Effects of Vasoactive Agents and Diuretics on Isolated Superfused Interlobar Renal Arteries[1] (36771)

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Many diuretic agents alter renal blood flow by changing renal vascular resistance (1, 2). However, little information is available concerning the actions of diuretics and other drugs on renal vascular smooth muscle, uncomplicated by reflex, hormonal or other extraneous influences. The purpose of this study therefore was to isolate a segment of the renal vascular tree for evaluation *in vitro* of direct actions of diuretics and other vasoactive agents. Renal arterioles constitute the major control of vascular resistance, however, the preafferent arteriolar portion of the renal vasculature accounts for a significant amount of the total resistance (3). For this reason, and because of the inaccessibility of the arterioles for work *in vitro*, interlobar renal arteries were chosen for study.

Methods. Mongrel dogs weighing from 16 to 31 kg were anesthetized with pentobarbital sodium (30 mg/kg) and tracheotomized. Kidneys were approached by a retroperitoneal flank incision and quickly excised after clamping the vessels. The excised kidney was immediately immersed in a warmed (38°) oxygenated (95% O_2–5% CO_2) physiological salt solution identical to that used by Bohr and Goulet (4) and by Carlson and Sparks (5) on interlobar renal aeteries in constant temperature baths. Excess blood was removed from the kidney by gentle manual pressure and the kidney was sectioned into halves in a coronal plane. The renal artery was traced into the pelvis and medulla where it branched into several interlobar arteries, averaging about 1 mm in external diameter. A portion of this vascular tree was excised and the adhering connective tissue carefully removed under a dissecting microscope. An interlobar artery was sectioned longitudinally and opened to expose the endothelial lining. Transverse sections were made of this vessel yielding strips approximately 1 mm wide. Surgical silk (6-0) was used to secure one end of the strip to a rigid metal hook in the superfusion apparatus and the other end to a force displacement transducer (Statham). This mounting procedure allows orientation of smooth muscle fibers in a vertical plane facilitating maximal displacement of the transducer arm. Responses to drugs were recorded as changes in tension (mg). Tissues were superfused, with the above mentioned solution, according to the technique of Gaddum (6). A flow rate of 6 ml/min was maintained by a Sigmamotor pump (Model T-8). Superfusate was directed onto the tissue through a small funnel-shaped bipolar electrode. Initial tensions of 500–700 mg were placed on the preparations, and the tissues allowed to stabilize for about 2 hr. During this time period a gradual loss of tension occurred and the preparations increased in sensitivity. Changes in tension during this time period have been suggested to be due to lengthening of undefined structural elements rather than to loss of spontaneous tone (7). Base line tension was then varied for each strip to obtain maximal tissue contractile responsiveness for a particular dose of agonist. This procedure established the length-tension optimum for each piece of tissue. This base line tension averaged 200 mg. Once tension and sensitivity had stabilized, these parameters remained constant with regard to drug responses and base line tension for approx-

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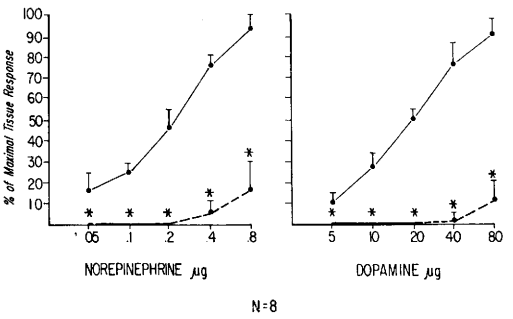

FIG. 1. Contractile response of the superfused interlobar renal artery to norepinephrine and dopamine, alone and in the presence of phentolamine. (●–) Control; (●- - -) treated; (*) significant difference.

imately 4 hr.

Agonists were introduced, by injection, into the superfusate at a point just above the funnel electrode. When drugs were tested for their ability to antagonize norepinephrine-induced vasoconstriction (furosemide, hydrochlorothiazide and phentolamine), they were added to the solution reservoir, so as to provide a constant concentration of antagonist superfusing the tissue. Sympathetic nerves within the vessel wall were stimulated at 12 V, 0.02 msec duration, with variable frequency, via the funnel-shaped bipolar electrode connected to a Grass stimulator (S-4).

Data were analyzed by an analysis of variance, randomized complete block design, and Duncan's new multiple range test (8). A p value of less than .05 was used as the criterion for significance.

Results. Norepinephrine, dopamine, sympathetic nerve stimulation, KCl and angiotensin were used in an attempt to characterize the reactivity of this preparation. Since tension produced by agonists varied between these small strips, data are expressed as the percentage of the maximum tension produced by the particular agonist on that piece of tissue.

Norepinephrine, in doses of 0.05 to 0.4 µg, produced dose-related contractions of the vascular strips. Dopamine, in doses ranging from 5 to 40 µg, produced dose-related contractions of similar magnitude (Fig. 1). Thus, the potency ratio was approximately 100. Phentolamine (2 µg/ml) added to the superfusate, antagonized the contractile responses of these two agonists.

Dose-related increases in tension of the preparation to sympathetic nerve stimulation and KCl are shown in Table I. Angiotinsin was found to be inactive as a vasoconstrictor, throughout a wide range of concentrations (0.01–10 µg). Two diuretics, furosemide which decreases renal vascular resistance and hydrochlorothiazide which increases renal vascular resistance, were evaluated for activity on the interlobar artery. In this experiment base line tension was elevated an additional 200–300 mg by the addition of norepinephrine to the superfusion solution. This procedure allowed visualization of either contractile or relaxant effects in the same preparation. The ability of the preparation to show relaxant effects was confirmed with small doses of acetylcholine. Furosemide did not alter tension in these preparations in single doses up to 8 mg. Hydrochlorothiazide produced dose-related increases in tension at doses of 1, 2, 4 and 8 mg (Table II). No relaxant effects were observed with either agent.

In order to test the ability of furosemide and hydrochlorothiazide to alter the increase in tension produced by norepinephrine, the

TABLE I. Response of the Isolated, Superfused Interlobar Renal Artery to KCl and Sympathetic Nerve Stimulation.

	KCl dose (mg)				
	1	2	4	8	
Mean	5.1[a]	33.4	54.1	91.2	
± SE	2.4	1.8	1.4	2.6	
	Nerve stimulation (Hz)				
	2.5	5	10	20	40
Mean	8.3[b]	19.8	37.1	69.5	84.2
± SE	2.4	2.8	5.3	5.8	2.8

[a] Values represent increases in tension (mg) expressed as a percentage of the maximal response. Tissues responded maximally to 10 mg KCl; $n = 5$.

[b] Values represent increases in tension (mg) expressed as a percentage of the maximal response. Tissues responded maximally to 60 Hz; $n = 5$.

TABLE II. Responses of the Isolated, Superfused Intralobar Renal Artery to Hydrochlorothiazide.[a]

	Hydrochlorothiazide dose (mg)			
	1	2	4	8
Mean	*35*[b]	*70*	128	224
± SE	13	34	59	80

[a] Data represent increases in tension (mg) exerted by the vascular strips after drug addition. Since data are not expressed as percentage of tissue maximum, variability is greater. Vehicle had no effect.

[b] Means italicized are not different from each other ($p < .05$). Statistics used were an analysis of variance, randomized complete block design, and Duncan's new multiple range test. Data from arteries obtained from 4 dogs.

diuretics were added to the superfusate reservoir and norepinephrine was used as the agonist. When furosemide (400 μg/ml), was added to the superfusate, no change in the ability of norepinephrine (0.05 to 0.4 μg) to contract the tissue was observed (Fig. 2). In addition, the maximum increase in tension produced by norepinephrine was unchanged after furosemide.

In a similar experiment, hydrochlorothiazide was added to the superfusate in concentrations up to 125 μg/ml. Above this concentration, chemical incompatibility and precipitation occurred. No change in base line tension was observed. Hydrochlorothiazide significantly attenuated the responses of 0.05 to 0.2 μg of norepinephrine but did not attenuate the 0.4 μg dose or prevent maximal contraction of the tissue to approximately 1 μg of norepinephrine (Fig. 3).

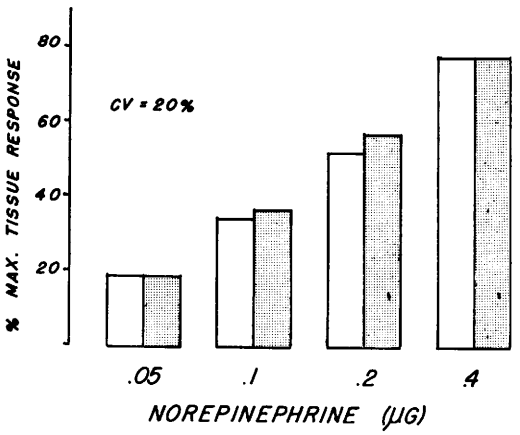

FIG. 2. Effects of furosemide on the contractile response of the superfused interlobar renal artery to norepinephrine. Clear bars = control; stippled bars = during furosemide superfusion; CV = coefficient of variation, $n = 6$.

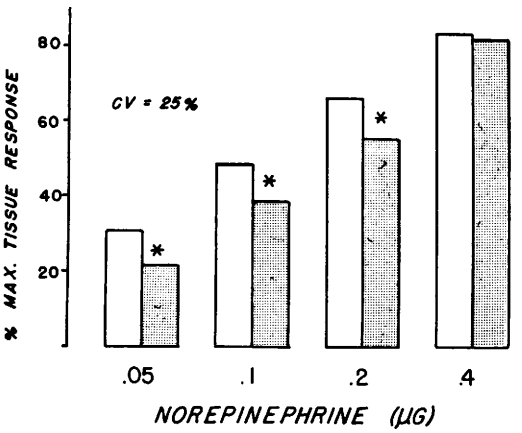

FIG. 3. Effects of hydrochlorothiazide on the contractile response of the superfused interlobar renal artery to norepinephrine. Clear bars = control; stippled bars = during hydrochlorothiazide superfusion; CV = coefficient of variation, $n = 5$, (*) significant difference.

Discussion. Experiments have been described that characterize the reactivity of an isolated, superfused interlobar artery preparation to various vasoactive agents. The interlobar artery was chosen for several reasons: (a) this artery lies totally within the kidney and is the smallest renal vessel accessible for excision and superfusion, (b) it contributes significantly to the total renal vascular resistance (3), and (c) if drug effects on this artery differed from those of extra renal arteries, this preparation would suggest intrinsic differences in the renal vessels.

The isolated intralobar artery was found to contract in response to KCl and sympathetic nerve stimulation. Both norepinephrine and dopamine were found to constrict the interlobar artery and this action was antagonized by phentolamine, suggesting an interaction with alpha adrenergic receptors. These findings are consistent with those of Zaroslinski and Browne (9) who used segments of

the main renal artery. Renal vasodilatation has been reported following small doses of dopamine *in vivo* (10, 11). No evidence for a relaxant action of dopamine was found in this or other studies (9). Thus it appears that the larger arteries of the kidney do not contribute to the renal vasodilatory action of dopamine.

The renal vasculature appears to be unique in that furosemide causes a rapid vasodilation in this organ but has no direct effect upon the vasculature of the hind limb (2, 12). On the isolated interlobar artery, furosemide did not alter tension nor attenuate the constrictor action of norepinephrine. Thus, no evidence was found to indicate that tension of larger renal arteries is decreased in response to furosemide.

Superfusion of the tissue with hydrochlorothiazide (125 μg/ml) was found to attenuate the contractile responses of norepinephrine. The highest doses of norepinephrine used (0.4 and 1 μg) were not antagonized significantly suggesting a competitive depression of reactivity. Thiazides have been reported to reduce vascular reactivity in other preparations (13–17). In this respect, this preparations appears to be similar to other vascular beds.

Thiazides have been reported to decrease renal blood flow (2, 18). Hydrochlorothiazide was found to produce dose-related contractions of the interlobar strips. High doses were necessary. However, since the drug is in contact with the tissue for only a short period of time in the superfusion technique it is possible that penetration into the tissue may be slower thus necessitating higher doses. No relaxant action of hydrochlorothiazide was observed on this preparation. This may be due to the fact that the norepinephrine concentrations in the superfusate were high enough to competitively overcome any relaxant acion of the hydrochlorothiazide.

Summary. The isolated, intralobar renal artery appears to be a useful preparation to study the actions of agents upon renal vasculature. It may be concluded that the interlobar arteries contribute to the renal vasoconstriction produce by norepinephrine and dopamine, but do not contribute to the renal vasodilatation produced by furosemide or small doses of dopamine. Hydrochlorothiazide may interfere with adrenergic vasoconstriction to an extent noted for other vascular beds. It is equivocal whether the larger renal arteries contribute to the renal vasoconsticton produced by thiazides.

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