Mechanism of Antidiuretic Effect of Beta Adrenergic Stimulation

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ABSTRACT The effect of beta adrenergic stimulation on renal-diluting capacity was examined in the dog. Beta adrenergic stimulation with intravenous isoproterenol significantly increased urinary osmolality (U_{Osm}) and decreased free water clearance (C_{H_2O}), and these effects were rapidly reversible with cessation of the infusion. This antidiuretic effect of systemic beta adrenergic stimulation was comparable in innervated and denervated kidneys and was not associated with alterations in glomerular filtration rate or renal vascular resistance. Renal perfusion pressure was maintained constant in all of the experiments. The same dose of isoproterenol, which produced the antidiuretic effect and markedly stimulated cardiac beta adrenergic receptors when infused intravenously, was not found either to increase U_{Osm} or to decrease C_{H_2O} when infused directly into the renal artery. Removal of the source of production and release of antidiuretic hormone (ADH) was, however, found to abolish the effect of intravenous isoproterenol on U_{Osm}. A small effect on C_{H_2O} persisted and appeared to be related to an increase in arterial hematocrit. Thus, the results of the study exclude a major role of alterations in renal hemodynamics and renal innervation in the antidiuretic response to beta adrenergic stimulation with isoproterenol. They also provide no support for the hypothesis that beta adrenergic stimulation may directly alter the water permeability of the renal tubular epithelium. Rather the results suggest that the primary mechanism of the antidiuretic effect of beta adrenergic stimulation involves the integrity of the hypothalamo-neurohypophyial system and the release of ADH.

INTRODUCTION

There is now a considerable amount of experimental evidence which suggests that the adrenergic nervous system is involved in the control of water metabolism. The results of studies in humans (1–4), rats (5, 6), and the toad bladder (7, 8) have suggested that alpha adrenergic stimulation may antagonize the action of vasopressin at the cellular level. This is an attractive hypothesis since the effect of vasopressin appears to be mediated by adenyl cyclase, an enzyme which in several tissues has been shown to be inhibited by alpha adrenergic stimulation and activated by beta adrenergic stimulation (9, 10). Further support for this hypothesis is derived from studies in the rat (11–13) and cat (14), which have demonstrated that beta adrenergic stimulation with intravenous isoproterenol is associated with an antidiuretic response. In most of these studies, however, the interpretation of the mechanism responsible for this antidiuretic effect has been complicated by simultaneous decreases in renal perfusion pressure and glomerular filtration rate, which by themselves may influence renal-diluting capacity (15, 16).

The present investigation was therefore undertaken to examine the mechanism of the antidiuretic effect of beta adrenergic stimulation and in particular to differentiate between a direct effect on the renal tubule, an effect mediated by alterations in renal hemodynamics and an effect mediated by an extrarenal mechanism involving the release of antidiuretic hormone (ADH).[1] The results demonstrate that an extrarenal reflex involving the integrity of the hypothalamo-neurohypophysial system and the release of ADH is primarily responsible for the antidiuretic effect of systemic beta adrenergic stimulation. The results also excluded a major role of alterations in renal hemodynamics and renal sympathetic tone in this antidiuretic response. Moreover, no experimental evidence was obtained to support a direct effect of beta

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[1] *Abbreviations used in this paper:* ADH, antidiuretic hormone; C_{H_2O}, free water clearance; FF, filtration fraction; GFR, glomerular filtration rate; PAH, *p*-aminohippuric acid; RPF, renal plasma flow; RVR, renal vascular resistance; U_{Osm}, urinary osmolality.

TABLE I

The Effects of Intravenous Isoproterenol on Systemic and Renal

Exp. No.	Dose	Cardiac output			Systemic arterial pressure		
		Pre-control	Iso	Post-control	Pre-control	Iso	Post-control
	μg/kg per min	*liters/min*			*mm Hg*		
1	0.018	3.9	5.6	4.1	163	157	155
2	0.018	4.2	6.7	4.4	157	164	168
3	0.018	5.0	8.5	5.5	157	144	146
4	0.072	5.9	7.5	5.7	138	133	152
5	0.018	3.8	4.3	3.1	143	141	144
6	0.036	3.1	5.9	3.8	163	144	162
7	0.036	3.8	5.5	3.7	162	160	158
8	0.036	2.5	6.1	2.2	147	131	149
9	0.036	2.2	4.4	2.3	149	133	139
10	0.036	2.3	3.5	2.3	139	127	135
Mean		3.7	5.8	3.7	152	143	151
±SE		±0.4	±0.5	±0.4	±1.7	±5.4	±2.0
P value		<0.001	<0.001		<0.005	<0.01	

L, left kidney; R, right kidney.
* These kidneys were denervated.

adrenergic stimulation on the water permeability of the renal tubule epithelium, since a dose of isoproterenol, which markedly stimulated cardiac beta adrenergic receptors when infused intravenously, was found not to produce an antidiuretic effect when infused directly into the renal artery.

METHODS

27 experiments were performed in 18 mongrel dogs of either sex weighing 20–30 kg. In these animals food was withheld 18 hr before study, but water was allowed ad lib. On the day of study the animals were anesthetized with intravenous pentobarbital (30 mg/kg), intubated, and ventilated with a Harvard respirator. Light anesthesia was maintained throughout the experiment by the intermittent administration of pentobarbital. All animals received 5 mg of deoxycorticosterone acetate intramuscularly. In five animals hypophysectomy and destruction of hypothalamic neural pathways[2] were performed through a buccal approach, after which these animals received 1 mg of dexamethasone intramuscularly. In all animals a solution of 2.5% glucose and water was then infused through a catheter in a foreleg vein at 20 ml/min for 50 min during which time the following surgery was performed. Polyethylene catheters were placed in both ureters and renal veins through bilateral flank incisions. Denervation was performed in some of the kidneys by stripping and severing the renal nerves and then applying 95% alcohol to the renal pedicle. In seven animals with an intact hypothalamo-neurohypophysial system, a 23 gauge needle was placed in the left renal artery.

[2] Histological sectioning of the brain in three of these animals demonstrated removal of greater than 90% of the supraoptic nuclei and tracts. Animals prepared in this manner responded normally to the exogenous administration of vasopressin.

Renal perfusion pressure			Arterial hematocrit volume			Arterial protein concentration				Glomerular filtration rate		
Pre-control	Iso	Post-control	Pre-control	Iso	Post-control	Pre-control	Iso	Post-control		Pre-control	Iso	Post-control
mm Hg			*%*			*g/100 ml*				*ml/min*		
140	139	140	41.7	45.5	43.9	5.9	5.6	5.3	L	76	66	74
									R*	77	73	73
133	136	134	47.8	49.3	47.4	6.4	6.4	6.1	L*	58	70	64
									R	56	70	64
136	135	135	46.9	48.3	46.1	5.1	4.7	4.5	L*	66	74	73
									R*	65	63	72
115	116	115	45.8	49.3	47.4	4.4	4.3	4.3	L*	65	67	72
									R*	66	59	64
143	141	144	42.2	45.8	42.5	5.8	5.8	5.5	L	57	56	65
									R	52	54	61
143	140	142	41.5	42.2	38.5	5.6	5.6	5.1	L	41	45	44
									R	39	48	44
142	141	140	38.5	39.2	35.9	5.1	4.6	4.5	L	44	51	54
									R	44	47	40
120	119	119	47.3	49.5	44.5	6.0	5.7	5.0	L*	47	46	45
									R	45	40	45
119	120	120	44.5	46.3	41.4	5.0	4.8	4.5	L*	45	51	51
									R	45	55	49
120	120	120	41.4	42.8	38.2	4.5	4.2	3.9	L*	51	59	51
									R	49	58	54
131	131	131	43.8	45.8	42.6	5.4	5.2	4.9		54	58	58
±3.6	±3.3	±3.5	±0.8	±1.2	±1.2	±0.2	±0.2	±0.2		±2.5	±2.2	±2.6
NS		NS	<0.001		<0.001	<0.01		<0.001		<0.05		NS

In 1 of these same animals and 11 other animals (including the five dogs with ablation of the hypothalamo-neurohypophysial system) an adjustable Blalock clamp was placed around aorta above the origin of both renal arteries. In all animals catheters were inserted into the aorta and inferior vena cava via the femoral artery and vein, respectively, for the continuous measurement of arterial and venous pressure with Statham transducers (Statham Instruments, Inc., Oxnard, Calif.) and a direct writing Gilson recorder (Gilson Medical Electronics, Inc., Middleton, Wis.). In the animals with the Blalock clamp around the aorta the arterial pressure was measured also in the aorta above the clamp via a catheter inserted in the brachial artery. In all animals a catheter was inserted also into the right atrium via the jugular vein to inject indocyanine green dye for determination of cardiac output by the dye dilution method with the use of a Gilson densitometer and a Lexington Instruments Cardiac Output Computer (Lexington Instruments Corp., Waltham, Mass.). Arterial blood was withdrawn from the brachial artery by means of a Lexington Instruments dye dilution pump. After completion of surgery, an intravenous infusion of 2.5% glucose (0.5 ml/min) was started which contained sufficient inulin and *p*-aminohippuric acid (PAH) to maintain blood levels of these substances between 15 and 25 and 1 and 3 mg/100 ml, respectively. After 1 liter of 2.5% glucose had been infused, the rate was decreased to 4 ml/min above urine flow. If within an hour after completion of the surgery the urine osmolality was not less than 100 mOsm/kg, an additional 600 ml of 2.5% glucose and water was administered over 30 min, and the infusion rate was again decreased to 4 ml/min above urine flow. No experiment was performed in those animals in which the urine osmolality remained above 100 mOsm/kg 1–1½ hr after the second water load, with the exception of one kidney each in two animals which had urinary osmolalities of 107 and 108 mOsm/kg H_2O. After stabilization of urine flow the experiment was started. Urine was collected at 5- or 10-min intervals throughout the experiment, and

TABLE I—(*Continued*)

Exp. No.	Renal vascular resistance			Filtration fraction		
	Pre-control	Iso	Post-control	Pre-control	Iso	Post-control
	mm Hg/(ml/min)					
1	0.331	0.367	0.385	0.321	0.329	0.378
	0.312	0.339	0.363	0.305	0.339	0.351
2	0.432	0.359	0.347	0.373	0.377	0.330
	0.416	0.325	0.309	0.343	0.343	0.290
3	0.297	0.284	0.263	0.293	0.329	0.285
	0.314	0.321	0.271	0.304	0.317	0.292
4	0.229	0.203	0.167	0.260	0.271	0.240
	0.229	0.241	0.201	0.266	0.282	0.257
5	0.405	0.416	0.384	0.283	0.310	0.310
	0.399	0.395	0.364	0.255	0.288	0.274
6	0.768	0.672	0.685	0.400	0.393	0.366
	0.810	0.640	0.679	0.401	0.393	0.364
7	0.685	0.534	0.530	0.366	0.344	0.338
	0.679	0.666	0.672	0.364	0.361	0.318
8	0.375	0.451	0.491	0.298	0.367	0.348
	0.371	0.553	0.482	0.288	0.392	0.345
9	0.491	0.487	0.479	0.348	0.409	0.365
	0.482	0.434	0.473	0.345	0.393	0.351
10	0.479	0.442	0.430	0.365	0.397	0.312
	0.473	0.426	0.432	0.351	0.370	0.360
Mean	0.449	0.428	0.420	0.326	0.350	0.319
±SE	±0.04	±0.03	±0.03	±0.01	±0.01	±0.01
P valve	NS	NS		<0.005	<0.001	

L, left kidney; R, right kidney.
* These kidneys were denervated.

arterial and renal venous blood samples were collected at the midpoint of alternate collections of urine. Cardiac output measurements were made every third period during the experiment. The effect of beta adrenergic stimulation on the water diuresis was examined by either the intravenous or intrarenal administration of isoproterenol by the following protocols.

Intravenous administration of isoproterenol. The dose of intravenous isoproterenol used in the present investigation ranged from 0.018 to 0.036 μg/kg per min in the initial experiments in all of the animals. In two of these animals three additional experiments were performed using intravenous doses ranging up to 0.144 μg/kg per min. In all but two studies renal perfusion pressure was maintained constant throughout the experiment by the appropriate adjustment of the suprarenal aortic clamp. In one study in an intact dog (Table I, experiment 5), an aortic clamp was not used, and arterial pressure did not diminish during the isoproterenol infusion. In one study in a dog with ablation of the hypothalamo-neurohypophysial system (Table II, experiment 5), the aortic clamp was used only to prevent an increase in renal perfusion pressure after cessation of the infusion.

The following experimental protocol was used for both the studies in animals with and without an intact hypothalamo-neurohypophysial tract. After three to five control periods, isoproterenol was infused intravenously and after an equilibration period of 10–20 min, three to five experimental urine collections were made. The infusion of isoproterenol was then discontinued, and after an equilibration period of 10–20 min three to five postcontrol urine collections were made.

Unilateral infusion of isoproterenol into the renal artery. Preliminary experiments were performed in an effort to find a dose of isoproterenol infused into the renal artery which would not alter systemic hemodynamics (as judged by changes in cardiac output and arterial pressure) and yet deliver a concentration of the drug to the renal circulation, which was at least as high, and preferably higher, than the concentration reaching the renal circulation during

Urinary sodium excretion			Urinary potassium excretion			Free water clearance			Urinary osmolality		
Pre-control	Iso	Post-control	Pre-control	Iso	Post-control	Pre-control	Iso	Post-control	Pre-control	Iso	Post-control
μEq/min			*μEq/min*			*ml/min*			*mOsm/kg H₂O*		
8	5	24	53	38	56	6.17	1.82	4.50	31	78	48
10	13	36	49	49	60	5.64	2.78	4.52	41	66	55
9	31	26	38	70	62	2.22	0.62	2.16	77	169	92
9	22	20	37	64	56	1.67	0.41	1.32	84	185	119
41	35	37	40	40	42	4.12	−0.07	4.20	61	270	64
49	35	43	39	39	40	5.52	1.74	5.28	49	114	60
27	14	15	39	32	47	1.95	−1.01	4.16	108	402	88
29	19	37	42	35	48	3.06	1.05	6.83	84	173	70
14	12	33	35	34	41	4.57	3.16	4.47	58	72	61
5	5	12	27	27	33	2.77	1.71	2.95	73	98	71
14	25	16	20	28	28	2.91	−0.28	1.42	42	365	86
9	11	6	21	24	26	2.22	−0.34	1.01	55	466	93
16	25	49	28	33	34	1.42	−0.29	0.26	86	315	199
6	6	10	26	25	28	1.01	−0.34	0.09	93	409	210
22	4	2	15	8	11	5.67	0.31	3.28	25	173	37
7	1	2	9	6	12	4.44	−0.13	2.42	28	389	46
2	1	2	11	22	25	3.28	−0.03	2.79	37	261	67
2	1	2	12	16	20	2.42	−0.42	2.12	46	604	75
2	17	7	25	47	38	2.79	−0.41	1.18	67	305	141
2	1	1	20	30	27	2.12	−0.67	0.39	75	454	188
14	14	19	29	33	37	3.30	0.48	2.77	61	268	94
±3.0	±2.6	±2.5	±2.9	±3.6	±3.4	±0.4	±0.3	±0.4	±5.3	±34.8	±11.3
NS	NS		NS	NS		<0.001	<0.001		<0.001	<0.001	

the studies with intravenous administration of the drug. In these preliminary experiments it was found that the doses ranging from 0.009 to 0.036 μg/kg per min could be infused into the renal artery without influencing cardiac output and total peripheral resistance. This was in contrast to the increase in cardiac output and decrease in total peripheral resistance observed during the intravenous administration of comparable doses of the drug. This finding thus allowed for similar doses of the drug to be used during the intrarenal infusion studies as that used in the studies during the intravenous infusion of the drug. Of the nine experiments with the intrarenal infusion of isoproterenol, the dose used was 0.018 μg/kg per min in six experiments 0.009 μg/kg per min in two experiments and 0.036 μg/kg per min in one experiment. Except that the drug was infused into the renal artery rather than a peripheral vein, the experimental protocol was the same as in the studies with the intravenous infusion of isoproterenol. In one of these intrarenal studies (experiment 7) a hypophysectomy was performed before the study, and alpha-adrenergic blockade with an infusion of phenoxybenzamine into the renal artery (0.1 μg/kg per min) was maintained through the experiment.

The analytical procedures and calculations used in the present experiments have been referred to elsewhere (17). The following abbreviations will be used; glomerular filtration rate (GFR), renal plasma flow (RPF), renal vascular resistance (RVR), filtration fraction (FF), free water clearance (C_{H_2O}), and urinary osmolality (U_{Osm}).

RESULTS

Experiments during the intravenous administration of isoproterenol (Figs. 1–3, Tables I and II). In Fig. 1 are shown the results of an experiment which illustrates the effect of intravenous isoproterenol on systemic and renal hemodynamics and renal-diluting capacity in a dog with an intact hypothalamo-neurohypophysial tract. In this experiment the increase in cardiac out-

TABLE II

The Effects of Intravenous Isoproterenol on Systemic and Renal Hemodynamics and Renal-Diluting

Exp. No.	Dose	Cardiac output			Systemic arterial pressure		
		Pre-control	Iso	Post-control	Pre-control	Iso	Post-control
	μg/kg per min	*liters/min*			*mm Hg*		
1	0.018	5.0	6.4	4.8	114	100	113
2	0.144	4.8	6.7	4.3	113	108	183
3	0.144	4.3	5.4	4.1	183	110	130
4	0.018	3.4	4.0	3.4	145	123	140
5	0.036	3.6	5.0	3.6	120	113	128
6	0.018	2.9	4.5	2.5	130	120	133
7	0.018	2.5	4.4	2.6	133	121	138
8	0.036	2.6	3.2	2.8	138	113	145
Mean		3.6	4.9	3.5	135	114	139
±SE		±0.4	±0.4	±0.3	±6.7	±3.0	±6.5
P value		<0.001	<0.001		<0.05	<0.02	

put and decrease in total peripheral resistance during the intravenous isoproterenol infusion were such that systemic arterial pressure remained constant throughout the study. The U_{osm} and C_{H_2O} returned to the control level after cessation of the infusion. These effects of intravenous isoproterenol on U_{osm} and C_{H_2O} were not accompanied by either a decrease in glomerular filtration rate or an increase in renal vascular resistance. The results of all of the experiments in the intact animals are shown on Table I and are qualitatively quite similar. In some of these experiments, the intravenous isoproterenol infusion was associated with a fall in systemic arterial pressure; however, in all of the studies the renal perfusion pressure was constant throughout the experiments. In Fig. 2 is shown a representative experiment of the effect of an intravenous infusion of isoproterenol in an animal with ablation of the hypothalamo-neurohypophysial tract. In contrast to the experiments in the intact animals, the intravenous infusion of isoproterenol was not associated with either an increase in U_{osm} or a decrease in C_{H_2O} in this experiment. The results of all the experiments in the animals with ablation of the hypophysial-hypothalamic neural system are shown in Table II. Although the mean control cardiac output in these animals was similar to the intact animals, the mean control systemic arterial pressure and thus total peripheral resistance was lower. This lower systemic arterial pressure was also associated with lower control levels of glomerular filtration rate and renal blood flow. These control blood pressures and renal hemodynamics in this group of animals were however within a range frequently observed in the normal dog. Although a small but significant increase in mean U_{osm} occurred during the intravenous infusion of isoproterenol in this group of animals, the U_{osm} did not decrease after cessation of the infusion. A small and reversible effect on C_{H_2O} was observed during the intravenous infusion of isoproterenol in these animals with ablation of their hypothalamo-neurohypophysial system. However, a marked difference was observed between the effect of intravenous isoproterenol on U_{osm} and C_{H_2O} in these animals versus the intact animals, and this difference is illustrated in Fig. 3. The only significant effect on renal hemodynamics in both groups of animals was a small increase in

Renal perfusion pressure			Arterial hematocrit volume			Arterial protein concentration				Glomerular filtration rate		
Pre-control	Iso	Post-control	Pre-control	Iso	Post-control	Pre-control	Iso	Post-control		Pre-control	Iso	Post-control
mm Hg			%			*g/100 ml*				*ml/min*		
100	100	100	37.9	38.8	36.9	4.2	4.1	3.9	L*	52	51	52
									R	38	33	39
100	101	100	36.9	42.0	38.0	3.9	3.8	3.7	L*	52	52	50
									R	39	36	42
100	100	100	38.0	43.5	38.8	3.7	3.9	3.6	L*	50	53	54
									R	42	41	40
123	123	127	46.7	50.4	48.5	5.2	5.2	4.7	L*	39	36	33
									R	32	32	30
120	113	115	45.8	50.6	44.7	4.9	4.6	4.2	L*	32	30	32
									R*	27	24	27
113	114	117	34.0	40.6	34.9	3.2	3.4	3.1	L*	25	26	26
									R*	24	25	24
117	118	118	34.9	40.3	36.8	3.1	3.3	3.3	L*	26	26	25
									R*	24	25	24
118	113	113	36.8	42.8	39.3	3.3	3.5	3.2	L*	25	24	23
									R*	24	24	22
112	110	111	38.9	43.6	39.7	3.9	4.0	3.7		35	34	34
±2.6	±4.2	±4.6	±1.6	±1.7	±1.7	±0.3	±0.2	±0.2		±2.6	±2.6	±2.7
NS	NS		<0.001	<0.001		NS	<0.005			NS	NS	

filtration fraction, which was accompanied by a rise in hematocrit. The magnitude of these changes in hematocrit and filtration fraction were comparable in the two groups of animals and thus did not explain the different effects on U_{Osm} and C_{H_2O}.

Experiments during the infusion of isoproterenol into the renal artery (Fig. 4, Table III). In contrast to the significant effects of intravenous isoproterenol on cardiac output and total peripheral resistance, when the same dose of the drug was infused into the renal artery, these systemic effects of the drug were not observed (Fig. 4). In these experiments neither cardiac output nor arterial pressure were altered by intrarenal isoproterenol. Cardiac output was 3.9 ±0.4, 3.8 ±0.3, and 3.6 ±0.3 liters/min before, during, and after the intrarenal infusion of isoproterenol, and the arterial pressures during these same periods were 137 ±6, 133 ±6, and 133 ±7 mm Hg. Arterial hematocrit was not affected by the intrarenal infusion of isoproterenol. The plasma protein concentration declined slightly throughout the experiment from 5.3 to 5.1 to 4.9 g/100 ml before, during, and after the intrarenal infusion, respectively. Despite the higher intrarenal concentration of the drug in these experiments, the infusion of isoproterenol into the renal artery was not associated with significant and reversible effects on renal hemodynamics, C_{H_2O}, and U_{Osm} of the infused kidney (Table III). A small increase in C_{H_2O} did occur during the infusion but did not diminish significantly after cessation of the infusion. Analysis of the ratio of the infused over the contralateral kidney revealed a small but significant and reversible effect on C_{H_2O}, GFR, and RVR (Table III).

DISCUSSION

Robison, Butcher, and Sutherland (18) recently proposed that adenyl cyclase may be a beta adrenergic receptor. This suggestion was based on the findings that beta adrenergic stimulation increases adenyl cyclase activity in certain tissues (9, 10). In this context, the adrenergic nervous system could be an important regulator of the water permeability of the diluting segment of the mammalian nephron since the effect of vasopressin appears to be mediated by increasing the activity of adenyl cyclase which catalyzes the formation of

TABLE II—(*Continued*)

Exp. No.	Renal vascular resistance			Filtration fraction		
	Pre-control	Iso	Post-control	Pre-control	Iso	Post-control
	mm Hg/(ml/min)					
1	0.371	0.382	0.362	0.318	0.338	0.331
	0.485	0.535	0.427	0.307	0.309	0.294
2	0.362	0.375	0.435	0.331	0.372	0.392
	0.427	0.562	0.491	0.294	0.364	0.371
3	0.435	0.423	0.384	0.392	0.441	0.380
	0.491	0.501	0.438	0.371	0.400	0.318
4	0.388	0.495	0.602	0.237	0.307	0.315
	0.417	0.537	0.655	0.210	0.296	0.308
5	0.487	0.489	0.477	0.261	0.290	0.261
	0.579	0.609	0.553	0.266	0.292	0.254
6	0.661	0.654	0.705	0.302	0.337	0.325
	0.764	0.752	0.788	0.325	0.370	0.340
7	0.705	0.624	0.650	0.325	0.311	0.296
	0.788	0.700	0.730	0.340	0.337	0.322
8	0.650	0.703	0.752	0.296	0.346	0.328
	0.730	0.725	0.797	0.322	0.354	0.338
Mean	0.546	0.567	0.578	0.306	0.342	0.323
±SE	±0.04	±0.03	±0.04	±0.01	±0.01	±0.01
P value		NS NS			<0.001 <0.01	

TABLE III

The Effects of Intrarenal Isoproterenol on Renal Hemodynamics,

	Glomerular filtration rate			Renal vascular resistance			Filtration fraction		
	Pre-control	Iso	Post-control	Pre-control	Iso	Post-control	Pre-control	Iso	Post-control
	ml/min			*mm Hg/ml per min*					
Infused kidney (n = 9)									
Mean	44.3	47.0	45.8	0.503	0.474	0.512	0.295	0.303	0.314
±SE	±2.8	±3.4	±3.5	±0.05	±0.05	±0.05	±0.03	±0.03	±0.03
P value	NS	NS		NS	NS		NS	NS	
Noninfused kidney (n = 9)									
Mean	45.2	44.5	45.1	0.489	0.501	0.498	0.291	0.305	0.305
SE	±3.4	±3.0	±3.1	±0.05	±0.05	±0.05	±0.03	±0.03	±0.03
P value	NS	NS		NS	NS		NS	NS	
Ratio of infused/noninfused kidney*									
Mean	0.99	1.06	1.02	1.03	0.95	1.02	1.02	1.00	1.04
SE	±0.03	±0.02	±0.04	±0.03	±0.04	±0.05	±0.03	±0.04	±0.04
P value	<0.02	<0.05		<0.05	<0.01		NS	NS	

* Dimensions of measurements (ml/min, mmHg/ml per min, μEq/min, and mOsm/kg H_2O) apply only to infused and noninfused kidneys and not to the ratios between these kidneys.

Urinary sodium excretion			Urinary potassium excretion			Free water clearance			Urinary osmolality		
Pre-control	Iso	Post-control	Pre-control	Iso	Post-contro	Pre-control	Iso	Post-control	Pre-control	Iso	Post-contro
μEq/min			*μEq/min*			*ml/min*			*mOsm/kg H_2O*		
1	1	1	29	24	24	5.20	4.18	4.71	51	52	39
5	4	4	21	14	16	3.10	1.78	2.31	62	67	50
1	1	1	24	21	25	4.71	3.52	3.19	39	65	87
4	3	2	16	10	19	2.31	0.96	1.78	50	96	96
1	2	10	25	32	33	3.19	2.95	3.56	87	108	104
2	2	3	19	18	18	1.78	1.06	1.06	96	142	148
11	2	4	20	14	13	3.20	2.53	2.29	86	92	76
1	1	1	15	11	10	1.39	0.58	0.81	107	147	103
35	13	11	22	18	21	3.87	3.07	3.15	97	103	104
9	3	2	19	13	16	3.03	1.81	2.00	94	115	117
10	14	20	16	18	19	2.43	2.16	2.56	69	81	79
6	12	18	16	17	17	2.15	2.21	2.46	71	77	81
20	31	40	19	20	19	2.56	2.80	3.14	79	87	87
18	32	47	17	18	17	2.46	2.95	3.15	81	85	89
40	40	45	19	20	19	3.14	2.55	2.56	87	116	120
47	38	43	17	19	19	3.15	2.49	2.58	89	118	117
13	12	16	20	18	19	2.98	2.35	2.58	78	97	94
±3.7	±3.6	±4.4	±1.0	±1.4	±1.3	±0.3	±0.2	±0.2	±4.6	±6.6	±6.3
NS	<0.01		NS	NS		<0.001	<0.01		<0.001	NS	

Electrolyte Excretion, and Renal-Diluting Capacity

Urinary sodium excretion			Urinary potassium excretion			Free water clearance			Urinary osmolality		
Pre-control	Iso	Post-control	Pre-control	Iso	Post-control	Pre-control	Iso	Post-control	Pre-control	Iso	Post-control
μEq/min			*μEq/min*			*ml/min*			*mOsm/kg H_2O*		
23	36	29	27	30	30	3.48	3.82	3.52	57	59	60
±5.4	±7.8	±5.7	±3.5	±3.4	±4.4	±0.26	±0.33	±0.30	±3.2	±3.8	±5.4
<0.005	NS		<0.02	NS		<0.05	NS		NS	NS	
16	21	24	27	27	30	3.17	3.08	3.18	55	58	58
±4.8	±6.0	±6.1	±3.0	±2.9	±3.8	±0.27	±0.33	±0.34	±6.0	±6.6	±7.8
<0.02	NS		NS	NS		NS	NS		NS	NS	
1.93	2.62	1.51	1.02	1.14	1.02	1.12	1.28	1.15	1.14	1.12	1.15
±0.27	±0.56	±0.20	±0.04	±0.02	±0.04	±0.06	±0.08	±0.06	±0.14	±0.13	±0.16
NS	<0.05		<0.05	<0.02		<0.02	<0.02		NS	NS	

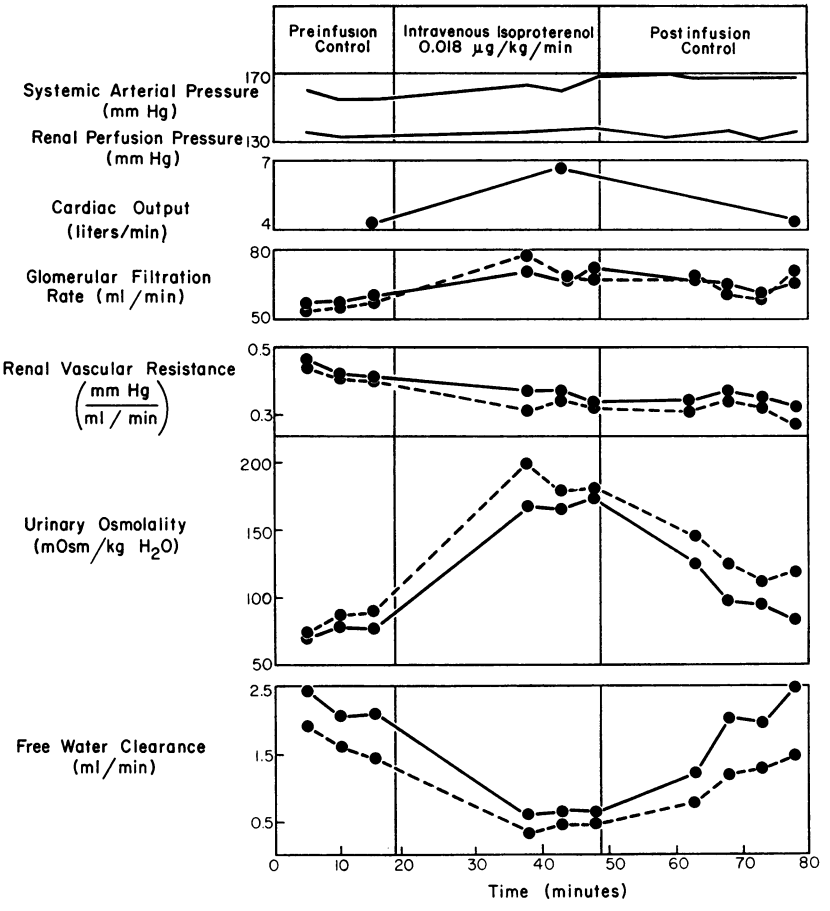

FIGURE 1 Antidiuretic effect of systemic beta adrenergic stimulation with intravenous isoproterenol. The increase in urinary osmolality and decrease in free water clearance were associated with an increase in cardiac output as renal perfusion pressure, glomerular filtration rate, and renal vascular resistance were not significantly altered. The left kidney was denervated and is denoted by the solid line, and the right innervated kidney is denoted by the broken line.

3',5'-adenosine monophosphate (cyclic AMP) (19–21). In support of the possibility that renal adenyl cyclase is a beta adrenergic receptor is the finding that the intravenous administration of the potent beta adrenergic-stimulating substance, isoproterenol, is known to be associated with an antidiuresis (11–14), while the intravenous administration of the alpha adrenergic-stimulating catecholamine, norepinephrine, is known to inhibit the antidiuretic effect of vasopressin (1–6). Moreover, the effect of norepinephrine in humans has been shown to occur in the absence of changes in filtration rate and renal plasma flow and has been shown to be dose related, thus suggesting a competitive inhibition at the site of the renal tubule epithelial cell (1). On the other hand, in most studies in which an antidiuretic effect of isoproterenol has been demonstrated, a fall in renal perfusion pressure has been consistently observed (11–14), and when measured, glomerular filtration rate has also frequently been observed to diminish (11–14). Since the simultaneous decrease in renal perfusion pressure and glomerular filtration rate are known to affect renal-diluting ability (15, 16), the results of these previous studies do not distinguish between a direct effect of isoproterenol on the water permeability of the distal nephron and an effect mediated by alterations in renal hemodynamics. Moreover, in the presence of a decrease in systemic arterial pressure the release of ADH may account entirely for, or at least contribute to, the antidiuretic effect of beta adrenergic stimulation. The purpose of the present investigation was to examine these different possible mechanisms which might account for the antidiuretic effect associated with beta adrenergic stimulation.

In the present study a considerably smaller dose by body weight of intravenous isoproterenol than has been previously used in the rat and cat (11–14) was found

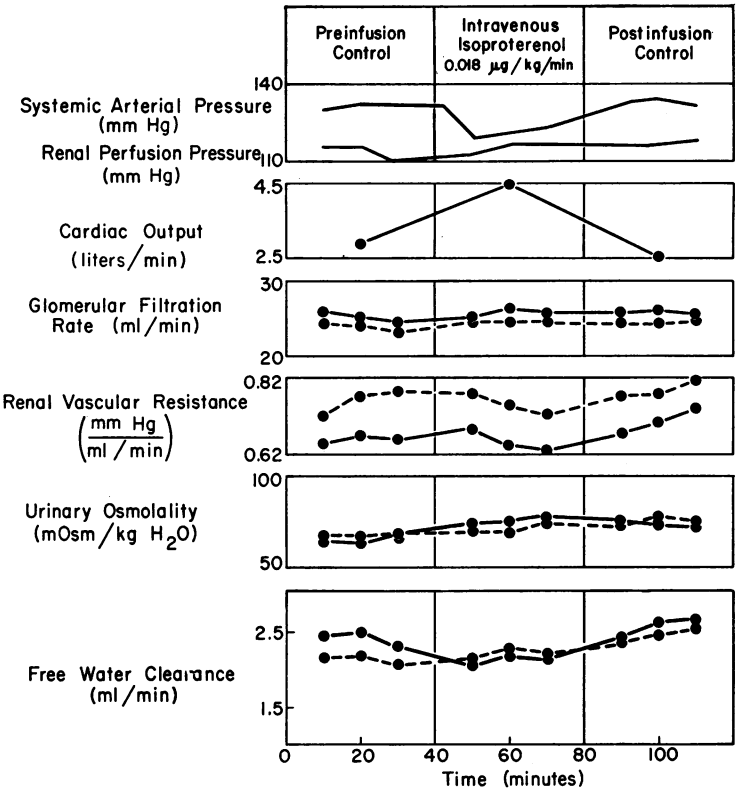

FIGURE 2 Absence of antidiuretic effect of intravenous isoproterenol in an animal after ablation of hypothalamo-neurohypophysial system. The left kidney is denoted by the solid line and the right kidney by the broken line. Both kidneys were denervated.

to be associated with a consistent antidiuretic effect in the dog. Even with this dose of intravenous isoproterenol either a transient or sustained fall in systemic arterial pressure was frequently observed. In order to avoid any direct renal effect of these changes in arterial pressure, the renal perfusion pressure was maintained constant in all of the present experiments. With this experimental design, the antidiuretic effect of systemic beta adrenergic stimulation was clearly demonstrated to occur independent of any decrease in renal perfusion pressure. It should be emphasized however, that if allowed to occur, any decrease in renal perfusion pressure during an intravenous isoproterenol infusion could contribute to the resulting antidiuretic effect, particularly by decreasing the rate of free water clearance. A role of the renal nerves in the antidiuretic effect of beta adrenergic stimulation was also excluded by the present results, since no consistent quantitative differences in the antidiuretic effect could be observed between the innervated and denervated kidneys (Fig. 3, Table I). We can not exclude, however, the possibility that the fall in systemic arterial pressure during intravenous isoproterenol was associated with a baroreceptor-mediated increase in renal sympathetic tone which was obscured by the direct renal-vasodilating effect of the drug (22, 23). If present, such an effect of increased renal sympathetic tone would have to be mediated by circulating catecholamines in the denervated kidneys. In any case, since no significant change in renal vascular resistance was observed in either the denervated or innervated kidneys, the antidiuretic effect associated with the intravenous infusion of isoproterenol does not seem to require renal innervation.

An influence of alterations in glomerular filtration rate was also found not to be important in the antidiuretic effect associated with systemic beta adrenergic stimulation. In a number of individual experiments glomerular filtration rate actually increased during the intravenous infusion of isoproterenol, and in the group as a whole a small mean increase was observed (Table I). This finding established that a decrease in glomerular filtration rate leading to a diminution in distal fluid delivery is not a factor in the antidiuretic effect associated with intravenous isoproterenol.

The only significant and reversible alteration in renal hemodynamics which occurred during the intravenous

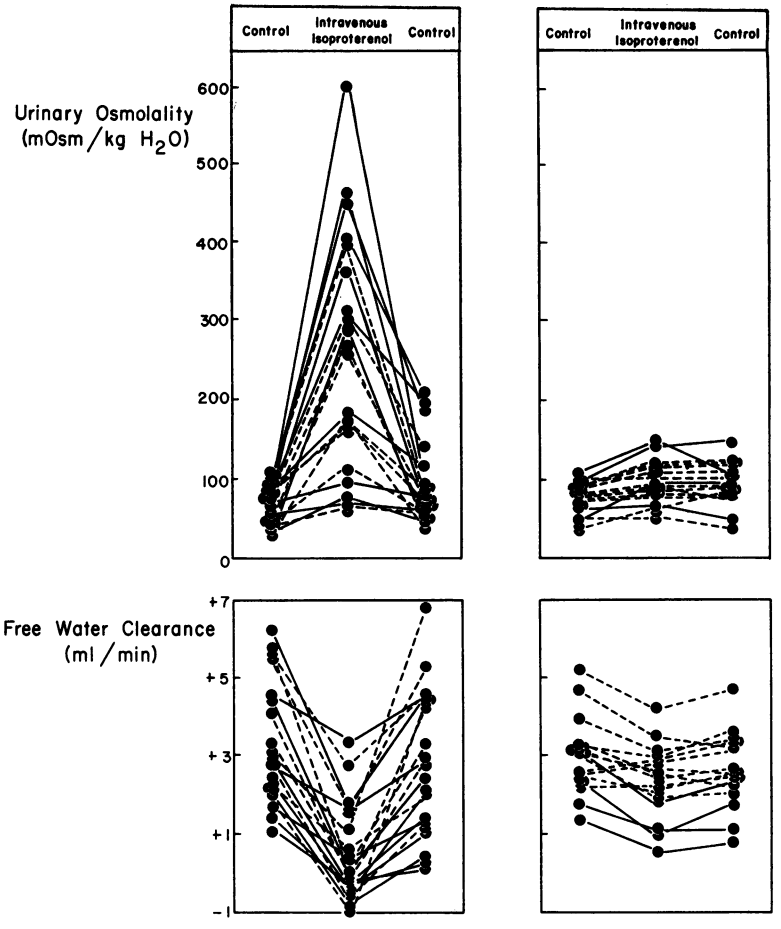

FIGURE 3 Effect of intravenous isoproterenol on urinary osmolality and free water clearance in the intact dog (left) vs. the dog with ablation of the hypothalamo-neurohypophysial system (right). The broken lines indicate the results in the denervated kidneys, and the solid lines indicate the results in the innervated kidneys. Each point is the mean of three to five periods.

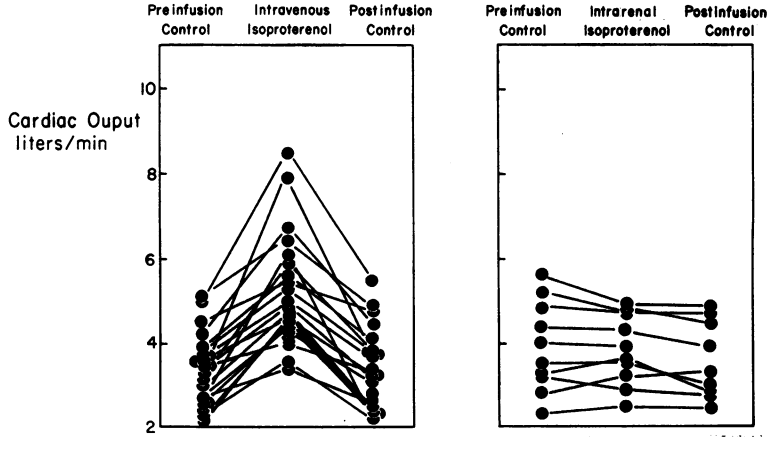

FIGURE 4 Effect of the same dose of intravenous vs. intrarenal isoproterenol on cardiac output.

infusion of isoproterenol was a small change in filtration fraction (Table I). This increase in filtration fraction could have been due to the concomitant rise in arterial hematocrit (17, 24). Recently, Schrier and Earley (17) have demonstrated that an increase in arterial hematocrit is associated with both an increase in filtration fraction and a decrease in solute-free water reabsorption, and it was suggested that these effects of hematocrit were due to an increase in postglomerular viscosity and enhanced sodium reabsorption in the proximal tubule, respectively. Micropuncture studies have recently confirmed that an increase in hematocrit is associated with an increase in proximal tubular reabsorption (25, 26). However, in a quantitative sense, the small increase in hematocrit and filtration fraction that was observed in the present study during the intravenous infusion of isoproterenol would seem only to contribute in a very minor way to the resulting antidiuretic effect (17).

The finding that alterations in renal hemodynamics and renal innervation did not contribute in any major way to the antidiuresis suggested an importance of either a direct effect of beta adrenergic stimulation on the renal tubular epithelial cell or an extrarenal reflex mechanism involving the release of ADH. Two approaches were used to differentiate between these two potential mechanisms. One approach was to examine the effect of intravenous isoproterenol on the water diuresis in animals in which the source of production and release of ADH had been ablated. The second approach was to investigate the effect of isoproterenol infused into the renal artery at a dose which was not associated with any systemic effects of the drug but yet delivered a concentration of drug to the renal circulation, which was at least as high as the renal arterial concentration achieved during the intravenous infusion of isoproterenol.

In the group of animals with ablation of the hypothalamo-neurohypophysial system, the intravenous infusion of isoproterenol was associated with no reversible effect on urinary osmolality. However, a small, reversible effect on free water clearance was observed in this group of animals. This small effect on free water clearance seemed most likely to be related to the effect of beta adrenergic stimulation to increase arterial hematocrit and filtration fraction. Although the changes in hematocrit and filtration fraction were similar in the normal animals and the animals with ablation of their hypothalamo-neurohypophysial tract, the antidiuretic effect was markedly different in these two groups of animal (Fig. 3). This finding further supports the conclusion that any role of the increase in hematocrit in the antidiuretic effect is a relatively minor one. These results, however, primarily emphasize that the presence of an intact hypothalamo-neurohypophysial tract is of major importance in the antidiuretic effect of beta adrenergic stimulation. This conclusion of our studies in dogs is different from that of a recent preliminary communication in which the antidiuretic effect of intravenous isoproterenol was demonstrated to occur in rats with hereditary diabetes insipidus, and thus presumably with no endogenous production of ADH (27). In these studies in rats the dose of isoproterenol was severalfold by body weight of the dose used in the present study. Although the effect of this larger dose of intravenous isoproterenol on systemic arterial pressure and renal hemodynamics was not reported, on the basis of the present results the relatively small increase in urinary osmolality which occurred in the diabetes insipidus rats (from 102 to 184 mOsm/kg H_2O) could have been due to effects on systemic and renal hemodynamics.

The present group of experiments in which isoproterenol was infused directly into the renal artery provided further support for the conclusion that the major antidiuretic effect of beta adrenergic stimulation is an extrarenal reflex mechanism. We found that doses of isoproterenol that markedly increased cardiac output and decreased total peripheral resistance when infused intravenously did not exert either of these effects on systemic hemodynamics when infused directly into the renal artery (Fig. 4). The pathway(s) whereby the kidney so efficiently inactivates isoproterenol is currently under study in our laboratory. This efficient renal inactivation of the drug allowed us to examine the direct effect of intrarenal beta adrenergic stimulation on water diuresis in the absence of concomitant alterations in systemic hemodynamics. While previous investigators have infused isoproterenol into the renal artery (22, 23, 28), in none of these studies have the systemic effects of the drug been adequately evaluated. In some of these studies arterial pressure has been shown to decrease (22, 23) while in other studies the arterial pressure has been unchanged during the intravenous administration of isoproterenol (28). However, as demonstrated in the present study, arterial pressure may remain constant in the presence of substantial systemic effects of beta adrenergic stimulation to increase cardiac output and to decrease total peripheral resistance. In the present study the documentation of the absence of any systemic effects during the intrarenal infusion of the drug (which if present could potentially activate extrarenal reflex mechanism[s] and account for any observed effects on water diuresis) provided the experimental circumstance to examine the direct intrarenal effect of beta adrenergic stimulation on water diuresis. Since the concentration of isoproterenol in the renal circulation in these experiments would be con-

siderably greater than the level achieved during the intravenous infusion of the drug,[8] the antidiuretic effect might be expected to be more pronounced if such an effect is due to a direct action of beta adrenergic stimulation on the renal tubular epithelial cell. No antidiuretic effect was observed, however, when isoproterenol was infused directly into the renal artery. In fact when the infused kidney was compared with its contralateral control kidney, the intrarenal isoproterenol was found to produce a relative increase in free water clearance. This finding is compatible with the recent results of Gill and Casper (28); however, in contrast to their results the effect on free water clearance found in our study was associated with parallel changes in glomerular filtration rate and renal vascular resistance when compared with the contralateral kidney (Table III).

The possibility should be considered that since isoproterenol is rapidly cleared by the kidney that the infused isoproterenol may not reach effective concentrations at any potential beta adrenergic receptor sites on either the luminal or peritubular border of the collecting duct. However, since the vasa recti surrounding collecting ducts derive directly from postglomerular arterioles without bathing cortical proximal and distal tubules, the concentration of drug in these vasa recti should be comparable with that reaching the glomerulus. In this regard preliminary studies with radioisotope-labeled isoproterenol indicate that the clearance of the drug is at least comparable with the rate of glomerular filtration.[4] This finding thus suggests that the drug reaches both the peritubular and luminal border of the collecting duct. Another possibility must also be considered that larger intrarenal doses of isoproterenol than used in the present experiments may be needed to alter tubular permeability. If so, such intrarenal beta adrenergic receptors must be much less sensitive than the beta adrenergic receptors in the heart and peripheral vasculature which were significantly stimulated during the intravenous infusion of isoproterenol in the present study. Moreover, the in vivo demonstration of such an insensitive intrarenal beta adrenergic receptor would be very difficult since larger intrarenal doses will produce systemic effects and thereby not allow the differentiation between an antidiuretic effect initiated by extrarenal vs. intrarenal pathways.

[8] For example, during the intravenous dose of isoproterenol of 0.018 μg/kg per min in the intact animals, the mean cardiac output and renal blood flow per kidney for four experiments were 6.2 liters/min and 393 ml/min, respectively. On the basis of this data the calculated mean rate of isoproterenol reaching the renal circulation of each kidney would be 0.001 μg/kg per min, a rate for less than the doses (0.009–0.0036 μg/kg per min) of isoproterenol infused into the renal artery.

[4] Lifschitz, M. D., A. Goldfein, and R. W. Schrier. Unpublished observation.

In summary, the present results have demonstrated that an extrarenal reflex involving the integrity of the hypothalamo-neurohypophysial system and the release of ADH is primarily responsible for the antidiuretic effect of systemic beta adrenergic stimulation. The results also excluded a major role of alterations in renal hemodynamics or renal sympathetic tone in this antidiuretic response. Moreover, a dose of isoproterenol, which was associated with a marked stimulation of the cardiac beta adrenergic receptors when infused intravenously, was not found to produce an antidiuretic effect when infused directly into the renal artery. This latter result thus provided no support for a direct effect of beta adrenergic stimulation to increase the permeability to water of the renal tubular epithelium.

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