intraparenchymal injections the experimental conditions described above included direct observation of carbon circulating within the kidney. That such animals were capable of developing a histamine-type response in susceptible vessels was confirmed by the regular occurrence of labeling in the venules of the lower urinary tract. As shown in Table I, we used a wide range of doses for intravenous, intracystic and intraparenchymal injections of histamine. Thus, it is unlikely that the lack of vascular labeling in our experiments is related to the doses used.

Other factors may be responsible for the failure of intraparenchymal renal vessels to respond to the histamine-type liberators. In a previous publication it was postulated that renal vessels did not exhibit labeling due to the tightness of the renal capsule, since this property was shared with the vessels of the testis (8). However, Gabbiani, Badonnel and Majno (7) could not obtain vascular labeling with histamine in the testis even after splitting the tunica albuginea over a length of 5 mm surrounding the local injection. Another possible explanation for the lack of histamine effect may be the presence of histaminase ac-

tivity within the kidney (12); however, this could not explain the lack of response of serotonin and bradykinin. The possibility that local vasoconstriction or shunting would impede the delivery of vasoactive substances and carbon to the intrarenal vessels would also be an unlikely explanation, since perfusion of the parenchyma with circulating carbon was directly observed and there was regular labeling of capsular and lower urinary tract vessels.

It is possibile that some peculiarity of renal anatomy and microcirculatory physiology prevents vascular leakage. The renal cortical interstitial pressure, once presumed to be high, is now believed to be no greater than 5 mm Hg (13). We have induced water diuresis in rats by adding 5% glucose to the drinking water (14). The urine osmolality fell from approximately 1000 to approximately 300. Histamine and horseradish peroxidase injected during diuresis again failed to result in labeling of vessels. It is unlikely, therefore, that medullary hypertonicity alone accounts for the lack of vascular response.

The structural features of renal microvasculature deserve consideration. The vast majority of small blood vessels within the renal parenchyma are the peritubular capillaries. It is assumed that small veins and venules are present in connection with the interlobular vessel. Particular attention was given to perivascular connective tissue which could conceivably contain labeled small venules but these were not found. The peritubular capillaries in the cortex and the ascending capillaries of the medullary rete are of the fenestrated type, but the arterial capillaries of the vascular bundles have a continuous although specialized endothelium (6). The response of fenestrated-type endothelium to histamine-type mediators has not been studied in great detail. Clementi and Palade (15) reported vascular leakage of carbon in intestinal fenestrated capillaries after local intra-arterial perfusion of histamine. Hurley found no histamine leakage in the fenestrated vessels of the intestinal mucosa whereas the venules of the serosa were responsive (J. V. Hurley, personal communication). The intestinal fenestrated vessels did, however, respond to chemical injury. It seems likely that the lack of response of intraparenchymal renal vessels to vasoactive substances may be related to peculiarities of their structure. In this respect, since recent work by Majno, Shea and Leventhal (3) strongly suggests that histamine-type mediators cause contraction of endothelial cells, it may be that intrarenal small vessels contain endothelial cells that lack a contractile mechanism.

Gabbiani, Badonnel and Majno (7) reported several other organs in which blood vessels do not respond to intra-arterial injections of histamine, serotonin or bradykinin: these include the testis, brain, cerebellum and Gasserian ganglion. They refer to the fact that at least two of these organs, the brain and the testis, are devoid of mast cells. Mast cell stains of the kidney also show a paucity of mast cells within the renal parenchyma, as opposed to their frequent occurrence in the renal capsule and the serosa of the lower urinary tract (11). The latter finding accounts for the localization of vascular leakage principally in the adventitia of the lower urinary tract after systemic injections of the mast cell damaging enzyme, horseradish peroxidase.

The failure of histamine-type mediators to induce vascular leakage in the renal parenchyma leads to the larger question of renal vascular response to other forms of inflammatory renal injury, for example bacterial infection. Although local edema is described as a component of the histological reaction in acute pyelonephritis, there have been no specific studies on the presence of localization of increased vascular permeability in either hematogenous or ascending pyelonephritis. Whereas there is considerable evidence of differences between the renal cortex and renal medulla as regards the rapidity and severity of neutrophilic exudation in response to local injury (16), little is known of vascular leakage at these two sites. Finally, it is to be emphasized that the lack of vascular response in renal vessels was determined only with respect to leakage of carbon particles, and our experiments

did not consider other possible hydrodynamic or permeability effects of the vasoactive agents. In this regard studies using Evans Blue and other *protein* tracers suggest that renal medullary vessels are freely permeable to proteins and that this permeability is altered by such pharmacologic agents as antidiuretic hormone (17). It remains to be seen whether renal blood vessels will respond to other presumed mediators of the inflammatory response or whether direct injury plays the most important role in vascular leakage in the kidney.

Summary. Histamine, serotonin, bradykinin and the mast cell damaging enzyme, horseradish peroxidase, when delivered to the kidney by various routes, failed to cause venular leakage of intravenously injected colloidal carbon within the renal parenchyma. This finding was accompanied by typical venular leakage in the peripelvic, periureteral and pericystic fat and submucosa. Failure of parenchymal renal vessels to respond to vasoactive substances is probably the result of some functional or structural peculiarity of the renal microvasculature.

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