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Research Article

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Since it has been shown that a decrease in filtration fraction and presumably peritubular capillary protein concentration will decrease proximal tubular sodium reabsorption, studies were performed to determine whether the fall in total kidney filtration fraction seen with both vasodilators is paralleled by a similar change in the circulation of superficial nephrons. The results of these studies indicate that neither agent altered superficial nephron capillary protein concentration, hematocrit, or filtration fraction.

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The Effect of Bradykinin on Proximal Tubular Sodium Reabsorption in the Dog: Evidence for Functional Nephron Heterogeneity

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ABSTRACT In a previous study we have found that acetylcholine, a renal vasodilator, inhibits fractional and absolute reabsorption of sodium in the proximal tubule of the dog. To delineate whether this effect on proximal tubular sodium reabsorption was related to alterations in renal hemodynamics or to a direct tubular action of the drug, free-flow micropuncture studies were performed in the dog in which the tubular fluid to plasma inulin ratio and nephron filtration rate were determined before and during the administration of a structurally different renal vasodilator, bradykinin. This agent increased sodium excretion from 12 to 96 μEq/min and decreased total kidney filtration fraction from 0.35 to 0.25. However, sodium reabsorption in the proximal tubule of the superficial nephrons was unchanged during bradykinin administration.

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In contrast, a decrease in capillary protein concentration, hematocrit, and filtration fraction was consistently demonstrated during the intrarenal infusion of 7.5–15 ml/min of Ringer's solution while an increase in these parameters occurred during the i.v. administration of norepinephrine, 60 μg/min. In the Ringer's infusion studies, both fractional and absolute sodium reabsorption in the proximal tubule were decreased concomitant with the fall in capillary protein concentration and hematocrit.

This data suggests that: (*a*) the hemodynamic effect of renal vasodilatation is not the same in the circulation of all nephrons; (*b*) the inhibitory effect of acetylcholine on proximal tubular sodium reabsorption is due to a direct tubular action; (*c*) a decrease in capillary protein concentration and/or hematocrit does decrease proximal tubular sodium reabsorption; (*d*) although proximal reabsorption of sodium is unchanged in the superficial nephrons during bradykinin administration, a decrease in reabsorption may be present in deeper nephrons in which filtration fraction is decreased.

INTRODUCTION

Recent micropuncture studies from our laboratory have indicated that acetylcholine, a renal vasodilator, causes a decrease in fractional and absolute sodium reabsorption in the proximal tubule (1). There are two possible explanations for this inhibitory action of acetylcholine. First, Earley and Friedler (2) have proposed that the hemodynamic effect of this drug alters Starling forces in the peritubular capillary circulation which in some manner decreases the reabsorption of sodium. Second, Parmelee and Carter (3) and May and Carter (4) have performed studies in the chicken which suggest that acetylcholine and other cholinergic agents have a direct inhibitory effect on sodium reabsorption independent of any hemodynamic action of the drug.

To differentiate these two possibilities, proximal tubular sodium reabsorption was determined before and during the administration of a noncholinergic vasodilator, bradykinin. This vasodilator caused a natriuresis and the

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same hemodynamic alterations as acetylcholine but was found to have no effect on superficial nephron proximal tubular sodium reabsorption. Furthermore, the superficial nephron circulation was not altered by either agent in the manner that would be predicted from whole kidney clearance data. These findings indicate that the inhibitory effect of acetylcholine on superficial nephron proximal tubular sodium reabsorption is due to a direct tubular effect, and that the hemodynamic effect of renal vasodilatation is not the same in the circulation of all nephrons.

METHODS

Studies were performed on mongrel dogs weighing between 12 and 22 kg. All animals were deprived of food and water for 18–24 hr before the study. The dogs were anesthetized with pentobarbital (30 mg/kg) and were subsequently given small maintenance doses as necessary. An endotracheal tube was inserted and the animals were ventilated with a Harvard respirator (Harvard Apparatus Co., Inc., Millis, Mass.). Cannulas were inserted in a leg vein for infusions and in the femoral artery for blood pressure measurement and blood collection. Both ureters were cannulated through a suprapubic incision. A 23-gauge hooked needle was placed in the orifice of the left renal artery and kept open with an infusion of Ringer's solution at a rate of 0.2 ml/min. A catheter was placed in the left renal vein for measurement of para-aminohippurate (PAH)[1] extraction in all studies in which renal blood flow was determined.

The animal was prepared for micropuncture as previously described (1). An infusion was given to establish and maintain a plasma inulin concentration of 100 mg/100 ml and a PAH concentration in plasma of 2 mg/100 ml. The maintenance infusion was given at a rate of 1 ml/min. Samples of proximal tubular fluid were obtained with sharpened micropipettes containing colored mineral oil. Each tubule was blocked with a long column of oil and all fluid reaching the puncture site was collected in a precisely timed interval to permit the determination of tubular flow rate. Minimal suction was used and the majority of samples were collected spontaneously after initial gentle aspiration to begin the collection. In 11 experiments the recollection micropuncture technique was used, and samples were collected before and during the administration of bardykinin, 5 μg/min, in the left renal artery. In the first nine studies, the proximal tubules were chosen randomly while in the latter two studies, end proximal tubules were identified with the intrarenal injection of 0.5 ml of 5% Lissamine Green. The first collections were obtained at least thirty minutes after the Lissamine Green injections had been completed. Experimental period collections were obtained 15 min after the vasodilator solution had been started.

In studies in which postglomerular capillary protein concentration or hematocrit were determined, blood samples were collected from the center of the vascular star which is the terminal portion of the efferent arteriole. These capillaries are easily discernable from the remaining capillaries by their greater size and typical appearance. It was also found that after puncture of these large capillaries, blood would spontaneously enter the collecting pipette without suction while this did not occur in smaller peritubular capillaries. Blood samples were collected with sharpened micropipettes with an outer tip diameter of 10–14 μ. After puncture of the capillary, a small column of colored mineral oil was injected to determine proper placement of the pipette. If any oil was found to enter a surface convolution, no blood collection was obtained. In the protein collections, the internal surface of the pipettes was coated with a 1/200 silicone solution while in the hematocrit studies the pipettes were coated with an aqueous solution of heparin.

The protein samples were transferred and analyzed by the method described by Brenner, Falchuk, Keimowitz, and Berliner (5). For hematocrit determination, the blood was immediately transferred to a constant bore quartz capillary which had been previously coated with a dilute silicone solution and was sealed in a larger glass capillary. The capillary was initially filled with a small volume of mineral oil followed by the blood sample, and then another column of oil was added. The capillary tube was then sealed with a microflame with special precaution being taken to prevent heating the blood. The constant bore tubes were then placed inside a larger glass capillary, sealed with adhesive cement and spun in a hematocrit centrifuge for 30 min. The hematocrit was determined optically with the Vernier attachment of a Brinkmann micro-manipulator (Brinkmann Instruments Inc., Westbury, N. Y.). Concomitant with each peritubular capillary collection, a femoral artery blood sample was obtained for determination of arterial protein concentration or hematocrit by the same technique used for the capillary samples.

The following studies were performed using these methods: (*a*) In 14 studies, capillary samples were obtained before and during the administration of bradykinin, 5 μg/min, in the left renal artery. The experimental period collections were obtained 15 min after the vasodilator infusion had been started. In six of these studies, the peritubular capillary protein concentration was determined, while in eight, hematocrits were measured.

(*b*) 10 studies were performed in which capillary samples were obtained before and during the administration of acetylcholine, 40 μg/min, in the left renal artery. Protein data was obtained in four experiments and capillary hematocrits in the remaining six studies.

In all studies, an infusion of Ringer's solution was given at a rate of 0.2 ml/min in the left renal artery throughout the entire experiment with the appropriate vasodilator being added to the infusion solution in the experimental period.

(*c*) In these studies, the animal was prepared for micropuncture in an identical manner to that in the free-flow studies. After an initial 30 sec tubular fluid collection had been obtained, Ringer's solution with an inulin concentration of approximately 100 mg/100 ml was infused at a rate between 7.5 and 15 ml/min in the left renal artery. 15–30 sec after the infusion had begun, a repeat 30 sec collection was obtained from the same tubule. In addition, superficial nephron capillary blood was obtained before and during the Ringer's infusion concomitant with 10 of the tubular fluid recollection pairs. The total perfusion time in which both the tubular fluid and capillary sample were obtained was never greater than 3 min and the repeat tubular fluid collection was always completed within 1 min of starting the infusion. A blood sample was also obtained from the femoral artery and renal vein before and during each infusion. This same procedure was repeated two to four times at 20-min intervals in a given experiment. In another group of four studies, the same protocol was followed except that only capillary blood was collected and protein determinations were obtained with these samples. A maximum of 150 ml of Ringer's solution was given in any one experiment and the

[1] *Abbreviations used in this paper:* GFR, glomerular filtration rate; PAH, para-aminohippurate.

systemic hematocrit did not decrease by more than 2 ml/100 ml in any study.

(*d*) In five studies, three or more capillary blood samples were obtained before and during the i.v. administration of norepinephrine, 60 μg/min.

The clearance data presented is a mean of three 15-min collections in both the control and experimental periods. PAH was determined by the method of Bratton and Marshall (6). Plasma and urine inulin concentration were determined by the diphenylamine method (7). The concentration of inulin in tubular fluid was measured by the fluorometric method of Vurek and Pegram (8). Tubular fluid volume was measured in a constant bore capillary of known internal diameter. Sodium and potassium concentration in urine and plasma was measured by an Instrumentation Laboratory Flame Photometer (Instrumentation Laboratory Inc., Watertown, Mass.).

The data was analyzed by standard statistical methods and all results are presented as the mean ±1 SEM.

Calculations. (1) Nephron filtration rate (V_O) = $(TF/P)_{In}$ (V_F), where $(TF/P)_{In}$ is the tubular fluid-to-plasma inulin ratio and V_F equals the tubular flow rate in nanoliters per minute.

(2) Absolute reabsorption (nl/min) = $V_O - V_F$.

(3) Whole kidney filtration fractions = glomerular filtration rate/renal plasma flow. Glomerular filtration rate (GFR) was determined from the clearance of inulin and renal plasma flow from PAH clearance corrected for PAH extraction ratio.

(4) Filtration fraction of superficial nephrons was calculated from arterial and capillary protein concentration by the equation of Bresler (9):

$$(FF_p) = 1 - \frac{P_A}{P_C},$$

where P_A is the protein concentration in femoral artery blood and P_C is the superficial nephron capillary protein concentration.

(5) The filtration fraction was also calculated from the arterial and capillary hematocrit by the following equation:

$$(FF_{Hct}) = \frac{1 - \frac{Hct_A}{Hct_C}}{1 - Hct_A},$$

where Hct_A is the femoral artery hematocrit and Hct_C is the superficial nephron capillary hematocrit.

RESULTS

Bradykinin free-flow micropuncture studies. In Table I is shown the data of 11 studies in which proximal tubular sodium reabsorption was determined before and during the administration of bradykinin. In these studies the mean glomerular filtration rate was unchanged during bradykinin administration but sodium excretion increased in each with a mean change from 12 to 96 μEq/min. Fractional sodium excretion increased from 0.2 to 1.7%. There was no change in sodium excretion in the contralateral kidney nor was there any change in systemic blood pressure during bradykinin administration.

The values for all proximal tubule sample pairs are shown in Fig. 1. The mean TF/P_{In} ratio for the 53 collections was 1.57 in both the control and experimental period. When the data was analyzed using the mean value for each of the 11 studies, the TF/P_{In} ratios were also unchanged at 1.59 and 1.60 in the control and experimental period, respectively.

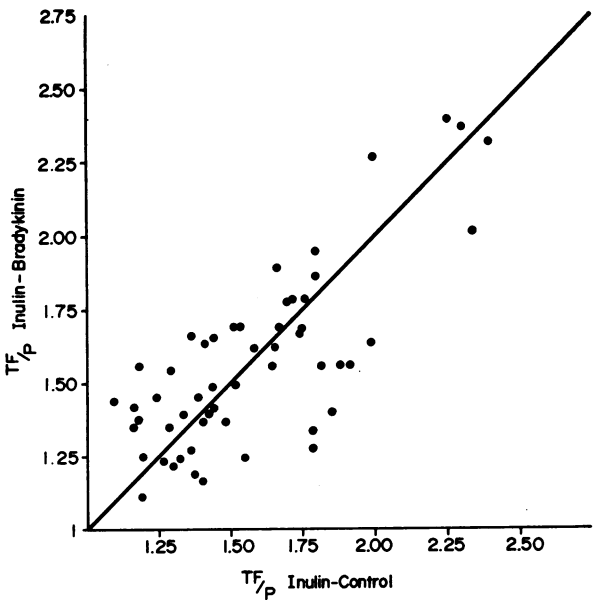

FIGURE 1 Effect of bradykinin on the tubular fluid to plasma inulin ratio in the proximal tubule.

Fig. 2 contrasts the results of these studies with data previously obtained during the administration of acetyl-

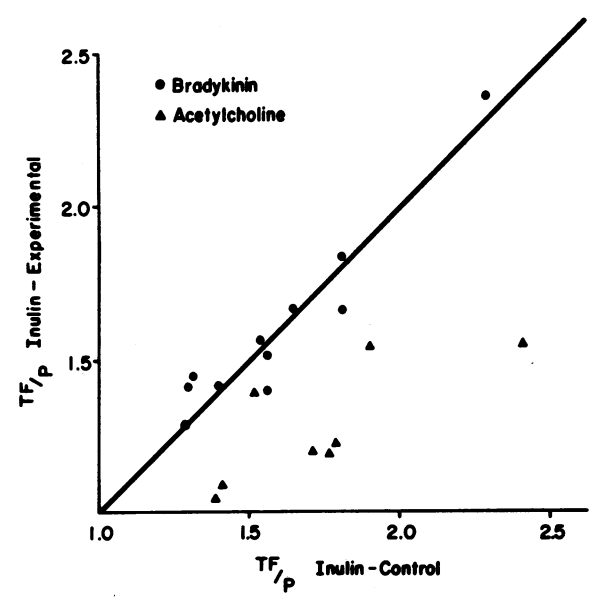

FIGURE 2 Comparison of the effect of bradykinin and acetylcholine on the tubular fluid to plasma inulin ratio in the proximal tubule. ● = bradykinin. ▲ = acetylcholine.

TABLE I

Summary of Bradykinin Free-Flow Studies

Exp. No.	GFR*		Sodium excretion		Fractional sodium excretion		TF/P inulin ratio		Nephron filtration rate		Absolute reabsorption		Distal nephron delivery		Renal plasma flow*		Filtration fraction	
	C	B	C	B	C	B	C	B	C	B	C	B	C	B	C	B	C	B
	ml/min		*μEq/min*		%		‡		*nl/min*		*nl/min*		*nl/min*		*ml/min*			
1	53	54	33	162	0.4	2.1	1.56 ±0.08 (5)§	1.51 ±0.08	155 ±9	185 ±13	56 ±8	62 ±10	99 ±4	123 ±8	155	184	0.34	0.29
2	39	40	8	72	0.1	1.3	1.28 ±0.18 (3)	1.28 ±0.07	51 ±8	81 ±8	12 ±5	16 ±5	39 ±4	65 ±6	101	156	0.38	0.26
3	40	35	20	126	0.4	2.6	1.56 ±0.11 (5)	1.40 ±0.08	70 ±8	63 ±3	24 ±4	17 ±3	46 ±7	46 ±4	112	182	0.35	0.20
4	46	50	3	35	0.1	0.5	1.81 ±0.13 (5)	1.83 ±0.07	71 ±4	87 ±4	31 ±2	39 ±2	39 ±3	48 ±3	164	264	0.28	0.19
5	47	40	8	91	0.1	1.6	1.81 ±0.07 (5)	1.66 ±15	105 ±16	134 ±9	47 ±8	50 ±4	58 ±8	84 ±10	117	138	0.40	0.29
6	47	47	12	50	0.2	0.8	1.30 ±0.09 (7)	1.42 ±0.05	94 ±7	85 ±9	20 ±5	25 ±4	74 ±7	60 ±7	129	181	0.36	0.26
7	49	56	10	150	0.2	1.9	1.40 ±0.08 (6)	1.41 ±0.06	64 ±8	66 ±13	17 ±2	20 ±5	47 ±7	46 ±8	127	225	0.39	0.25
8	22	28	6	83	0.3	2.1	1.31 ±0.20 (4)	1.45 ±0.09	107 ±14	94 ±15	25 ±5	29 ±7	82 ±10	65 ±9	68	93	0.33	0.26
9	32	32	10	92	0.2	2.0	1.54 ±0.07 (6)	1.57 ±0.09	81 ±3	82 ±4	30 ±3	31 ±3	50 ±4	50 ±4	—	—	—	—
10	35	37	14	105	0.3	2.1	1.65 ±0.10 (4)	1.67 ±0.15	99 ±8	96 ±4	39 ±6	38 ±6	60 ±6	58 ±5	—	—	—	—
11	36	39	6	85	0.1	1.6	2.29 ±0.07 (3)	2.36 ±0.03	107 ±8	119 ±7	60 ±3	69 ±4	47 ±4	50 ±5	—	—	—	—
Mean	41	42	12	96	0.2	1.7	1.59	1.60	91	99	33	36	58	63	120	177	0.35	0.25
SEM	2	2	3	11	0.04	0.19	0.09	0.09	9	11	5	5	6	7	10	17	0.01	0.01

Abbreviations: C = control period, B = bradykinin period.

* Mean of three collections in each period.

‡ Mean ±SEM.

§ Number in parenthesis indicates the number of recollected pairs.

TABLE II

Summary of Bradykinin and Acetylcholine Capillary Protein Studies

Experiment number	Capillary protein concentration		Arterial protein concentration		Capillary filtration fraction		Total kidney filtration fraction	
	C	E	C	E	C	E	C	E
	g/100 ml		*g/100 ml*					
Bradykinin studies								
B_1	9.9±0.6* (4)‡	9.2±0.3 (5)	7.0	6.4	0.29	0.30	0.35	0.19
B_2	7.5±0.5 (3)	7.8±0.3 (5)	5.3	5.3	0.29	0.32	0.37	0.23
B_3	8.6±0.3 (4)	7.7±0.2 (4)	6.2	5.6	0.28	0.27	0.34	0.29
B_4	8.4±0.3 (4)	8.5±0.2 (3)	5.8	5.8	0.31	0.32	0.38	0.25
B_5	7.5±0.2 (5)	8.3±0.4 (3)	5.3	5.7	0.30	0.30	—	—
B_6	7.0±0.3 (3)	6.7±0.3 (3)	5.1	5.0	0.27	0.25	0.44	0.28
Mean	8.2	8.0	5.8	5.6	0.29	0.29	0.38	0.25
SEM	0.4	0.3	0.3	0.3	0.01	0.01	0.02	0.02
Acetylcholine studies								
A_1	6.3±0.3 (4)	6.7±0.4 (5)	4.1	4.6	0.34	0.32	0.44	0.31
A_2	8.1±0.4 (5)	8.1±0.6 (4)	6.0	6.0	0.26	0.26	0.38	0.30
A_3	8.2±0.2 (5)	7.6±0.2 (3)	6.0	5.6	0.27	0.26	0.27	0.17
A_4	6.6±0.3 (4)	6.7±0.2 (4)	4.7	4.7	0.29	0.30	0.36	0.26
Mean	7.3	7.3	5.2	5.2	0.29	0.29	0.36	0.26
SEM	0.4	0.4	0.4	0.4	0.02	0.01	0.04	0.03

Abbreviations: C = control period, E = experimental period, B = bradykinin, A = acetylcholine.

* Mean±SEM.

‡ Number in parenthesis represents numbers of observations.

choline (1). Each point is the mean TF/P_{In} ratio in the control and experimental period. In the 11 bradykinin studies, the ratio of the experimental to the control period was unchanged at 1.00±0.02. In contrast, the TF/P_{In} ratio fell in each of the eight acetylcholine studies with a mean ratio of 0.74±0.04.

Nephron filtration rate increased in five, decreased in three, and was essentially unchanged in three studies during bradykinin administration. The mean change from 91±9 to 99±11 nl/min was not statistically significant.

Absolute reabsorption was 33±5 nl/min in the control period and 36±5 nl/min during bradykinin administration. These values were not statistically different.

Using the tubular flow rate to the puncture site as an index of distal nephron delivery, there was a variable response from experiment to experiment but the mean change from 58±6 to 63±7 nl/min was not statistically significant.

Renal plasma flow was measured in eight studies and increased in each from a mean of 120 ml/min to 177 ml/min during bradykinin. Filtration fraction fell in each of these studies with a mean change from 0.35 to 0.25.

Therefore, bradykinin caused a natriuresis in association with a fall in total kidney filtration fraction but no alteration in proximal tubular sodium reabsorption in superficial nephrons. To determine whether both bradykinin and acetylcholine altered superficial nephron filtration fraction in a similar manner to the change in whole kidney filtration fraction, further studies were performed in which the superficial nephron protein concentration and hematocrit were measured before and during the administration of both drugs.

Capillary protein data. Initial studies were performed to validate the ultramicro method for protein determination. In 20 unknown samples, the ratio of the observed to predicted protein concentration was 1.00±0.02. The coefficient of variation of 10 replicate samples was 4%.

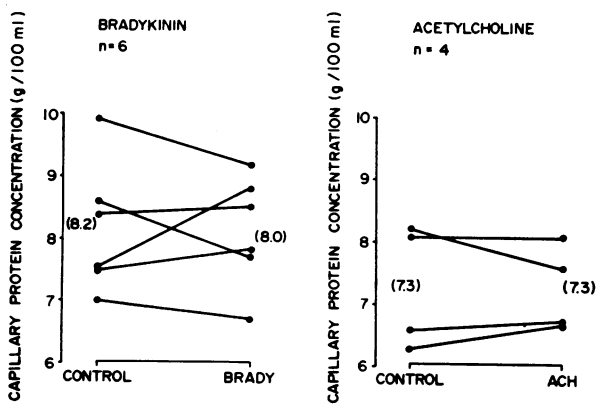

FIGURE 3 Effect of bradykinin and acetylcholine on superficial nephron protein concentration. Each point is the mean of at least three determinations. Left hand panel = bradykinin studies. Right hand panel = acetylcholine studies.

In Table II and Fig. 3 are presented the capillary protein data in both the bradykinin and acetylcholine studies. In the six bradykinin studies the mean superficial nephron capillary protein concentration was 8.2 g/100 ml in the control period and was unchanged at 8.0 g/100 ml during bradykinin, while the mean arterial protein concentration was 5.8 and 5.6 g/100 ml in the control and experimental periods, respectively. The filtration fraction calculated from this data was unchanged, 0.29 in both the control and experimental periods (Fig. 4). In contrast, total kidney filtration fraction fell in each of the five studies in which it was measured from 0.38 to 0.25.

Four studies were performed in which capillary protein concentration was determined before and during acetylcholine administration. As with the bradykinin studies, there was no change in the mean capillary protein concentration. Filtration fraction was also unchanged at 0.29 in both periods while total kidney filtration fraction fell in each of these studies with a mean fall from 0.36 to 0.26.

Capillary hematocrit studies. Preliminary studies were performed to evaluate the ultra-micro method for hematocrit determination. Samples of heparinized whole blood with hematocrits varying from 30 to 60 ml/100 ml were prepared and transferred to micropipettes of the same size used for in vivo collections and a comparison was then made of the results obtained with the micro and standard macro methods. The ratio of the micro to macro methods in 26 comparisons was 1.01 ±0.01. The coefficient of variation of 10 replicate determinations of a sample with a hematocrit of 45 ml/100 ml was 1%.

In two studies, blood samples were obtained from capillaries on the renal capsule and compared with the simultaneous arterial hematocrit. Both the capillary and arterial hematocrit were 46 ml/100 ml in the first study and 38 ml/100 ml in the second study.

This data indicates that an accurate and reproducible hematocrit can be obtained with this method and that the collection technique does not artifactually alter the capillary hematocrit.

In Table III and Fig. 5 are presented the data of eight studies in which the capillary hematocrit was

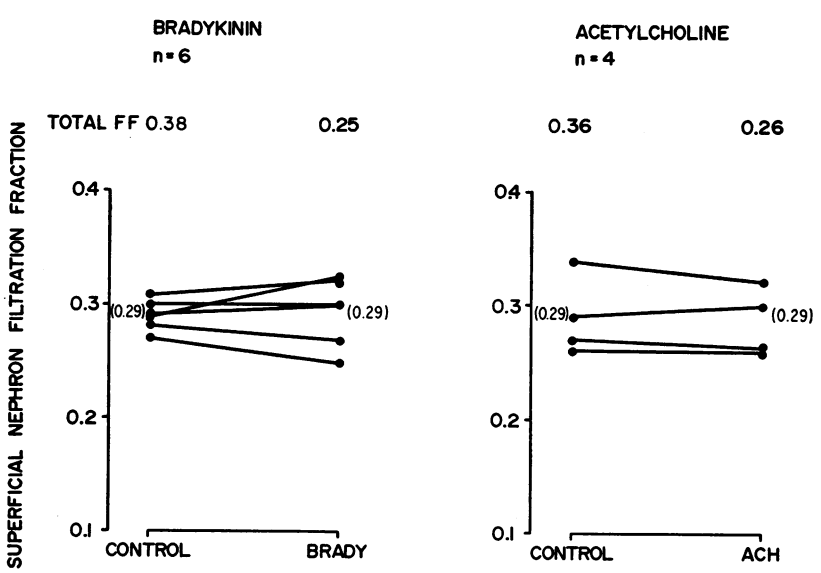

FIGURE 4 Effect of bradykinin and acetylcholine on superficial nephron and total kidney filtration fraction. Total FF = total kidney filtration fraction. Left hand panel = bradykinin studies. Right hand panel = acetylcholine studies.

TABLE III

Summary of Bradykinin and Acetylcholine Capillary Hematocrit Studies

Experiment number	Capillary hematocrit		Arterial hematocrit		Capillary filtration fraction	
	C	E	C	E	C	E
	ml/100 ml		*ml/100 ml*			
Bradykinin studies						
B_1	55±1.0* (5)‡	55±1.1 (5)	45	47	0.34	0.29
B_2	57±1.2 (6)	52±1.7 (4)	49	48	0.26	0.16
B_3	43±1.3 (4)	43±1.2 (5)	35	35	0.28	0.28
B_4	34±1.2 (4)	38±1.7 (4)	27	29	0.28	0.36
B_5	40±1.0 (4)	48±1.3 (4)	35	38	0.19	0.33
B_6	45±2.0 (3)	46±0.9 (5)	38	38	0.23	0.26
B_7	54±1.2 (4)	49±1.0 (5)	45	45	0.31	0.15
B_8	46±1.8 (5)	45±0.9 (5)	37	37	0.31	0.28
Mean	47	47	39	40	0.27	0.26
SEM	3	2	3	2	0.01	0.02
Acetylcholine studies						
A_1	40±0.8 (5)	47±2.0 (3)	34	37	0.23	0.33
A_2	53±0.7 (5)	56±1.9 (4)	44	49	0.28	0.26
A_3	56±1.1 (5)	56±1.4 (4)	49	48	0.26	0.27
A_4	55±1.0 (5)	55±0.7 (5)	45	47	0.34	0.29
A_5	59±1.1 (5)	57±1.3 (5)	51	50	0.25	0.24
A_6	42±1.4 (4)	42±1.1 (5)	36	36	0.26	0.26
Mean	51	52	43	44	0.27	0.28
SEM	3	3	3	3	0.01	0.01

Abbreviations: C = control period, E = experimental period, B = bradykinin, A = acetylcholine.

* Mean ±1 SEM.

‡ Number in parenthesis represents number of observations

determined before and during the administration of bradykinin. The mean capillary hematocrit rose in two studies, fell in two studies, and was essentially unchanged in the remaining four studies. The mean capillary hematocrit was 47 ml/100 ml in both periods while the arterial hematocrit was 39 and 40 ml/100 ml in the control and bradykinin periods, respectively. In addition, the mean calculated filtration fraction was 0.27 in the control period and unchanged at 0.26 in the experimental period. The results of six acetylcholine studies are also shown in Table III and Fig. 5. As in the bradykinin studies, there was no change in the capillary hematocrit. The mean value was 51 ml/100 ml in the control period and 52 ml/100 ml during acetylcholine administration while the arterial hematocrit was essentially unchanged. The calculated filtration

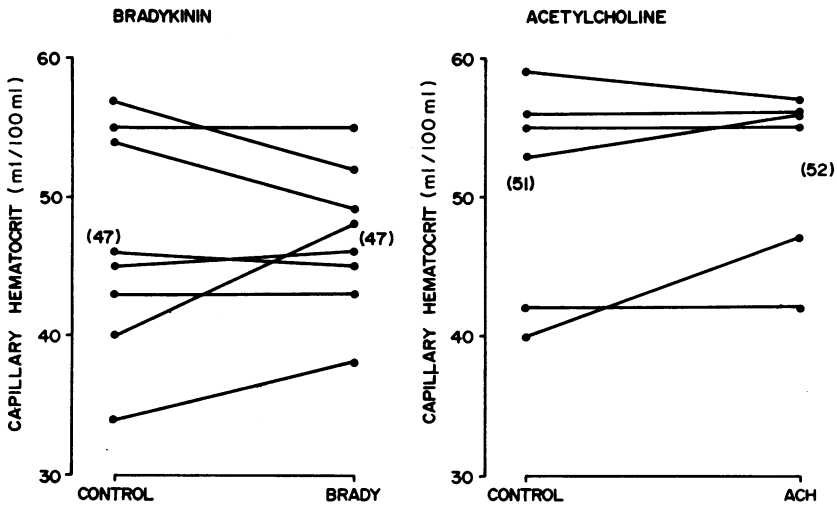

FIGURE 5 Effect of bradykinin and acetylcholine on superficial nephron capillary hematocrit. Each point is the mean of at least three determinations. Left hand panel = bradykinin studies. Right hand panel = acetylcholine studies.

fraction was also constant at 0.27 and 0.28 in the control and experimental periods, respectively.

Intrarenal Ringer's studies. The results of these studies are shown in Table IV. Tubular flow rates were obtained before and during Ringer's infusion in 18 paired collections and rose in all but one of these samples. There was a mean increase in flow rate from 82 to 111 nl/min ($P < 0.001$). Tubular fluid to plasma

TABLE IV

Summary of Intrarenal Ringer's Infusion Studies

Exp. No.	Tubule No.	Tubular flow rate		TF/P inulin		Nephron filtration rate		Absolute reabsorption		Capillary hematocrit		Renal vein hematocrit		Superficial nephron filtration fraction*	
		C	E	C	E	C	E	C	E	C	E	C	E	C	E
		nl/min				*nl/min*		*nl/min*		*ml/100 ml*		*ml/100 ml*			
1	1	58	83												
	2	54	91							56	48	49	45	0.25	0.10
	3	56	75												
2	1	72	80	1.93	1.57	139	126	67	46	53	46	45	40	0.27	0.24
	2	89	100	1.69	1.44	150	144	61	44	52	44	45	40	0.24	0.18
	3	95	111	1.50	1.38	143	153	48	42	50	44	43	39	0.25	0.18
	4	80	120	1.43	1.18	114	143	34	21	50	42	43	37	0.25	0.19
3	1	48	65												
	2	67	76												
	3	42	52	1.38	1.18	58	61	16	9	55	47	44	40	0.36	0.25
4	1	99	121	1.40	1.30	130	157	40	36	44	40	36	33	0.28	0.27
	2	126	113	1.43	1.29	180	146	54	33	45	40	35	33	0.33	0.27
5	1	109	150	1.09	1.00	119	150	10	0	63	59	52	48	0.35	0.36
	2	81	152	1.74	1.44	140	219	59	67						
	3	98	160	1.42	1.16	139	185	41	25	63	59	52	49	0.35	0.33
6	1	125	146	1.69	1.28	211	186	86	40						
	2	95	129	1.30	1.00	124	129	29	0						
7	1	76	98	1.61	1.35	122	132	46	34						
Mean		82	111	1.51	1.27	136	148	45	31	53	46	44	40	0.29	0.24
SEM		6	5	0.06	0.05	12	12	6	6	2	2	2	2	0.02	0.02

Abbreviations: C = Control period, E = Ringer's infusion.

* The superficial nephron filtration fraction (FF) was calculated from the formula: $FF = (1 - [A_{Hct}/C_{Hct}])/(1 - A_{Hct})$, where A_{Hct} is the renal vein hematocrit and C_{Hct} is the superficial nephron capillary hematocrit.

inulin ratios were considered valid only on those recollections in which the Ringer's infusate had an inulin concentration that was ±10 mg/100 ml of the plasma inulin concentration at the time of the infusion. This criteria was met in 13 of these recollection pairs and the ratio of the infusate to the plasma inulin concentration in these collections was 0.99±0.03. The TF/P_{In} ratio fell in all 13 pairs with a mean change from 1.51 to 1.27 (Fig. 6). This change was significant at the $P < 0.001$ level. Nephron filtration rate rose in nine and fell in four collections and the mean change from 136 to 148 nl/min was not statistically significant. Absolute reabsorption fell in 12 of the 13 pairs with a mean change from 45 to 31 nl/min ($P < 0.001$). In addition, the capillary hematocrit which was obtained before and during Ringer's infusion in 10 pairs fell in each with a mean change of 7 ml/100 ml ($P < 0.001$). This was associated with a mean decrease in the renal venous hematocrit of 4 ml/100 ml. Superficial nephron filtration fraction was also calculated before and during the Ringer's infusion using the renal venous hematocrit as the preglomerular value in the calculation of this parameter. This was considered valid since the urinary flow rate was 0.5 ml/min or less in each study. Using this value there was a mean fall in the calculated superficial nephron filtration fraction of 0.05 ($P < 0.005$).

In Table V are presented the data of 10 capillary pairs obtained in four studies with the identical protocol to that of the previous data, but in which only the capillary protein concentration was determined before and during the Ringer's infusion. The capillary protein concentration fell in each of the 10 pairs with a mean fall of 1.1 g/100 ml ($P < 0.001$). The renal vein protein concentration fell 0.5 g/100 ml and the calculated filtration fraction fell in 8 of the 10 pairs with a mean decrease of 0.04 ($P < 0.005$).

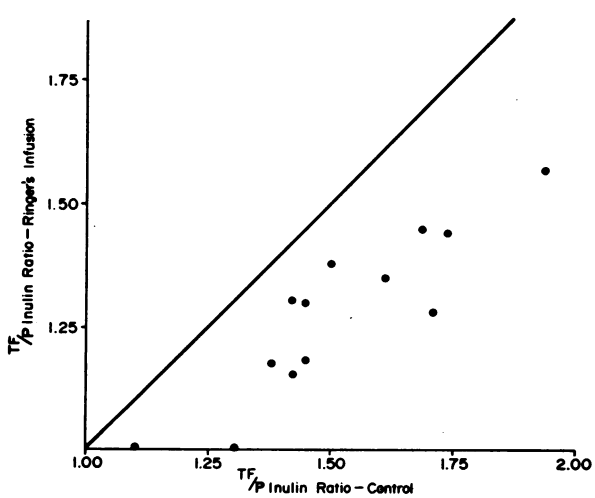

FIGURE 6 Effect of intrarenal Ringer's infusion on the tubular fluid to plasma inulin ratio in the proximal tubule.

TABLE V

Change in Superficial Nephron Capillary Protein Concentration During Intrarenal Infusion of Ringer's Solution

Exp. No.	Sample	Capillary protein concentration		Renal vein protein concentration		Superficial nephron filtration fraction*	
		C	E	C	E	C	E
		g/100 ml		*g/100 ml*			
1	1	6.7	5.6	4.8	4.3	0.28	0.23
	2	6.5	5.3	4.8	4.3	0.29	0.19
2	1	6.6	5.6	4.7	4.2	0.29	0.25
	2	7.2	5.7	4.6	4.0	0.37	0.30
3	1	8.6	7.8	6.1	5.5	0.29	0.30
	2	8.0	7.2	5.8	5.4	0.28	0.25
	3	7.9	7.3	5.8	5.3	0.27	0.27
4	1	9.1	7.3	6.3	5.8	0.31	0.21
	2	8.9	7.7	6.1	5.7	0.32	0.26
	3	8.2	7.6	6.0	5.7	0.27	0.25
Mean		7.8	6.7	5.5	5.0	0.29	0.25
SEM		0.3	0.3	0.2	0.2	0.02	0.01

Abbreviations: C = control period, E = Ringer's infusion.

* The superficial nephron filtration fraction (FF) was calculated from the formula: FF = 1 − (P_A/P_C), where P_A is the renal vein protein concentration and Pc is the superficial nephron capillary protein concentration.

Norepinephrine studies. These studies were performed to determine whether an increase in superficial nephron hematocrit and filtration fraction could be discerned in a model known to increase both the systemic hematocrit and the total kidney filtration fraction. There was a marked increase in mean arterial pressure in each study with a mean increase of 47 mm Hg. As is shown in Table VI, the systemic hematocrit rose in each study with a mean increase of 7 ml/100 ml while the total kidney filtration fraction rose from 0.36 to 0.52. There was a mean increase in the capillary hematocrit of 13 ml/100 ml and the calculated superficial nephron filtration fraction rose from 0.26 to 0.46 during norepinephrine.

DISCUSSION

The data presented in this paper indicates that superficial nephron proximal tubular sodium reabsorption is not affected in the same manner by two structurally different renal vasodilators, bradykinin and acetylcholine, even though their hemodynamic effects were similar. A similar conclusion was reached by Dirks and Seely in a preliminary publication of free-flow micropuncture studies in the dog (10) and by Heller and Nováková in split droplet studies in the rat (11). In a previous study we demonstrated that acetylcholine significantly depressed fractional and absolute reabsorption in the proximal tubule (1) while in the present

TABLE VI

Summary of Norepinephrine Studies

Exp. No.	Capillary hematocrit		Arterial hematocrit		Superficial nephron filtration fraction		Kidney filtration fraction	
	C	E	C	E	C	E		
	ml/100 ml		*ml/100 ml*					
1	48±1.3* (5)‡	65±4.0 (3)	40	52	0.27	0.54	0.36	0.52
2	54±1.6 (5)	66±0.8 (4)	49	52	0.19	0.43	—	—
3	54±1.7 (4)	64±0.7 (3)	46	51	0.26	0.41	0.34	0.47
4	51±3.3 (3)	63±3.1 (3)	44	52	0.25	0.36	0.40	0.56
5	51±1.8 (4)	67±1.5 (3)	40	48	0.35	0.56	0.35	0.53
Mean	52	65	44	51	0.26	0.46	0.36	0.52
SEM	1.1	0.7	1.7	0.8	0.02	0.03	0.01	0.02

Abbreviations: C = control period, E = norepinephrine infusion.

* Mean±1 SEM.

‡ Number in parenthesis represents the number of abbreviations.

experiments it was found that bradykinin did not alter proximal tubular sodium reabsorption. However, both agents decreased the total kidney filtration fraction and, therefore, presumably the peritubular capillary protein concentration. Since a decrease in peritubular protein concentration has been shown by Brenner and associates (5) and Spitzer and Windhager (12) to decrease proximal tubular sodium reabsorption, the reason for the difference in effect of the two vasodilators was not clear. Although there is considerable data in the literature suggesting that acetylcholine may act as a direct inhibitor of sodium transport (3, 4, 13), this alone would not seemingly explain the differences found if the superficial nephron filtration fraction and protein concentration did indeed fall as would be predicted from the whole kidney clearance data. Therefore, one possible explanation for the difference in superficial nephron proximal tubular sodium reabsorption was that the two agents had divergent effects on the superficial nephron circulation. To further evaluate this possibility, studies were performed in which the superficial nephron protein concentration and hematocrit were determined with both agents. The results of these studies indicate that neither agent altered the superficial nephron protein concentration, hematocrit or filtration fraction.

This failure to demonstrate an alteration in the capillary protein concentration or hematocrit was not due to a lack of sensitivity of the methods. The analytical techniques used to measure both the protein concentration and hematocrit were shown to be quite accurate and reproducible, and the variation in protein concentration or hematocrit between samples in a given experimental period was also quite small. In addition, both an increase and a decrease in these parameters were easily demonstrable in the appropriate experimental model. As is shown in Table IV, the capillary hematocrit was shown to fall in all 10 collection pairs obtained during the intrarenal infusion of Ringer's solution. Similar findings were found with the identical protocol when protein concentration was determined before and during Ringer's infusion as is shown in Table V. It should also be noted that the mean decrease of 1.1 g/100 ml is quite similar to the change in protein concentration in the superficial nephron circulation that would be predicted from the change in total kidney filtration fraction during renal vasodilatation. In addition, although the differences were small, there was a statistically significant fall in the calculated filtration fraction during Ringer's infusion in both the hematocrit and protein studies. This could be due to a decrease in efferent arteriolar viscosity. The norepinephrine studies (Table VI) demonstrated a model in which an increase in both superficial nephron capillary hematocrit and filtration fraction was clearly demonstrable. Therefore, we feel that this data strongly indicates that the methods used can discern both increases and decreases in the superficial nephron protein concentration and

hematocrit and that the constancy of both parameters in the circulation of superficial nephrons during renal vasodilatation is a true reflection of an unchanged filtration fraction in these nephrons.

There are two other sources of information which tend to confirm these results. First, Daugharty, Ueki, Troy, and Brenner have noted similar disparate changes in superficial and total kidney filtration fraction during saline loading in the rat (14). Since renal vasodilatation also occurs during saline loading these findings in the rat may be closely linked to the results of the present study. Second, data obtained with the radioactive microsphere method is also in agreement with the present findings (15). Since both nephron filtration rate and filtration fraction were unchanged during renal vasodilatation, renal plasma flow must have remained constant in superficial nephrons even though total renal blood flow increased 50%. This would be possible only if both agents caused a redistribution of blood flow to inner cortical nephrons. Studies performed with the radioactive microsphere method to measure intrarenal blood flow indicate that the per cent of blood flow to outer cortical nephrons does indeed decrease and that absolute blood flow remains relatively constant during renal vasodilatation. Therefore, data obtained with two different methods indicates that outer cortical blood flow remains constant during vasodilatation by either saline loading or the administration of acetylcholine or bradykinin.

As is shown in Table II, the superficial nephron filtration fraction was consistently lower than the total kidney filtration fraction in the control hydropenic period. There are several possible explanations for this finding. First, the capillary samples may have been contaminated by tubular fluid during the collection of the capillary sample. Special care was taken to avoid this by injecting colored mineral oil in the capillary and watching for any to enter a surrounding tubule. In addition, it would be extremely unlikely for contamination to occur systematically only during hydropenia.

Second, it is possible that at the point of collection of the blood sample, some tubular reabsorbate would have already re-entered the capillary circulation. There are two points against this being a major factor in the results. First, as is shown in Tables II and III, the superficial nephron filtration fraction was the same with both vasodilators during the experimental period. Since acetylcholine depressed reabsorption and bradykinin had no effect on tubular reabsorption, the similar filtration fraction in the face of a divergent effect on reabsorption argue against tubular reabsorbate significantly affecting the results obtained with these methods. Secondly, it should be pointed out that the superficial nephron filtration fraction was the same or even slightly higher than the whole kidney filtration fraction during renal vasodilatation and closely approximated the individual kidney filtration fraction in three of the four studies in which they were compared during norepinephrine infusion. Therefore, the discrepancy was only present in the control hydropenic period, and it is possible that the superficial nephron filtration fraction is indeed slightly lower than that of the whole kidney in the dog during hydropenia. Preliminary studies in our laboratory in which the blood flow per glomerulus has been estimated with the radioactive microsphere method and glomerular couting concomitant with simultaneous nephron filtration rates suggest that this may be correct.[2] In any case, definite changes in the superficial nephron filtration fraction were readily demonstrable in appropriate experimental models and were not found during renal vasodilatation with either bradykinin or acetylcholine.

It is also of interest that the calculated filtration fractions obtained with the two methods were quite similar with mean values of 0.29 and 0.27 for the protein and hematocrit method, respectively. These values are not statistically different. These results are at variance with the cell separation hypothesis of Pappenheimer and Kinter (16) in which it was proposed that red cells would be progressively concentrated in the center of the interlobular artery as blood traversed the cortex and that the outer cortical hematocrit would be considerably higher than the systemic hematocrit. If this were the case, the filtration fraction obtained with the hematocrit method should be higher than that with the protein method. Since this is not the case, the present data is evidence against the cell separation hypothesis, at least under the circumstances of these experiments, and is in agreement with similar findings by Brenner and Galla (17) in hydropenic rats. Although these authors did find slightly higher filtration fractions with the hematocrit method as the velocity of blood flow was increased by saline loading the rat, no such difference was found in our vasodilatation studies.

Since neither drug caused a discernible change in protein concentration or hematocrit in superficial nephrons, another explanation must be sought to explain the difference in effect of these two agents on proximal tubular sodium reabsorption. First, it is possible that the two drugs had consistently different effects on superficial nephron filtration rate. However, although there was considerable scatter in the nephron GFR data, this parameter was not significantly altered with either drug. In addition, it should be noted that the major factor responsible for the diminution in fractional reabsorption of sodium during acetylcholine administra-

[2] Stein, J. H., and T. F. Ferris. Unpublished observations.

tion was not an increase in nephron GFR but rather a decrease in absolute reabsorption (1). Second, acetylcholine may have a direct effect on proximal tubular sodium reabsorption. Studies by Parmelee and Carter (3) and May and Carter (4) in the chicken demonstrate that the renal portal infusion of various cholinergic agents caused an increase in urine flow and sodium excretion without any alteration in filtration rate or renal blood flow. In addition, Schilb has shown that mecholyl, a cholinergic agent similar in structure and action to acetylcholine, causes a decrease in net sodium flux, short-circuit current, and potential difference when added to the serosal surface of the turtle bladder (13). Therefore, there is ample ancillary evidence that cholinergic agents might indeed have a direct effect on sodium transport.

Since delivery of filtrate out of the proximal tubule of superficial nephrons was not increased during bradykinin administration, an explanation must be sought to explain the natriuresis seen with this agent. Although superficial nephron filtration fraction was unchanged, the fall in total kidney filtration fraction indicates that the filtration fraction and the peritubular protein concentration was decreased in the circulation of the more inner cortical nephrons. Since it has been shown that a decrease in peritubular capillary protein concentration inhibits sodium reabsorption in the proximal tubule (5, 12), it is likely that proximal tubular reabsorption is decreased and delivery out of the proximal tubule is increased in the deeper cortical nephrons during bradykinin administration. Secondly, Daugharty, Belleau, Martino, and Earley have performed studies during water diuresis which indicate that vasodilator agents such as acetylcholine and prostaglandin inhibit sodium reabsorption in the ascending limb of the loop of Henle (18). Similar studies performed in our laboratory have also shown an inhibitory effect of bradykinin on sodium reabsorption in the ascending limb of the loop of Henle,[3] which may be related to the increase in inner cortical blood flow and presumably medullary blood flow demonstrated by the radioactive microsphere method (15).

As is shown in Table V, the infusion of Ringer's solution in the renal artery depressed fractional and absolute reabsorption of sodium in the proximal tubule of superficial nephrons. Since the maximum volume infused by the time of completion of the repeat puncture in any recollection was 15 ml, it is inconceivable that changes in extracellular fluid volume accounted for the fall in fractional and absolute sodium reabsorption in the proximal tubule demonstrated. The fall in reabsorption was also independent of changes in nephron GFR. Therefore, it is felt that these results are consistent with the view that proximal tubular sodium reabsorption is inhibited when the peritubular capillary protein concentration and/or hematocrit are decreased and confirm similar studies by Brenner and associates in the rat (5). The failure to demonstrate a change in proximal tubular reabsorption during bradykinin is further evidence against a change in filtration fraction in these nephrons. Although the experimental design of these studies does not allow us to differentiate between the change in protein concentration or hematocrit as the predominant force in altering sodium reabsorption, recent studies by Brenner and Galla indicate that the changes in the former bear a more direct relationship to alterations in sodium reabsorption (17).

In summary, these studies indicate that the inhibitory effect of acetylcholine on proximal tubular sodium reabsorption in superficial nephrons is most likely due to a direct tubular effect. In addition, the demonstration of a constant superficial nephron capillary protein concentration, hematocrit and filtration fraction accompanying the fall in total kidney filtration fraction during the administration of bradykinin and acetylcholine is further evidence that these vasodilators cause a redistribution of blood flow to inner cortical nephrons. As previously discussed (15), the mechanism of this redistribution is not clear but may be related to the local release of renin in outer cortical nephrons or to differences in the steady state resistance of afferent and efferent arterioles in different portions of the renal cortex.

Lastly, the constant superficial nephron filtration fraction during renal vasodilatation is strong evidence for functional nephron heterogeneity. Although Nissen initially suggested that the filtration fraction may not be the same in all nephrons (19), the present data is the first direct evidence of a variable alteration in nephron filtration fraction in a given experimental model. The physiologic significance of this finding is demonstrable in the bradykinin studies. Although proximal tubular sodium reabsorption was not altered in superficial nephrons with this agent, this may not be true in the more inner cortical nephrons where peritubular capillary protein concentration must have decreased.

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