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Vasodilators, Intrarenal Distribution of Blood Flow, and Renal Function in Isolated Perfused Canine Kidneys[1] (39502)

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Introduction. Vasodilator compounds influence the renal excretion of salt ($U_{Na}V$) and water (V) in addition to their effects on renal hemodynamics (1–3). It is difficult to delineate *in vivo* the mechanisms by which such compounds alter renal physiology since they may affect the kidneys directly or indirectly alter systemic hemodynamics and extrarenal organ functions. In the present investigations, we have attempted to simplify such analyses of the actions of seven different vasodilator compounds by measuring their effects on renal hemodynamics and function in isolated blood-perfused canine kidneys. The isolated preparation permits control of extrarenal hemodynamics (stroke volume, pulse rate, and perfusion pressure) in the absence of an intact nervous system and extrarenal metabolic or endocrine influences. By eliminating these variables, it is often possible to bring out patterns of response to vasodilators which are not easily demonstrable *in vivo*. The vasoactive agents chosen for the present studies included directly acting vasodilators of natural origin (bradykinin, PGE_2, histamine, and acetylcholine) and several compounds which cause vasodilatation indirectly by altering the activities of other naturally occurring vasoactive substances (SQ 20881, saralasin) (4, 5). One additional vasodilator, eledoisin, a polypeptide derived from snake venom, was also included in these investigations for comparative purposes.

Materials and methods. Our method of isolated renal perfusion has been described in previous reports (6, 7). Kidneys and autologous blood perfusate (800 ml) were obtained from male mongrel dogs (15–20 kg) which had received heparin (4 mg/kg) and pentobarbital anesthesia (65 mg/kg, iv). Isolated perfusion was instituted within 2 min of nephrectomy using a Water's membrane oxygenator and perfusion system. Electrolytes (sodium, potassium, calcium, magnesium, chloride, phosphate, and bicarbonate) and urea were added to the perfusate at rates to replace urinary losses. Glucose, lactate, and pyruvate were dissolved in a 0.45% saline solution to final concentrations of 2 mg/ml, 9.0 mg/ml, and 0.9 mg/ml, respectively. In addition, this solution contained regular insulin (0.02 units/ml), antidiuretic hormone (2 munit/ml), and creatinine and *para*-aminohippurate (PAH) for clearance purposes. The solution was delivered into the renal venous perfusate at a rate (0.5 ml/min) which maintained the perfusate concentration of glucose in a range of 75–110 mg/dl and which provided sufficient or excess metabolic substrates, insulin, and antidiuretic hormone to the kidney for its energy needs and physiologic functions (8–10). Hemodynamic measurements included renal perfusion pressure (Narco manometer), total renal blood flow (RBF), and the intrarenal distribution of blood flow (radioactive microsphere method of McNay and Abe as adapted for the isolated kidney) (7). By the latter technique, the absolute and percentage of blood flows may be measured to the outer and inner halves of the renal cortex with that fraction of blood flow to the inner half of the canine renal cortex also reflecting medullary blood flow. Parameters of renal functions were assessed by measurement of clearances of creatinine (C_{Cr}), PAH, sodium, and osmolality.

Each vasodilator was infused into the renal arteries of five isolated kidneys during 15- to 30-min clearance periods at times of stable renal function. The rates of infusion were adjusted to increase renal blood flow by approximately 20–40% over control measurements with maintenance of renal

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perfusion pressure at a constant systolic level of 130 mm Hg. The dose range to accomplish this for each vasodilator is specified in the legend to Fig. 1. Hemodynamic and renal functional measurements were compared for the periods prior to and during the infusion of each vasodilator.

Results. Figure 1 and Table I summarize hemodyanmic and renal functional data obtained before and during infusions of vasodilator substances into 35 isolated canine kidneys. Each of seven vasodilators produced comparable increases in mean RBF (20 to 40%) at constant renal perfusion pressure. In all cases, no matter which vasodilator was infused, the fraction of RBF which perfused the inner renal cortex and medulla was increased to a greater extent as a result of vasodilatation than was the fraction of RBF which perfused the outer renal cortex. These data were consistent whether the intrarenal distribution of blood flow was measured by the microsphere technique or by the extraction ratio of PAH (E_{PAH}), which was diminished during the infusion of vasodilators.

In contrast to similar effects of each vasodilator on RBF, renal perfusion pressure, and intrarenal distribution of blood flow, the effects on the glomerular filtration rate (GFR) as measured by C_{Cr} varied widely (Table I). Eledoisin and PGE_2 caused significant reductions of C_{Cr} whereas the effect of bradykinin was insignificant. Infusions of two inhibitors of the renin angiotensin system, SQ 20881 (inhibitor of angiotensin I

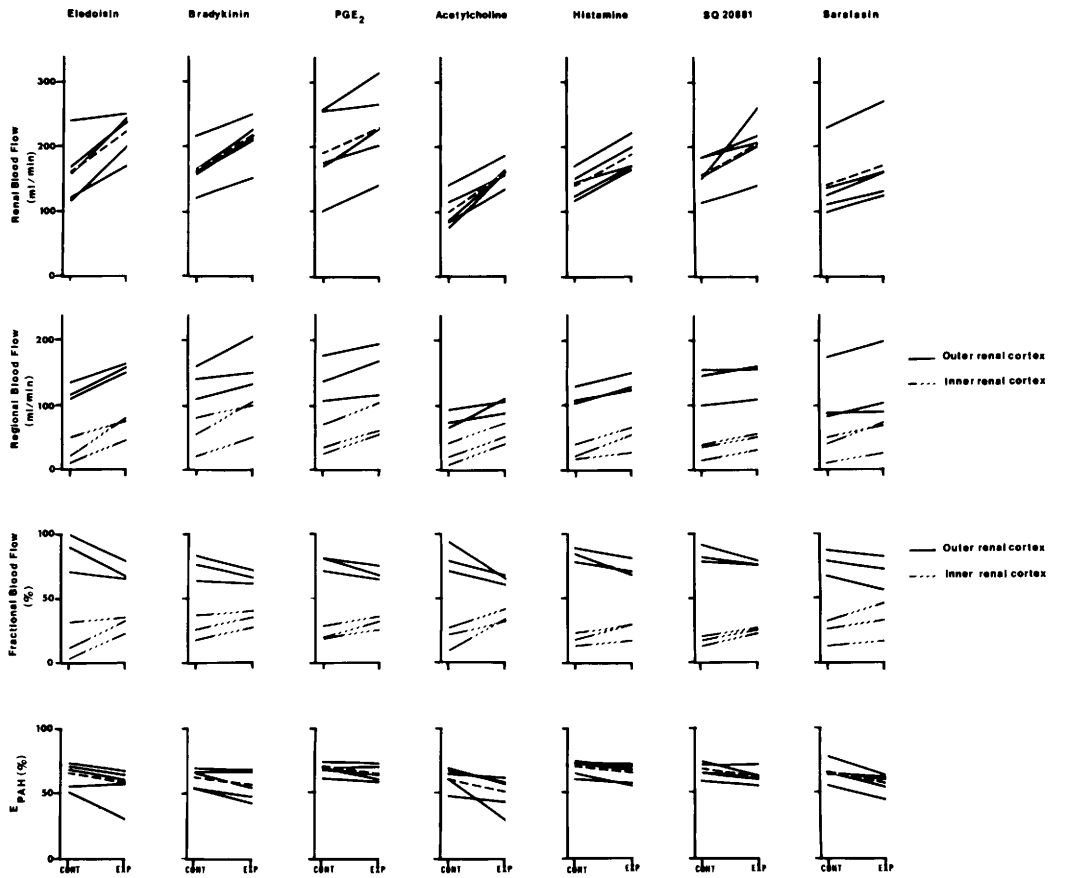

FIG. 1. Effects of vasodilators on renal hemodynamics. Solid lines signify changes in individual kidneys; broken lines signify mean changes. Infusion rates of vasodilators were ($\mu g\ kg^{-1}\ min^{-1}$): eledoisin, 0.10–0.40; bradykinin, 0.02–0.09; PGE_2, 0.01–0.11; acetylcholine, 0.25–5.0; histamine, 0.50–4.60; SQ 20881, 2.50–5.00; saralasin, 2.20–8.10. Note that each vasodilator caused increase in fractional blood flow to inner zone of kidneys.

TABLE I. EFFECTS OF VASODILATORS ON RENAL FUNCTION.[a]

	C_{Cr}[b] (ml/min)	C_{Cr}/C_{PAH}[c] (%)	C_{Cr}/RBF[d] (%)	FE_{Na}[e] (%)	FE_{H_2O}[f] (%)	$U_{Na}V$[g] (μEq/min)	V[h] (ml/min)
Eledoisin							
Control	20.5 ± 3.0	33.3 ± 4.8	14.8 ± 2.8	4.0 ± 0.5	5.6 ± 1.7	116 ± 17	0.99 ± 0.20
Experimental	13.3 ± 2.8	19.1 ± 1.4	6.4 ± 1.1	2.6 ± 0.6	5.4 ± 1.3	54 ± 16	0.62 ± 0.13
P	<0.05	<0.05	<0.05	<0.025	N.S.	<0.01	<0.025
Bradykinin							
Control	19.0 ± 0.8	29.4 ± 3.0	12.5 ± 1.3	3.6 ± 1.3	6.8 ± 2.8	112 ± 50	1.31 ± 0.56
Experimental	16.7 ± 1.4	21.6 ± 1.8	7.9 ± 0.2	4.4 ± 1.7	9.4 ± 4.0	128 ± 54	1.65 ± 0.78
P	N.S.	<0.02	<0.025	N.S.	N.S.	N.S.	N.S.
PGE_2							
Control	23.7 ± 3.3	26.7 ± 5.1	13.6 ± 2.8	2.9 ± 0.9	4.4 ± 1.0	104 ± 38	0.98 ± 0.29
Experimental	18.3 ± 2.4	20.8 ± 3.3	8.8 ± 1.2	2.8 ± 1.0	5.6 ± 1.3	79 ± 29	0.95 ± 0.24
P	<0.05	N.S.	<0.05	N.S.	<0.02	N.S.	N.S.
Acetylcholine							
Control	14.4 ± 2.6	36.0 ± 4.3	15.8 ± 3.1	6.5 ± 0.3	5.9 ± 0.7	124 ± 20	0.84 ± 0.16
Experimental	16.1 ± 2.4	23.4 ± 1.9	10.4 ± 1.1	16.8 ± 4.4	16.4 ± 4.7	332 ± 69	2.53 ± 0.57
P	N.S.	<0.05	N.S.	<0.05	<0.05	<0.05	<0.025
Histamine							
Control	16.3 ± 1.4	23.9 ± 2.8	11.7 ± 1.4	3.6 ± 0.9	3.8 ± 0.8	76 ± 19	0.58 ± 0.11
Experimental	17.2 ± 1.5	19.7 ± 2.2	9.2 ± 0.9	2.7 ± 0.6	3.2 ± 0.5	61 ± 12	0.52 ± 0.06
P	N.S.	<0.01	<0.01	<0.05	N.S.	N.S.	N.S.
SQ 20881							
Control	16.6 ± 0.6	27.1 ± 2.3	11.0 ± 0.7	4.4 ± 1.2	4.0 ± 1.4	98 ± 31	0.67 ± 0.21
Experimental	16.8 ± 0.7	20.3 ± 1.5	9.0 ± 1.0	4.8 ± 1.3	5.4 ± 1.5	124 ± 35	0.90 ± 0.25
P	N.S.	<0.02	N.S.	N.S.	<0.05	N.S.	N.S.
Saralasin							
Control	15.9 ± 2.6	31.3 ± 4.0	11.4 ± 1.4	8.0 ± 1.7	9.2 ± 1.3	174 ± 36	1.42 ± 0.25
Experimental	16.7 ± 3.2	24.8 ± 1.0	10.0 ± 0.8	8.8 ± 1.3	10.4 ± 2.0	212 ± 69	1.95 ± 0.78
P	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.

[a] Mean values ± standard errors are compared before (control) and during (experimental) infusions of vasodilators into each of five isolated kidneys. Statistical significance of effects is calculated on basis of Student's paired *t* test. N.S. = not significant.

[b] C_{Cr} = clearance of creatinine.

[c] C_{PAH} = clearance of PAH.

[d] RBF = renal blood flow.

[e] FE_{Na} = fractional excretion of sodium.

[f] FE_{H_2O} = fractional excretion of water.

[g] $U_{Na}V$ = excretory rate of sodium.

[h] V = urine flow.

converting enzyme) and saralasin (competitive antagonist for receptors of angiotensin II), caused little change in C_{Cr}. Similarly, histamine and acetylcholine did not reduce the C_{Cr}. The latter agents, if anything, tended to increase the C_{Cr}, although an insufficient number of studies was performed to draw definite conclusions. Despite the different effects of vasodilators in respect to *GRF*, each compound except saralasin reduced the ratio of GFR to renal blood flow (filtration fraction) in the isolated kidney. These results were obtained by calculating the filtration fraction in two ways (C_{Cr}/RBF on the basis of the total renal blood flow or C_{Cr}/C_{PAH} on the basis of clearance of PAH). Saralasin tended to reduce the filtration fraction also, but this effect was not significant ($P < 0.2$) relative to the few experiments which were conducted, possibly since the actions of saralasin may have varied widely dependent on the state of renin production by the individual kidneys.

Salt and water reabsorption and excretion were affected by the several vasodilators in different ways (Table I). These differences could be attributed to the dissimilar responses of the vasodilators on the GFR and/or the tubular reabsorption of salt and water. Eledoisin caused the most marked reductions in $U_{Na}V$ and V since it diminished the GFR as it enhanced the tubular reabsorption of salt (decreased FE_{Na}). There was little change in $U_{Na}V$ and V during the infusion of histamine. Small reductions in the reabsorption of water occurred during infusions of bradykinin, PGE_2, and SQ 20881. This effect allowed a slight diuresis to occur in those cases where these agents did not reduce GFR to a marked degree. In general, FE_{Na} (fractional excretion of sodium) was affected less by these compounds than

FE_{H_2O}. Acetylcholine differed from all other vasodilators presently investigated since it caused a marked natriuresis and diuresis primarily by its ability to reduce the tubular reabsorption of salt and water. The effect of saralasin on sodium and water metabolism has been inconsistent in our studies to date in the isolated kidney.

Discussion. Present investigations in isolated, perfused kidneys indicate that vasodilators uniformally increase fractional RBF to the inner renal cortex (Fig. 1). This response was obtained consistently in 21 experiments using seven different vasodilators. Our previous studies in isolated kidneys demonstrated an opposite effect of vasoconstrictors to diminish fractional RBF to this same inner zone of the kidney (7, 11). These results indicate relatively greater alterations of vascular resistance in the deep renal circulation compared with the outer renal circulation in response to vasoactive compounds. Although the mechanism for this differential effect is unknown, the fact that all vasoconstrictors tested in the isolated kidney act one way and all vasodilators act another way indicates a common mechanism and suggests that an inherent anatomic feature of the deep renal vasculature might be involved in these responses. It may be pertinent that vascular resistance in the inner renal circulation has been reported to be much greater than in the outer renal circulation (12) as a consequence of the great length of arterioles in the deep renal cortex and medulla (13). Therefore, the possibility is to be considered that these high resistance vessels may be especially responsive to the effects of circulating vasoconstrictors and vasodilators as one explanation for present results.

Although other investigators have infused vasodilator and vasoconstrictor hormones into kidneys *in vivo* without consistent effects on intrarenal blood blow (14, 15), there are several factors to explain these differences. First, it is not possible to control renal perfusion pressure as exactly *in vivo* as can be accomplished in isolated kidneys, and alterations of perfusion pressure influence of the intrarenal distribution of blood flow (16). Also, vasodilators stimulate compensatory adjustments of other regulatory systems *in vivo,* such as sympathetic nervous responses, renin or prostaglandin release which help determine the intrarenal distribution of blood flow (7, 11). The rates at which vasodilator or vasoconstrictor compounds were infused may be an additional factor. In present studies, doses of vasodilators were infused that caused only moderate increases in RBF. Normally, only a small fraction of the total RBF perfuses the deep cortical and medullary circulations (12, 15). If one infuses vasoactive agents in concentrations to produce marked changes in RBF, then of necessity a large part of the effect must involve the outer renal cortex since the capacity of inner cortical blood flow to change is limited. Thus, large doses of vasoactive compounds may mask a greater relative responsiveness of the deep renal vasculature to compounds, which is apparent only at a lower dose range.

It is of interest that different vasodilators had dissimilar effects on GFR despite comparable effects on other aspects of renal hemodynamics. Such differences can be caused in part by relatively greater effects of specific vasodilators on renal afferent or efferent arterioles to alter glomerular hydrostatic pressure (15). Other mechanisms may be that various compounds affect the glomerular capillary membrane differently to alter the ultrafiltration coefficient or cause different responses in intratubular pressure (15). Although our data demonstrated reductions of the filtration fraction after each vasodilator, indicative of greater efferent than afferent arteriolar relaxation, no additional information was obtained to allow an accurate analysis of why the C_{Cr} was altered differently by the vasodilators which we studied.

Despite comparable effects on RBF and perfusion pressure, the dissimilar patterns in response of $U_{Na}V$ and V to infusions of the different vasodilators are apparent in Table I. Several of these deserve further comment. Thus, the present data demonstrate the potency of acetylcholine relative to other vasodilators in diminishing the tubular reabsorption of salt and water. PGE_2 and

bradykinin had similar effects on salt and water metabolism in the isolated kidney. Since bradykinin has been shown to release PGE_2 in the kidney (17), a part of its effect could have been related to an increased release of endogenous PGE_2. It was reported recently that following inhibition of prostaglandin synthesis by indomethacin, bradykinin lost much of its ability to increase the renal excretion of free water (17). The ability of SQ 20881 to increase $U_{Na}V$ and V may have been related also to increased activity of endogenous bradykinin and PGE_2 since SQ 20881 not only inhibits the conversion of angiotensin I to angiotensin II but also inhibits the kininase responsible for the degradation of bradykinin (4). Finally, relatively consistent effects on $U_{Na}V$ and V were measured from experiment to experiment for each of the vasodilators except saralasin. Saralasin has both agonistic and antagonistic effects on angiotensin receptors which are dependent in part on rates of endogenous renin release (5, 18). Possibly, its inconsistent effects on salt and water metabolism in various isolated kidneys represented differences in its agonistic or antagonistic actions relative to the level of renin production by the isolated kdineys.

Summary. Each of the seven different vasodilators (acetylcholine, histamine, PGE_2, bradykinin, saralasin, SQ 20881, and eledoisin) infused into isolated blood-perfused canine kidneys at constant systolic perfusion pressure (130 mm Hg) at rates to cause 20–40% increases in RBF caused increases of fractional blood flow to the inner renal cortex and medulla as measured by radioactive microspheres. Despite similar hemodynamic effects of the different vasodilators on RBF, perfusion pressure and intrarenal distribution of blood flow, their effects on GFR, $U_{Na}V$, and V were dissimilar. In particular, eledoisin and PGE_2 reduced GFR and acetylcholine produced the greatest di-uresis and natriuresis by its effect to interfere with the tubular reabsorption of salt and water.

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