Journal of Clinical Investigation
Vol. 44, No. 11, 1965

Studies on the Mechanism of Natriuresis Accompanying Increased Renal Blood Flow and Its Role in the Renal Response to Extracellular Volume Expansion *

LAURENCE E. EARLEY † AND R. M. FRIEDLER

(From the Thorndike Memorial Laboratory, Second and Fourth [Harvard] Medical Services, Boston City Hospital, and the Department of Medicine, Harvard Medical School, Boston, Mass.)

In the dog the net tubular reabsorption of sodium may diminish during saline loading, independent of salt retaining hormones and even in the face of a decline in glomerular filtration rate (1–5). We have suggested that increased renal blood flow during saline loading may be one factor that contributes to the decreased tubular reabsorption of sodium (4, 5), since in the saline loaded dog when renal blood flow is decreased experimentally the net tubular reabsorption of sodium may increase (5). Recently, reports have appeared demonstrating that the infusion of several unrelated vasodilators [acetylcholine (6–8), dopamine (9), pyrogen (10), kallidin (11), and papaverine (12)] may increase the excretion of sodium in association with increased renal hemodynamics. The present studies were undertaken to determine *1*) the extent to which local renal vasodilatation may increase the excretion of sodium, *2*) whether such increases in sodium excretion may be due to decreased tubular reabsorption, and *3*) to what extent an effect of increased blood flow on tubular reabsorption may be operative during different degrees of saline loading.

The renal arterial infusion of acetylcholine resulted in large unilateral increases in renal plasma flow under all conditions studied. In animals not receiving a saline load increased renal plasma flow was invariably associated with increased sodium excretion that usually was not attributable to increased filtered sodium. Natriuresis accompanying increased blood flow was even greater in animals receiving small loads of saline, but was completely absent in animals receiving large saline loads and in animals undergoing mannitol diuresis.

* Submitted for publication June 16, 1965; accepted July 22, 1965.

Aided in part by grants AM-5401-04 from the National Institutes of Health and NsG595 from the National Aeronautics and Space Administration.

† Fellow of the Boston Medical Foundation.

Address requests for reprints to Dr. Laurence E. Earley, Thorndike Memorial Laboratory, Boston City Hospital, Boston, Mass. 02118.

Methods

Mongrel dogs of either sex ranging in weight from 17.3 to 30.0 kg were deprived of food and water for approximately 18 hours before experiments. Under light pentobarbital anesthesia the ureters were cannulated, a 23-gauge needle was placed in the proximal portion of the left renal artery in the direction of the kidney, and a plastic catheter was inserted into the left renal vein by way of the ovarian or spermatic vein (5). Isotonic saline was infused into the renal artery at a rate of 1.0 ml per minute throughout each experiment. A catheter inserted into the aorta through a femoral artery was used to record blood pressure and to collect arterial blood samples. Arterial blood pressure was measured with a Sanborn pressure transducer and recorder.

Three hours before experiments the animals received an intramuscular injection containing 5 U of Pitressin Tannate and 10 mg of desoxycorticosterone acetate (DOCA).[1] Two hours before experiments a maintenance infusion of inulin, *p*-aminohippurate (PAH), desoxycorticosterone (25 μg per minute), and vasopressin (40 to 60 mU per kg per hour) in isotonic saline (acidified to pH 5.0) was begun at 0.4 to 0.5 ml per minute. Blood samples were collected simultaneously from the aorta and the left renal vein at the mid-point of each clearance period. Blood samples were expelled into iced tubes, and plasma was separated by centrifugation within 7 minutes.

After collecting a minimum of three control clearance periods, the renal arterial infusion was changed to isotonic saline containing acetylcholine bromide.[2] The rate of drug infusion was either 20 or 40 μg per minute. After collecting a minimum of three clearance periods during the infusion of acetylcholine, the drug was discontinued, and in most experiments additional clearance periods were collected after the drug infusion. Collection

[1] Organon, West Orange, N. J.

[2] Eastman Organic Chemicals, Rochester, N. Y.

periods were from 5 to 20 minutes in duration, depending on the rate of urine flow.

Animals were studied under four conditions. *1) "Unloaded":* These 12 animals were receiving only the maintenance infusion containing hormones and clearance substances, the renal arterial infusion, and infusions of isotonic saline to maintain the patency of the femoral arterial and renal venous catheters (total rate of saline infusion, approximately 2.0 ml per minute). Four of the 12 animals studied under these conditions received an infusion of 250 ml of 0.9% saline approximately 90 minutes before experimental collections. *2) "Partially loaded":* In addition to the above "maintenance" infusions six animals received 400 ml of Ringer's solution (Na 145, K 3.5, Cl 128.5, HCO_3 20 mEq per L) at 50 ml per minute, and then throughout the experiment this solution was infused at 6 to 7 ml per minute. This partial loading was begun approximately 1 hour before experimental collections. *3) "Fully loaded":* These five animals were infused with 1,500 ml of the Ringer's solution at a rate of 50 ml per minute. The infusion was then slowed to a rate approximately 1 ml per minute greater than the rate of urine flow. Experimental collections were begun 10 to 20 minutes after completing the loading procedure. *4) Mannitol diuresis:* In five experiments acetylcholine was infused into the left renal artery at a rate of 20 or 40 μg per minute during mannitol diuresis. In addition to the above maintenance solutions these animals received intravenously either 15% mannitol in 40 mM NaCl (two experiments) or 10% mannitol in 30 mM NaCl at a rate that resulted in a urine flow between 5 and 15 ml per minute per kidney. When the rate of urine flow was stable, a minimum of three clearance periods was collected before the infusion of acetylcholine. After collecting three clearance periods during the drug infusion additional control collections were made in three of these studies.

Sodium and potassium were determined by internal standard flame photometry, and osmolality was measured cryoscopically. PAH was determined by the technique of Bratton and Marshall as adapted for the Technicon autoanalyzer (13). Inulin was determined colorimetrically by reaction with anthrone in H_2SO_4 (14) by a technique adapted for the Technicon autoanalyzer. Plasma protein was precipitated with $ZnSO_4$ and .75 N NaOH. The recovery of inulin added to plasma averaged 98.6 ± 1.9% (SD) (N = 36), and clearances averaged 97 ± 7% (SD) (N = 50) of those determined simultaneously by the method of Walser, Davidson, and Orloff (15).

Extraction ratios (E), renal plasma flow (RPF), and renal blood flow (RBF) were calculated by conventional formulae (5). In addition, "noncortical plasma flow" (NCPF) was calculated as the difference between RPF and the clearance of PAH or Diodrast.[3]

[3] In two experiments of the series Diodrast-^{131}I (Abbott Laboratories, Oak Ridge, Tenn.) was infused at approximately 0.25 μc per minute, and no PAH was included in the maintenance solution. In these experiments calculations of extraction and plasma flows were made from measurements of Diodrast-^{131}I in a well type scintillation counter (Nuclear of Chicago, Des Plaines, Ill.).

Results

Unloaded animals. The effects of the renal arterial infusion of acetylcholine on renal hemodynamics and sodium excretion in 12 experiments are summarized in Table I, and details of a representative experiment are given in Table II. Before the infusion of acetylcholine RPF averaged 159 ml per minute (range, 99 to 239 ml per minute), and E_{PAH} averaged 0.848 (range, 0.668 to 0.924, left kidney). During the renal arterial infusion of acetylcholine RPF increased by an average of 51% (range, + 18 to + 163 ml per minute), and absolute values of E_{PAH} decreased by an average of 0.074 (range, + 0.008 to − 0.186). This increased RPF was accompanied by increased clearance of PAH (C_{PAH}). Due to decreases in E_{PAH} proportionately larger increases occurred in NCPF. In control right kidneys C_{PAH} was usually unchanged. Glomerular filtration rate (GFR) and filtered sodium in the left kidney decreased in six experiments, increased slightly in four experiments, and were unchanged in two experiments. The excretion of sodium by the left kidney increased during the infusion of acetylcholine by an average of 149 μEq per minute (range, 11 to 310 μEq per minute). Sodium excretion by the control right kidney usually decreased slightly during the infusion of acetylcholine. After the infusion of acetylcholine had been discontinued, hemodynamics and sodium excretion returned towards the preinfusion control values. The effects of acetylcholine on renal hemodynamics and sodium excretion began abruptly when the infusion of drug was started and diminished abruptly when the drug was discontinued (Table II).

In 8 of these 12 experiments decreased net reabsorption of sodium accompanied increased RPF as sodium excretion increased despite no change, or decreased, filtered sodium (Tables I and II).

Partially loaded animals. The results of these six experiments are summarized in Table III. Before the infusion of acetylcholine RPF averaged 139 ml per minute (range, 87 to 200 ml per minute), and E_{PAH} (or $E_{Diodrast}$) averaged 0.867 (range, 0.801 to 0.922). These values are not distinctly different from those during the control periods in the unloaded animals described above. During the infusion of acetylcholine RPF increased by an average of 92% (range, 78 to 159

TABLE I

*The effects of renal arterial infusion of acetylcholine on renal hemodynamics and sodium excretion in the absence of saline loading**

Experiment		V		GFR		C_{PAH}		E_{PAH}	RPF	NCPF	$U_{Na}V$		U_{osm}		P_{Na}
		R	L	R	L	R	L	L	L	L	R	L	R	L	
		ml/min		*ml/min*		*ml/min*			*ml/min*	*ml/min*	*μEq/min*		*mOsm/kg*		*mEq/L*
1.	Control	0.52	0.95	40	51	104	128	0.815	157	29	93	264	775	776	151
	Acetylcholine	0.23	2.78	36	49	100	187	0.805	232	46	38	574	861	490	151
	Control	0.19	0.53	36	45	85	123	0.836	147	24	18	113	1,003	857	151
2.	Control	0.74	1.21	49	51	121	138	0.840	165	27	148	242	580	581	151
	Acetylcholine	0.40	2.17	49	51	129	167	0.824	204	36	83	368	954	443	149
	Control	0.34	0.75	52	47	117	119	0.867	137	18	83	162	1,033	702	148
3.	Control	0.35	0.40	41	28	128	95	0.668	143	48	104	83	1,157	939	160
	Acetylcholine	0.21	1.98	35	29	115	112	0.551	209	97	60	339	1,516	446	160
	Control	0.17	0.32	32	21	87	62	0.746	83	21	50	54	1,565	688	159
4.	Control	0.37	0.34	63	58	172	167	0.865	193	26	87	76	1,530	1,474	153
	Acetylcholine	0.32	1.25	60	55	181	253	0.724	350	97	81	262	1,590	766	151
	Control	0.36	0.36	67	62	201	214	0.795	269	55	114	108	1,656	1,491	152
5.	Control	0.75	0.90	55	52	139	149	0.792	188	40	115	135	601	531	143
	Acetylcholine	0.59	1.70	48	49	130	165	0.729	226	61	82	246	595	429	144
6.	Control	0.38	0.59	36	42	78	84	0.853	99	15	55	145	646	816	145
	Acetylcholine	0.24	1.54	36	43	80	114	0.816	140	26	29	295	733	508	145
	Control	0.23	0.45	37	43	83	95	0.861	110	15	24	117	793	943	146
7.	Control	0.26	0.25	66	73	158	168	0.899	186	18	57	68	1,342	1,581	153
	Acetylcholine	0.19	1.13	65	73	141	204	0.895	228	24	34	216	1,429	671	152
	Control	0.16	0.16	63	73	141	169	0.921	184	15	24	31	1,722	2,033	151
8.	Control	0.78	0.60	38	34	104	95	0.895	107	12	216	159	908	902	152
	Acetylcholine	0.50	1.09	35	35	90	112	0.903	125	13	150	228	1,035	602	151
	Control	0.38	0.31	38	34	87	80	0.907	89	9	123	89	1,233	1,123	151
9.	Control	0.59	0.62	44	45	100	105	0.924	113	8	140	160	849	838	147
	Acetylcholine	0.37	1.65	45	52	113	206	0.803	254	48	105	350	1,002	575	147
10.	Control	0.44	0.42	80	81	205	212	0.872	239	27	153	146	1,254	1,290	150
	Acetylcholine	0.24	0.81	76	75	187	257	0.825	312	55	98	229	1,478	909	148
11.	Control	0.44	0.33	51	50	149	145	0.862	168	23	39	34	578	683	144
	Acetylcholine	1.68	2.79	49	46	160	205	0.710	289	84	99	186	226	199	146
12.	Control	0.72	0.95	43	46	119	133	0.887	150	17	139	185	605	559	152
	Acetylcholine	0.49	1.16	43	43	130	191	0.701	272	81	90	196	660	475	151

* Abbreviations are as follows: V = rate of urine flow, GFR = glomerular filtration rate, C_{PAH} = clearance of *p*-aminohippurate, E_{PAH} = extraction ratio for *p*-aminohippurate, RPF = total renal plasma flow, NCPF = "noncortical" renal plasma flow (RPF − C_{PAH}), $U_{Na}V$ = rate of excretion of sodium, U_{osm} = concentration of total urinary solute, and P_{Na} = concentration of sodium in plasma.

Values for the left side are the means of three to five uniform consecutive collection periods during each phase of the experiment. In some experiments fewer individual collections were made on the right side during the same time periods. Transition periods of 10 to 15 minutes associated with changing urinary flow were not utilized for clearance calculations.

TABLE II

*The effects of renal arterial infusion of acetylcholine on renal hemodynamics and electrolyte excretion in experiment no. 4**

Time	V		GFR		C_{PAH}		E_{PAH}	RPF	NCPF	$U_{Na}V$		U_KV		P_{Na}
	R	L	R	L	R	L	L	L	L	R	L	R	L	
min	*ml/min*		*ml/min*		*ml/min*			*ml/min*	*ml/min*	*μEq/min*		*μEq/min*		*mEq/L*
0–15	0.38	0.36	61	61	173	178	0.867	205	27	93	81	53	57	153
15–35	0.36	0.32	64	55	171	155	0.862	180	25	81	71	54	54	153
Acetylcholine bromide 20 μg per minute into left renal artery														
35–45	0.38	1.96									378		145	
45–55		1.42		52		288	0.732	393	105	86	271	60	112	152
55–65	0.33	1.38	57	53	185	246	0.689	357	111	79	279	49	109	151
65–75		1.23		60		256	0.726	353	97		273		117	150
75–85	0.31	0.97	62	56	176	222	0.750	296	74	82	226	43	110	150
Discontinue acetylcholine bromide infusion														
85–110	0.38	0.28								96	78	37	45	
110–130	0.35	0.36	66	63	201	217	0.792	274	57	109	104	37	54	151
130–150		0.37		63		223	0.795	280	57		115		48	152
150–170	0.36	0.36	67	60	201	201	0.799	252	51	118	105	39	47	153

* Additional abbreviation as follows: U_KV = rate of excretion of potassium.

TABLE III

*The effects of renal arterial infusion of acetylcholine on renal hemodynamics and sodium excretion in the presence of "partial" saline loading**

Experiment		V R	V L	GFR R	GFR L	C_{PAH} R	C_{PAH} L	E_{PAH} L	RPF L	NCPF L	$U_{Na}V$ R	$U_{Na}V$ L	U_{osm} R	U_{osm} L	P_{Na}	Arterial pressure
		ml/min		*ml/min*		*ml/min*			*ml/min*	*ml/min*	*μEq/min*		*mOsm/kg*		*mEq/L*	*mm Hg*
13.	Control	3.50	3.56	43	39	125	118	0.810	146	28	471	424	334	297	148	129
	Acetylcholine	1.57	7.83	39	41	116	200	0.663	303	103	247	797	415	233	149	112
	Control	1.38	2.39	39	36	121	111	0.768	144	33	222	262	437	293	149	108
14.†	Control	1.92	2.39	42	39	116	110	0.801	138	28	479	531	615	529	150	142
	Acetylcholine	2.12	6.90	42	41	111	179	0.702	255	76	509	1,084	569	322	151	138
	Control	2.04	2.76	43	38	113	100	0.789	127	27	487	556	558	448		140
15.†	Control	2.01	3.34	30	32	67	74	0.847	87	13	391	550	445	361	140	135
	Acetylcholine	2.30	6.93	29	34	70	111	0.674	165	54	409	950	400	287	141	133
	Control	2.55	4.81	28	31	73	79	0.824	96	17	413	672	359	298	141	133
16.	Control	1.72	2.12	62	65	178	183	0.915	200	17	478	582	699	674	144	145
	Acetylcholine	0.64	3.18	59	64	193	253	0.837	302	49	205	685	967	510	143	129
	Control	0.81	1.44	56	60	182	183	0.919	199	16	242	394	869	717	142	141
17.	Control	1.73	1.98	62	60	137	133	0.905	147	14	497	530	734	658	148	156
	Acetylcholine	0.44	4.14	57	62	145	231	0.755	306	75	195	892	1,273	491	148	143
	Control	0.99	1.17	54	54	152	145	0.880	165	20	347	327	984	730	147	145
18.	Control	2.17	1.92	56	57	105	109	0.922	118	9	503	442	575	602	148	157
	Acetylcholine	1.35	2.99	55	60	105	187	0.820	228	41	354	575	676	482	149	142
	Control	1.11	1.09	56	59	101	119	0.899	132	13	302	271	721	689	146	130

* Abbreviations and representation of data are the same as in Table I.
† No PAH infused in these two experiments. Clearances, extraction ratios, and plasma flows calculated from measurement of tracer amounts of Diodrast-^{131}I.

ml per minute), and absolute values of E_{PAH} or $E_{Diodrast}$ decreased by an average of 0.125 (range, 0.078 to 0.173). These changes appear somewhat greater than those during the drug infusion in the unloaded animals. C_{PAH} also increased in the experimental kidney, but was unchanged in control right kidneys. The increases in sodium excretion by the left kidney during the infusion of acetylcholine averaged 320 μEq per minute (range, 103 to 553 μEq per minute). This increased excretion of sodium was in most experiments distinctly greater than that observed during the drug infusion in the unloaded animals. During the infusion of acetylcholine sodium excretion by the control right kidney was unchanged or slightly decreased.

GFR and filtered sodium by the experimental kidney increased slightly during the infusion of acetylcholine in five of these six experiments. However, the increase in sodium excretion was

TABLE IV

*The effects of renal arterial infusion of acetylcholine on renal hemodynamics and sodium excretion in the presence of "full" saline loading**

Experiment		V R	V L	GFR R	GFR L	C_{PAH} R	C_{PAH} L	E_{PAH} L	RPF L	NCPF L	$U_{Na}V$ R	$U_{Na}V$ L	U_{osm} R	U_{osm} L	P_{Na}
		ml/min		*ml/min*		*ml/min*			*ml/min*	*ml/min*	*μEq/min*		*mOsm/kg*		*mEq/L*
19.	Control	3.00	7.66	48	52	127	177	0.796	222	45	498	1,210	383	338	145
	Acetylcholine	2.98	8.43	48	58	127	246	0.659	373	127	530	1,298	399	323	145
	Control	2.68	6.32	59	64	118	219	0.816	268	49	477	1,119	422	377	146
20.	Control	8.38	7.97	73	74	230	246	0.659	373	127	1,676	1,562	421	428	146
	Acetylcholine	6.92	8.01	73	72	220	273	0.532	513	240	1,391	1,546	443	416	146
	Control	7.16	6.61	74	72	222	233	0.711	328	95	1,322	1,322	425	433	145
21.	Control	11.15	11.16	58	64	272	301	0.706	426	125	1,694	1,763	310	322	142
	Acetylcholine	11.14	9.64	56	59	272	335	0.557	601	266	1,693	1,513	309	320	139
	Control	12.69	10.55	56	60	264	305	0.654	466	161	1,827	1,656	297	316	138
22.	Control	6.09	10.60	47	52	268	280	0.681	411	131	920	1,654	346	330	149
	Acetylcholine	4.25	7.18	45	52	258	341	0.600	568	227	676	1,142	379	358	148
	Control	3.66	9.44	44	54	250	295	0.686	430	135	564	1,435	389	337	148
23.	Control	17.58	17.19	45	46	114	107	0.591	181	74	2,426	2,303	300	297	150
	Acetylcholine	15.06	16.93	41	44	103	136	0.356	382	246	2,063	2,235	302	291	149
	Control	18.00	13.85	43	41	103	96	0.641	150	54	2,430	1,884	290	297	151

* Abbreviations and representation of data are the same as in Table I.

clearly greater than the increased filtered sodium in five of the six studies.

Fully loaded animals. The results of these five experiments are summarized in Table IV. These animals were undergoing marked natriuresis with rates of sodium excretion ranging from 1,210 to 2,303 μEq per minute per (left) kidney during control collections. Before the infusion of acetylcholine RPF averaged 323 ml per minute (range, 181 to 426 ml per minute, left kidney), values distinctly greater than those observed during control periods in the unloaded and partially loaded animals. E_{PAH} averaged 0.687 (range, 0.591 to 0.796), and these extraction ratios were lower than those observed during control periods in the unloaded and partially loaded experiments. During the infusion of acetylcholine into the left renal artery RPF in the left kidney increased by an average of 165 ml per minute, and the absolute values of E_{PAH} decreased an average of 0.145. C_{PAH} in control right kidneys was unaffected by the infusion of acetylcholine. Despite the further large changes in RPF and E_{PAH} during the infusion of acetylcholine, sodium excretion by the left kidney did not increase further. In these experiments GFR was not changed consistently during the infusion of acetylcholine.

Mannitol diuresis. The results of these five experiments are summarized in Table V. During control periods RPF averaged 235 ml per minute (range, 186 to 271 ml per minute, left kidney), and E_{PAH} averaged 0.723 (range, 0.629 to 0.806). Before the infusion of acetylcholine sodium excretion in these animals ranged from 282 to 1,013 μEq per minute. During the infusion of acetylcholine into the left renal artery RPF increased and E_{PAH} (left kidney) decreased in each study. The range of increased RPF was from 57 to 229 ml per minute, and the absolute decreases in E_{PAH} ranged from 0.064 to 0.254. The infusion of acetylcholine was not associated with increased excretion of sodium by the left kidney in any of these studies. Relatively small decreases in the rate of sodium excretion occurred during the drug infusion in association with small decreases in GFR. However, GFR during the infusion of drug was similar to control values either before or after the infusion in four of the five experiments.

Discussion

The present observations demonstrate that acetylcholine, when infused into the renal artery, may result in large unilateral increases in renal plasma flow. This increased plasma flow was usually accompanied by decreased extraction of PAH. Since the extraction of isotopic tracer amounts of Diodrast-^{131}I was similarly decreased during acetylcholine infusion, it is unlikely that the diminished extractions were due to tubular loads in excess of the transport maximum, and the changes are consistent with the occurrence of a redistribution of blood flow away from the renal cortex as plasma flow increases during the infusion of acetylcholine (5). Although Vander has reported no decrease

TABLE V

*The effects of renal arterial infusion of acetylcholine on renal hemodynamics and sodium excretion during mannitol diuresis**

Experiment		V		GFR		C_{PAH}		E_{PAH}	RPF	NCPF	$U_{Na}V$		U_{osm}		P_{Na}
		R	L	R	L	R	L	L	L	L	R	L	R	L	
		ml/min		*ml/min*		*ml/min*			*ml/min*	*ml/min*	*μEq/min*		*mOsm/kg*		*mEq/L*
24.	Control	4.91	4.63	34	33	113	117	0.629	186	69	313	282	388	401	133
	Acetylcholine	5.14	4.25	33	30	121	129	0.494	257	128	326	214	378	384	135
	Control	5.27	4.23	31	29	106	114	0.613	185	71	320	215	376	405	133
25.	Control	7.68	5.89	35	33	180	167	0.708	236	69	596	426	376	398	134
	Acetylcholine	6.80	4.44	32	27	161	174	0.553	327	153	466	216	394	410	133
	Control	6.63	4.80	29	27	149	155	0.588	259	104	435	259	397	420	132
26.	Control	4.95	8.39	31	38	201	217	0.800	271	54	241	587	308	301	143
	Acetylcholine	4.85	6.58	29	36	202	241	0.736	328	87	211	391	311	315	145
	Control	5.13	7.02	29	34	179	192	0.833	231	39	217	405	309	315	143
27.	Control	6.53	12.36	26	39	166	201	0.806	249	48	340	1,013	348	367	132
	Acetylcholine	6.00	13.19	23	38	170	290	0.608	478	188	227	893	379	372	130
28.	Control	10.63	9.84	41	40	161	156	0.674	232	76	665	616	334	337	131
	Acetylcholine	11.45	10.73	39	38	162	179	0.420	428	249	619	530	363	363	128

* Abbreviations and representation of data are the same as in Table I.

TABLE VI

*Summary of changes in renal hemodynamics and sodium excretion produced by the infusion of acetylcholine into the left renal artery in the four groups of animals**

	GFR		C_{PAH}		E_{PAH}	RPF	NCPF	$U_{Na}V$	
	R	L	R	L	L	L	L	R	L
	ml/min		*ml/min*			*ml/min*	*ml/min*	*μEq/min*	
"Unloaded" (12)	−2.3	−0.5	−2	+47	−.074	+78	+31	− 33	+149
"Partial" load (6)	−2.3	+1.7	+2	+73	−.125	+121	+48	−150	+320
"Full" load (5)	−1.6	−0.6	−6	+45	−.145	+165	+120	−172	−152
Mannitol diuresis (5)	−2.2	−2.6	−1	+29	−.161	+129	+100	− 61	−136

* Abbreviations are the same as in preceding Tables.
Values are the means of changes from control periods preceding the infusion of acetylcholine into the left renal artery. Numbers in parentheses indicate the number of experiments in each group of studies.

in E_{PAH} during the renal arterial infusion of acetylcholine in two experiments (6), our observations are in agreement with those of Harvey, who reported that the renal arterial infusion of acetylcholine does decrease E_{PAH} (16).

In the hydropenic animals and those receiving relatively small loads of saline, increased renal plasma flow during the infusion of acetylcholine was uniformly associated with increases in the excretion of sodium. However, despite even larger increases in plasma flow during the infusion of acetylcholine, in the animals receiving relatively large saline loads no further increase in sodium excretion occurred. Likewise, increased renal plasma flow and decreased E_{PAH} were not associated with increased sodium excretion when the agent was infused during mannitol diuresis (Table VI). These observations of increased sodium excretion during the infusion of acetylcholine in the first two groups of animals are in agreement with observations previously reported by others (6–8). Vander reported that the natriuretic effect of acetylcholine was not different in dogs receiving saline infusions than in dogs receiving no saline (6). However, the saline loading employed by the latter author was somewhat less than that in the partially loaded animals of the present study. The present results demonstrate that this natriuresis accompanying renal vasodilatation may occur with little or no increase in filtered sodium, indicating that a net decrease in the reabsorption of sodium occurs during the infusion of acetylcholine, as has been suggested by others (6, 7). In the present study this decreased reabsorption of sodium was invariably associated with increased renal plasma flow, and in all but one instance (experiment 8, Table I) with decreased E_{PAH}.

Previous studies from this laboratory have demonstrated an inverse relationship between renal blood flow and the tubular reabsorption of sodium during saline loading (5). We suggested that increases in renal medullary blood flow, which may be included, in part in the noncortical fraction of plasma flow ($RPF - C_{PAH}$), may decrease medullary interstitial hypertonicity and secondarily increase the rate of flow of tubular fluid with a lowered concentration of sodium through Henle's loop. This, in turn, could decrease the absolute reabsorption of sodium by the ascending limb of the loop and thereby contribute to over-all diminished reabsorption of sodium during saline loading. This mechanism is entirely speculative, but the present results do lend support to the concept that changes in renal blood flow or renal vascular resistance may influence the tubular reabsorption of sodium through some intrarenal mechanism. The failure of acetylcholine to evoke increased sodium excretion in the markedly natriuretic fully loaded animals with already relatively high RPF and low E_{PAH} is consistent with the view that any role of blood flow in decreasing sodium reabsorption is already maximally effective in the presence of large saline loads.

Acetylcholine produced greater natriuretic effects in animals receiving small saline loads (partially loaded) than in those receiving no load (unloaded). In both these groups, before the infusion of acetylcholine, E_{PAH} was high and RPF was distinctly lower than in the fully loaded group. Thus, any effect of blood flow on sodium reabsorption may have been minimal or absent in the hydropenic and partially loaded animals. The present data do not allow an analysis of the mechanisms involved in the production of the control

levels of natriuresis in animals receiving the partial saline loads. However, since renal plasma flow was relatively low and E_{PAH} relatively high before the induced renal vasodilatation, it would not appear that these hemodynamic factors could have been contributing to the natriuresis in this group of animals. Dirks, Cirksena, and Berliner have reported recently that similarly small loads of saline may be associated with large decreases in fractional reabsorption in the proximal nephron of the dog (17). If reabsorption by the ascending limb of Henle's loop or more distal portions of the nephron is to some extent indirectly influenced by intrarenal hemodynamics, then much of the decreased proximal reabsorption that could be present in these partially loaded animals (with relatively low blood flow) may be reabsorbed at more distal sites. Therefore, increasing renal blood flow under these conditions could permit a larger part of the decreased proximal reabsorption to be excreted, as reabsorption at more distal tubular sites diminished in response to increased blood flow. Such a combination of factors could account for the greater natriuretic response to vasodilatation in this group of animals. Alternatively, the larger natriuretic effect in the partially loaded group could be due to a higher filtered load of sodium (as compared to the unloaded group) or to the somewhat greater increases in renal plasma flow during the infusion of acetylcholine.

Acetylcholine failed to produce increased sodium excretion during mannitol diuresis also. Mannitol diuresis increases renal plasma flow and decreases E_{PAH} (18), and in the present studies total and noncortical plasma flows were greater during mannitol diuresis before the infusion of acetylcholine than during control periods in the hydropenic and partially loaded animals. Therefore, it is possible that any natriuretic effect that increased blood flow may have was already present during mannitol diuresis, as suggested above for the fully loaded (saline) animals. The possibility cannot be excluded, however, that the filtered load of sodium was sufficiently low during mannitol diuresis to preclude any natriuretic response to the increased blood flow. If the mechanism whereby increased renal blood flow diminishes tubular reabsorption of sodium is dependent upon a reduction in medullary interstitial hypertonicity as discussed above, then as the solute concentration of the medullary interstitium approaches isotonicity, further increases in blood flow may not produce further decreases in the reabsorption of sodium. It could be expected, therefore, that the reduction of medullary interstitial hypertonicity (as reflected by urinary osmolality) during mannitol diuresis would minimize the effect of increases in blood flow to further reduce the interstitial solute concentration. Also any effect of changes in the movement of water from the descending limb on tubular fluid sodium concentration (and ultimately sodium reabsorption) would be minimized during mannitol diuresis, since the presence of nonreabsorbable solute would result in an already lowered concentration of sodium in the isotonic fluid delivered from the proximal convolution. Therefore, a significant fraction of the osmotically effective solute within the loop could be mannitol, and the absolute changes in sodium concentration due to diminished loss of water from the descending limb would be less than in the presence of high medullary interstitial osmolality with sodium as the only major osmotically "effective" solute in the tubular fluid.

In one experiment of the partially saline loaded group (experiment 13, Table III) urine was near isotonicity before the infusion of acetylcholine, yet a large increase in sodium excretion occurred as blood flow was increased by acetylcholine. If in this experiment urinary osmolality reflected medullary interstitial osmolality, then this observation is inconsistent with the concept that a reduction in medullary interstitial hypertonicity is a necessary intermediate step between increased renal blood flow and decreased reabsorption of sodium. However, in this latter experiment a relatively large decrease in urinary osmolality occurred during the infusion of acetylcholine, and the urine became distinctly hypotonic to plasma. Therefore, it is possible that for unknown reasons urinary osmolality was not reflecting medullary interstitial osmolality in this one study.

The possibility should be considered that increases in renal blood flow may decrease proximal tubular reabsorption of sodium through some intrarenal mechanism, since Dirks, Cirksena, and Berliner have reported relatively large decreases in fractional reabsorption in the proximal convolution of the dog during saline loading (17). However, this does not appear likely, since these latter

authors reported also that the decreased proximal reabsorption persisted in the presence of reductions in blood flow sufficient to produce large decreases in glomerular filtration (17), and on the other hand it has been observed that during saline loading reductions in blood flow insufficient to reduce glomerular filtration may be associated with increased net tubular reabsorption (5). Therefore, it would appear that any effect decreased blood flow may have to increase the reabsorption of sodium could be taking place distally to the proximal convolution.

The present studies do not exclude the possibility that the natriuretic effect of acetylcholine is due to some direct action on the renal tubule and that the increased renal plasma flow is only an incidental event. However, this appears unlikely for several reasons. The natriuretic effect was present only when control blood flow was relatively low and was absent in saline loaded animals with high rates of renal plasma flow and in animals undergoing mannitol diuresis. Even though the mechanisms involved are uncertain, renal vasodilatation by a variety of means is accompanied by increased sodium excretion (6–12). Acetylcholine may stimulate active sodium transport in other tissues (19, 20), but any effect to increase sodium transport in the kidney should be reflected as decreased excretion of sodium.

Summary

The effect of unilateral renal vasodilatation on sodium excretion was studied by infusing acetylcholine into the renal artery of dogs under conditions of *1*) hydropenia (no saline load), *2*) partial saline loading, *3*) extensive saline loading, and *4*) mannitol diuresis. Unilateral increases in renal plasma flow and decreases in the extraction of *p*-aminohippurate (or Diodrast) occurred during the infusion of acetylcholine in all four conditions. Unilateral increases in sodium excretion without equivalent increases in filtered sodium occurred during the infusion of acetylcholine in the hydropenic and partially loaded animals. These observations are consistent with the concept that increases in renal plasma flow per se may result in decreased net tubular reabsorption of sodium. In animals receiving relatively large saline loads and in animals undergoing mannitol diuresis similarly large increases in renal plasma flow during the infusion of acetylcholine did not result in further increases in sodium excretion, suggesting that any effect of increased blood flow to decrease the tubular reabsorption of sodium was already maximally operative under these conditions.

The natriuretic effect of increased plasma flow was greater in the animals receiving small saline loads than in the hydropenic group, although control rates of blood flow were similar in both groups. This latter observation is consistent with the view that relatively small saline loads may activate natriuretic factors other than blood flow, and thereby may condition the kidney for a greater (natriuretic) response to increased blood flow.

Acknowledgments

We are indebted to Susan Howard and Barbel Juergens for their assistance with these studies.

References

1. De Wardener, H. E., I. H. Mills, W. F. Clapham, and C. J. Hayter. Studies on the efferent mechanism of the sodium diuresis which follows the administration of intravenous saline in the dog. Clin. Sci. 1961, **21**, 249.
2. Levinsky, N. G., and R. C. Lalone. The mechanism of sodium diuresis after saline infusion in the dog. J. clin. Invest. 1963, **42**, 1261.
3. Rector, F. C., Jr., G. Van Giesen, F. Kiil, and D. W. Seldin. Influence of expansion of extracellular volume on tubular reabsorption of sodium independent of changes in glomerular filtration rate and aldosterone activity. J. clin. Invest. 1964, **43**, 341.
4. Earley, L. E., and R. M. Friedler. Observations on the mechanism of decreased tubular reabsorption of sodium and water during saline loading. J. clin. Invest. 1964, **43**, 1928.
5. Earley, L. E., and R. M. Friedler. Changes in renal blood flow and possibly the intrarenal distribution of blood during the natriuresis accompanying saline loading in the dog. J. clin. Invest. 1965, **44**, 929.
6. Vander, A. J. Effects of acetylcholine, atropine, and physostigmine on renal function in the dog. Amer. J. Physiol. 1964, **206**, 492.
7. Pinter, G. G., C. C. C. O'Morchoe, and R. S. Sikand. Effect of acetylcholine on urinary electrolyte excretion. Amer. J. Physiol. 1964, **207**, 979.
8. Williams, R. L., J. E. Pearson, Jr., and M. K. Carter. The saluretic effects of arecoline hydrochloride infused into the left renal artery of dogs. J. Pharmacol. exp. Ther. 1965, **147**, 32.
9. McDonald, R. H., Jr., L. I. Goldberg, J. L. McNay, and E. P. Tuttle, Jr. Effect of dopamine in man: augmentation of sodium excretion, glomerular fil-

tration rate, and renal plasma flow. J. clin. Invest. 1964, **43**, 1116.

10. Gombos, E. A., and T. H. Lee. Renal functional response to pyrogen induced renal hyperemia (abstract). Clin. Res. 1965, **13**, 306.
11. Webster, M. E., and J. P. Gilmore. Influence of kallidin-10 on renal function. Amer. J. Physiol. 1964, **206**, 714.
12. Meyer, M. B., and L. I. Goldberg. Natriuretic effect of papaverine. Fed. Proc. 1965, **24**, 258.
13. Harvey, R. B., and A. J. Brothers. Renal extraction of para-aminohippurate and creatinine measured by continuous *in vivo* sampling of arterial and renal-vein blood. Ann. N. Y. Acad. Sci. 1962, **102**, 46.
14. Davidson, W. D., and M. A. Sackner. Simplification of the anthrone method for the determination of inulin in clearance studies. J. Lab. clin. Med. 1963, **62**, 351.
15. Walser, M., D. G. Davidson, and J. Orloff. The renal clearance of alkali-stable inulin. J. clin. Invest. 1955, **34**, 1520.
16. Harvey, R. B. Effects of intra-arterial acetylcholine infusion on PAH extraction and RBF in the dog. Fed. Proc. 1965, **24**, 404.
17. Dirks, J. H., W. J. Cirksena, and R. W. Berliner. The effect of saline infusion on sodium reabsorption by the proximal tubule of the dog. J. clin. Invest. 1965, **44**, 1160.
18. Braun, W. E., and L. S. Lilienfield. Renal hemodynamic effects of hypertonic mannitol infusions. Proc. Soc. exp. Biol. (N. Y.) 1963, **114**, 1.
19. Fänge, R., K. Schmidt-Nielsen, and M. Robinson. Control of secretion from the avian salt gland. Amer. J. Physiol. 1958, **195**, 321.
20. Hokin, L. E., and M. R. Hokin. Evidence for phosphatidic acid as the sodium carrier. Nature (Lond.) 1959, **184**, 1068.