Effects of Dietary Sodium and of Acute Saline Infusion on the Interrelationship between Dopamine Excretion and Adrenergic Activity in Man

R. WAYNE ALEXANDER, JOHN R. GILL, JR., HIROHIKO YAMABE, WALTER LOVENBERG, and HARRY R. KEISER

From the Hypertension-Endocrine Branch, National Heart and Lung Institute, National Institutes of Health, Bethesda, Maryland 20014

ABSTRACT The effects of dietary sodium and of saline infusion on urinary dopamine and norepinephrine and on the relationship of these catecholamines to adrenergic activity were determined. In seven normal subjects on a 9-meq sodium intake, urinary dopamine and norepinephrine were 136±18 (SE) and 37.4±5.3 μg/day, respectively. When sodium intake was increased to 209 or 259 meq/day, urinary dopamine increased to 195±20 μg/day ($P < 0.01$) whereas urinary norepinephrine decreased to 21.1±3.0 μg/day ($P < 0.01$). Infusion of saline in seven subjects increased sodium excretion and urinary dopamine (from 2.18±0.22 to 2.79 ±0.19 μg/20 min, $P < 0.01$), but decreased plasma dopamine-β-hydroxylase by 33% and urinary norepinephrine insignificantly. The clearance of inulin and *p*-aminohippurate did not change significantly and filtration fraction was the same. The data indicate that an increase in dietary sodium or infusion of saline results in an apparent decrease in adrenergic activity and an increase in urinary dopamine. Dopamine excretion would thus appear to relate inversely to adrenergic activity and to parallel sodium excretion. These findings suggest a possible role for dopamine and norepinephrine in the regulation of renal sodium excretion.

INTRODUCTION

Dopamine, the precursor of the sympathetic neurotransmitter norepinephrine, is present in human urine in readily measurable quantities (1), but the factors which govern its excretion have not been determined.

Dietary sodium may be a determinant of dopamine excretion as suggested by a recent report of a direct and significant correlation between the concentration of urinary sodium and of urinary dopamine (2). As dietary sodium also reportedly affects norepinephrine excretion, presumably as a consequence of a change in sympathetic activity (3), it is possible that the sympathetic nervous system also mediates the effects of dietary sodium on urinary dopamine. The observation that dopamine excretion decreased and norepinephrine excretion increased in response to a change from supine to an upright position (2) suggests that dopamine excretion may be inversely related to sympathetic nervous activity.

An increase in renal sympathetic nerve discharge (4) and renal arterial infusion of norepinephrine (5) can increase the renal tubular reabsorption of sodium whereas the intravenous or renal arterial infusion of dopamine increases the renal excretion of sodium (6–8). This latter observation suggests that an increase in renal dopamine, physiologically induced, could decrease the tubular reabsorption of sodium possibly via specific dopaminergic receptors (9, 10). The opposite effects of norepinephrine and dopamine on the renal handling of sodium and the suggestion that there may be an inverse relationship between sympathetic nervous activity and urinary dopamine excretion suggests a possible role for both catecholamines in the physiologic regulation of sodium excretion. In the present studies, the effects of sodium intake and of rapid infusion of saline on adrenergic activity and dopamine excretion were determined to explore the interrelationships of sympathetic activity, dopamine metabolism, and sodium excretion in more detail.

METHODS

The experimental design consisted of two protocols to which the participants gave their informed consent. In one

Received for publication 24 October 1973 and in revised form 28 February 1974.

protocol seven normal subjects, one woman and six men, aged 19–29 yr, who had been taking a diet with an ad lib. sodium intake, were placed on a banana-free diet which contained 9 meq of sodium. They were given furosemide, 40 mg/day, for the first 3 days of the diet. After 8 days, sodium intake was increased to 259 meq/day (except for K. R. and M. S. who received 209 meq/day) by giving sodium chloride in a shaker for 10 days. Thus, the change in sodium intake did not involve a change in dietary constituents which may in themselves be a determinant of urinary dopamine (11). Urine was collected for 24 h on alternate days with 15 ml of 6 N hydrochloric acid and was refrigerated until assayed for catecholamines, creatinine, and sodium. Under similar collection and storage conditions the content of free epinephrine, norepinephrine, and dopamine are very stable (12). Blood was drawn every 2–3 days throughout the study for sodium, potassium, and creatinine. The data, which exclude those days immediately after a change in sodium intake, are presented as means of the last three collection periods during 9 meq sodium intake and as means of the last 4 days of high sodium intake.

In another protocol, seven normal women, aged 19–23 yr who had no history of urinary tract abnormality or disease, were given a diet which contained 59 meq of sodium/day for 3 days. On the morning of study, breakfast was withheld and an intravenous infusion of 5% dextrose solution was given at 3 ml/min. Urine was collected by indwelling bladder catheter. After urine flow had stabilized, approximately 50 min after the start of the infusion, urine was collected for 20-min periods and the bladder washed by water and air at the end of each period. An aliquot was acidified with 1 N hydrochloric acid and then refrigerated until catecholamines were determined. Venous blood was drawn through an indwelling Cournand needle at midperiod. After four periods, the infusate was changed to normal saline, 15 ml/min, and urine was collected for an additional eight periods. All infusates contained inulin and *p*-aminohippurate (PAH), and the clearances of inulin (C_{IN})[1] and of *p*-aminohippurate (C_{PAH}) were determined as previously described (13). The filtration fraction was calculated as C_{IN}/C_{PAH}. The infusion data presented are the means of the three periods immediately preceding infusion of saline (control) and the means of the last four periods of saline infusion (saline).

The nature and purpose of the above study and the details of the procedures were presented to the subjects in written form. After full review of this form with each subject, they indicated informed consent by signing the form. No subject was studied more than once, and urine cultures before, at the time of, and several days after study were obtained. The study protocol and precautions were approved by the Clinical Research Committee of the Medical Board of the National Institutes of Health.

Plasma dopamine-β-hydroxylase (DβH), the enzyme which converts dopamine to norepinephrine, was measured as previously described (14) and expressed in units such that 1 U of DβH activity is that amount of enzyme which converts 1 nmol of tyramine to octopamine in 20 min.

Free dopamine and norepinephrine were measured as follows. An aliquot of urine (25–110 ml) was adjusted to pH 8.6 in the presence of 400 mg of alumina, 10 ml of 2% EDTA, and 30 mg of sodium metabisulfite. The urine and the alumina with the adsorbed catecholamines were poured through a glass column which contained an additional 400 mg of alumina at pH 8.6. The catecholamines were eluted with 7.5 ml of 0.2 N acetic acid. Free urinary dopamine was measured by the method of Anton and Sayre (1). Internal standards of dopa and dopamine were used and samples were corrected for dopa which was occasionally present. Free norepinephrine was measured by the method of von Euler (15) after ferricyanide oxidation at pH 6.5. Epinephrine was determined after ferricyanide oxidation at pH 2.85 and norepinephrine values were corrected for epinephrine content. As dopamine interferes with the measurement of norepinephrine (1–2%), the amount of interference was calculated and used to correct the norepinephrine values. The average recovery rates for the dopamine and norepinephrine assays were 94 and 89%, respectively but the values were not corrected for recovery. The mean coefficient of variation for the dopamine assay is 13% and that for norepinephrine is 15%. The sensitivity of the dopamine and norepinephrine assays is such as to measure accurately 0.05 and 0.02 μg, respectively, concentrations considerably lower than those in the sample. As a 20-min collection of urine contains very little epinephrine, norepinephrine and epinephrine were measured together, in this case, after ferricyanide oxidation at pH 6.5 and expressed as norepinephrine + epinephrine. Serum and urinary creatinine were measured by the method of Bonsnes and Taussky (16) and sodium was determined by internal standard flame photometry.

Statistical analysis was performed utilizing a paired *t* test (17). A *P* of 0.05 or less was considered to be significant.

RESULTS

The data in Table I demonstrate the effects of a restricted sodium intake (9 meq/day) and of a high

TABLE I

Effect of Low and High Sodium Intakes on Urinary NE and DA

Subject	Sodium intake	$U_{Na}V$	C_{Cr}	NE	DA
		meq/day	*ml/min*	*μg/day*	*μg/day*
D. K.	9	1±1	84±6	25.2±3.6	180±41
	259	258±26	106±5	19.0±2.0	234±17
R. R.	9	14±11	108±9	50.6±2.3	91±5
	259	231±36	112±10	27.0±3.3	165±14
M. S.	9	24±22	60±0	14.4±2.3	66±6
	209	170±9	93±11	10.7±2.2	127±17
R. J.	9	2±1	118±11	48.7±11.3	185±21
	259	268±26	127±3	29.9±2.5	265±23
S. J.	9	2±3	77±1	37.4±8.9	156±12
	259	277±19	87±5	13.9±2.1	183±12
K. R.	9	7±3	108±1	34.7±1.9	107±18
	209	203±20	104±5	16.9±1.6	146±11
G. C.	9	14±12	90±4	50.8±2.4	166±17
	259	187±19	108±3	30.4±2.4	242±13
Mean	9	9±3	92±8	37.4±5.3	136±18
±SEM	209 or 259	228±18	105±5	21.1±3.0	195±20
P		<0.01	<0.01	<0.01	<0.01

$U_{Na}V$, sodium excretion in the urine; C_{Cr}, clearance of creatinine; NE norepinephrine excretion in urine; DA, dopamine excretion in the urine.

[1] *Abbreviations used in this paper:* C_{IN}, clearance of inulin; C_{PAH}, clearance of *p*-aminohippurate; DβH, dopamine-β-hydroxylase.

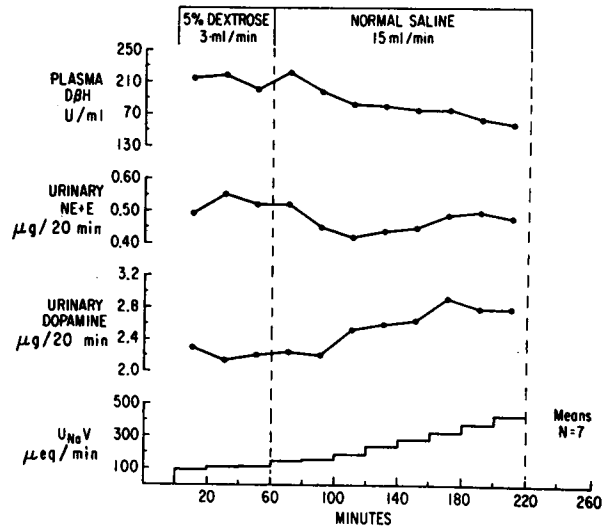

FIGURE 1 The effect of infusion of normal saline on plasma DβH, urinary norepinephrine + epinephrine (NE + E), and urinary dopamine and urinary sodium excretion ($U_{Na}V$). Note that urinary dopamine excretion tends to parallel urinary sodium excretion and to be inversely related to plasma DβH.

sodium intake (209 or 259 meq/day) on urinary norepinephrine and dopamine. After the initial 3 days on the 9 meq sodium diet, the excretion of norepinephrine (37.4±5.3 (SE) μg/day) and dopamine (136±18 μg/day) was relatively stable. The ratio of dopamine to norepinephrine ranged from 1.8 to 7.2 with a mean of 4.0. On a 209 or 259 meq/day sodium intake, mean urinary norepinephrine was 21.1±3.0 μg/day, a value significantly ($P < 0.01$) lower than that observed with a restricted sodium intake, whereas mean urinary dopamine was 195±20 μg/day, a value significantly ($P < 0.01$) higher than that observed during a restricted sodium intake. The changes in excretion of norepinephrine and dopamine resulted in a higher mean ratio of dopamine to norepinephrine of 9.8, with a range of 6.1–13.1. The clearance of creatinine was significantly ($P < 0.01$) higher during the high sodium intake than during the period of sodium restriction (105 ±5 vs. 92±8 ml/min).

Fig. 1 presents the experimental design and a summary of the results of the studies of the effect of saline infusion. The values shown for each 20 min period are means of the values from all seven studies and they are plotted to show the pattern of response of plasma DβH and urinary catecholamines to saline infusion. By the third period of saline infusion, plasma DβH and urinary norepinephrine + epinephrine had decreased appreciably and urinary dopamine and sodium

TABLE II
Effects of Infusion of Saline

Subject	Regimen	V	$U_{Na}V$	DA	NE + E	DβH	Hct	C_{IN}	C_{PAH}
		ml/min	*μeq/min*	*μg/20 min*		*U/ml*	%	*ml/min*	
D. J.	Control	8.5	182	2.93	0.58	492	38	86	463
	Saline	4.3	473	2.82	0.47	370	34	86	522
J. S.	Control	6.4	77	1.40	0.43	209	38	79	432
	Saline	4.8	486	2.12	0.36	171	35	92	542
T. G.	Control	5.2	98	1.56	0.44	57	41	92	378
	Saline	4.8	450	2.12	0.35	48	38	103	372
V. C.	Control	5.9	100	2.83	0.68	22	38	74	412
	Saline	4.7	447	3.35	0.58	19	36	86	565
S. B.	Control	6.5	84	2.45	0.70	173	36	83	446
	Saline	3.1	225	3.46	0.79	138	32	94	438
M. E.	Control	6.5	107	1.87	0.46	294	34	86	523
	Saline	3.6	271	2.52	0.42	250	31	74	453
F. K.	Control	7.9	98	2.24	0.34	230	35	104	516
	Saline	2.9	160	3.11	0.35	186	32	103	534
Mean	Control	6.7±0.44	107±14	2.18±0.22	0.52±0.05	211±67	37±1	86±4	453±21
±SE	Saline	4.0±0.27	359±47	2.79±0.19	0.47±0.06	169±50	34±1	91±4	489±28
			<0.01	<0.01	>0.10	<0.05	<0.01	>0.2	>0.2

V, urine flow; $U_{Na}V$, sodium excretion; DA, dopamine excretion; NE + E, norepinephrine plus epinephrine excretion; Hct, hematocrit; C_{IN}, clearance of inulin; C_{PAH}, clearance of PAH.

had started to rise. These initial changes progressed throughout the remainder of the saline infusion except for urinary norepinephrine + epinephrine which returned toward control values.

Table II summarizes the results of all the studies as means of the three periods before infusion of saline (control) and as means of the last four periods of infusion of saline. Control plasma DβH activity varied over a wide range from 22 to 492 U/ml, with a mean of 211±67 U/ml. With saline infusion, plasma DβH activity decreased significantly ($P < 0.05$) to a mean value of 169±50 U/ml, a change of 20% which, in part, may have been dilutional as hematocrit decreased 8%. By the final period DβH activity had decreased 33% from the mean control value (Fig. 1). Mean urinary norepinephrine + epinephrine (control, 0.52± 0.05 μg/20 min) tended to decrease with infusion (saline, 0.47±0.06 μg/20 min) but the change was not significant ($P > 0.1$). Urinary dopamine, however, increased significantly ($P < 0.01$) from a mean control value of 2.18±0.22 to 2.79±0.19 μg/20 min in association with an increase in urinary sodium from 107 ±14 to 359±47 μeq/min and a decrease in urine flow from 6.7±0.44 to 4.0±0.27 ml/min. The ratio of urinary dopamine to norepinephrine + epinephrine (4.2, control; 5.9, saline) increased with saline infusion as it did with an increase in dietary sodium. The C_{IN} and C_{PAH} tended to increase with saline infusion but the values were not significantly ($P > 0.2$) different from control values; filtration fraction did not change (control, 0.19; saline, 0.19).

DISCUSSION

A change in dietary sodium intake from 9 to 209 or 259 meq/day was associated with a significant decrease in urinary norepinephrine excretion and a significant increase in urinary dopamine excretion which was sustained throughout the several days of observation after the diet change (Table I). A qualitatively similar pattern of change in urinary norepinephrine + epinephrine and in dopamine excretion was also observed during rapid intravenous infusion of saline (Table II), and suggests that these changes in urinary norepinephrine and dopamine occur very quickly when the volume of extracellular fluid is increased. The specific intermediary events by which changes in sodium intake lead to changes in urinary catecholamines are unknown, but there is evidence that changes in circulating blood volume may play a role. In previous studies, a restriction of sodium intake which was associated with a contraction of plasma volume resulted in an increase in urinary norepinephrine excretion (3). In other studies, the effects of autonomic or adrenergic blockade on the circulatory response to expansion of intravascular or extracellular fluid volume (18, 19) suggest that one possible effect of infusion of saline in the present studies was to decrease adrenergic activity. Presumptive evidence that adrenergic activity did indeed decrease during saline infusion was the associated decrease in plasma DβH activity (Fig. 1, Table II). This enzyme is contained together with norepinephrine in neuronal secretory granules and the two are released proportionally from adrenergic neurons in response to nerve discharge so that the rate of release of DβH may reflect nerve activity (20–22). Whereas directional changes in plasma DβH activity may reflect similar directional changes in adrenergic nerve activity, the wide range of resting, preinfusion values for plasma DβH activity in the normal women, from 22 to 492 U/ml, suggests that basal DβH activity in plasma may not correlate very well with adrenegic activity, a conclusion similar to that arrived at in another study (14). The reasons for this apparent lack of correlation are not known but factors such as the rate of degradation of the enzyme and the ratio of norepinephrine to DβH released from the nerve terminals may be as important as adrenergic activity in the determination of plasma DβH activity (14).

The origin of urinary dopamine has not as yet been clearly elucidated. The content of the diet consumed has been suggested as a determinant of dopamine excretion (11). The effects of dietary manipulation on urinary dopamine have been attributed to a change in the dietary content of dopa which was presumably decarboxylated in the body to form dopamine (23). In that study (11), the possibility that sodium chloride intake changed in conjunction with the other changes in diet cannot be excluded, however. In any event, it is unlikely that changes in dietary constituents other than sodium chloride contributed to the changes in urinary dopamine in the present studies, as sodium was added to the diet as salt in a shaker or given as an infusion of saline to subjects in a fasting state.

Interpretation of the changes in dopamine excretion must begin with a consideration of the source of urinary dopamine. Dopamine, like norepinephrine and epinephrine, is found in a conjugated form in both plasma and urine (24–26). Whereas free norepinephrine is readily detectable in human plasma (27, 28),[2] free dopamine frequently cannot be detected (25, 12),[2] but when it is measurable, the values are usually less than those for free norepinephrine (24). As the excretion of free dopamine is severalfold greater than norepinephrine, however, glomerular filtration could contribute importantly to urinary dopamine only if hydrolysis of the filtered conjugate occurred during storage and assay of the catecholamines. This possibility seems unlikely, how-

[2] Kopin, I. J. Personal communication.

ever, since urinary dopamine conjugates require boiling in strong acid to be hydrolyzed (29). As the preliminary perchloric acid deproteinization step in the measurement of plasma dopamine does not appear to hydrolyze the conjugates, the conditions of storage, chromatography, and assay of free dopamine in the present study are not likely to hydrolyze the urinary conjugates of dopamine. Thus, it would seem that the free dopamine measured in urine cannot be accounted for on the basis of glomerular filtration of free or conjugated dopamine.

The major fraction of free dopamine in the urine appears to reach tubular fluid by tubular transport rather than by glomerular filtration in the case of renal arterial infusion of dopamine (30). The implications of a minimal amount of circulating free dopamine and excretion of administered dopamine by tubular secretion are that the bulk of urinary free dopamine originates in the kidney and is excreted by tubular transport. This formulation is consistent with the conclusions of others (25).

An inverse relationship between urinary dopamine and norepinephrine has been observed in response to a change in posture. In this case, a change from supine to an upright position was associated with a decrease in dopamine and an increase in norepinephrine (2). A comparison of the ratio of urinary dopamine to urinary norepinephrine on low and on high sodium diets indicates that in spite of large variations in the excretion of norepinephrine in the various subjects, the ratio of dopamine to norepinephrine was clearly higher on the high sodium diet than on the low sodium diet for each subject and this difference in ratio was the result of reciprocal changes in dopamine and in norepinephrine. In the case of saline infusion, however, an inverse relationship between urinary dopamine and norepinephrine was present only during the initial periods of infusion. It should be noted, however, that the concluding periods of infusion were collected between the hours of 11 a.m. and 3 p.m. when the circadian excretion of norepinephrine (and epinephrine) is the greatest (31), and this could explain the tendency of norepinephrine excretion to return toward control rather than to decrease as urinary dopamine increased further. However, an inverse relationship between urinary dopamine and plasma DβH activity was present throughout the study (Fig. 1). These findings of an inverse relationship between urinary dopamine and urinary norepinephrine and between urinary dopamine and plasma DβH activity suggest that urinary dopamine is inversely related to adrenergic nervous system activity.

There is little experimental evidence which might relate the present data to events at the adrenergic neuron. It has been shown, however, that pharmacologic inhibition of DβH in adrenergic nerves by disulfiram results in an accumulation of dopamine and a decrease in norepinephrine (32, 33) and that, in this circumstance, dopamine and norepinephrine are released with sympathetic nerve stimulation in the same ratio in which they are stored (33). These observations as well as the observation that DβH in adrenergic nerves increases with increased sympathetic discharge and thus increased catecholamine synthesis (34) have led to the conclusion that DβH may play an important regulatory role in catecholamine synthesis (35). There is, however, no experimental evidence that under physiologic conditions DβH activity might become rate-limiting in catecholamine synthesis or that dopamine is released from adrenergic neurons. Furthermore, although dopamine excretion consistently varied in an inverse relationship with presumed levels of sympathetic nervous activity, a nonadrenergic neuronal source of altered dopamine synthesis cannot be excluded.

Dopamine, when infused systemically (6, 7) or into a renal artery (7, 8) exerts a potent natriuretic effect. The increase in sodium excretion is usually associated with an increase in renal blood flow and glomerular filtration rate. These changes in renal hemodynamics occur, at least in part, as a result of a direct action of dopamine on the renal vasculature (36), and, as they may be selectively attenuated by haloperidol and chlorpromazine, they are probably mediated by specific dopamine receptors in the kidney (9, 10). Whereas changes in renal vasodilatation may be linked to the increase in sodium excretion by virtue of a decrease in colloid oncotic pressure and an increase in hydrostatic pressure in peritubular capillaries such that the tubular reabsorption of sodium is decreased (37–39), a direct effect of dopamine on tubular transport of sodium may also occur.

The observation of a decrease in the tubular reabsorption of sodium which could not be explained in terms of events in the peritubular capillary has led to postulation of the existence of a natriuretic substance or substances (40). This hypothesis has been supported by the findings that administration of sodium chloride is associated with the appearance in the urine of a peptide or peptides which can depress sodium transport (41). The report of a direct and significant correlation between the concentration of urinary sodium and that of urinary dopamine (2) and the present findings of an increase in urinary excretion of dopamine in association with an increase in the urinary excretion of sodium in response to an increase in dietary sodium or in response to saline infusion suggest that a role in the physiologic regulation of renal tubular sodium reabsorption be considered for dopamine. The demonstration of such a role for dopamine as well as explora-

tion of possible interrelationships between dopamine and norepinephrine and other modulators of sodium metabolism such as the renin-angiotensin system and, possibly, prostaglandins, await further study.

REFERENCES

1. Anton, A. H., and D. F. Sayre. 1964. The distribution of dopamine and dopa in various animals and a method for their determination in diverse biological material. *J. Pharmacol. Exp. Ther.* **145**: 326–343.
2. Cuche, J. L., O. Kuchel, A. Barbeau, R. Boucher, and J. Genest. 1972. Relationship between the adrenergic nervous system and renin during adaptation to upright posture: a possible role for 3,4-dihydroxyphenethylamine (dopamine). *Clin. Sci. (Oxf.)*. **43**: 481–491.
3. Kelsch, R. C., G. S. Light, J. R. Luciano, and W. J. Oliver. 1971. The effect of prednisone on plasma norepinephrine concentration and renin activity in salt-depleted man. *J. Lab. Clin. Med.* **77**: 267–277.
4. Gill, J. R., Jr., and A. G. T. Casper. 1969. Role of the sympathetic nervous system in the renal response to hemorrhage. *J. Clin. Invest.* **48**: 915–922.
5. Gill, J. R., Jr., and A. G. T. Casper. 1972. Effect of renal alpha-adrenergic stimulation on proximal tubular sodium reabsorption. *Am. J. Physiol.* **223**: 1201–1205.
6. McDonald, R. H., Jr., L. I. Goldberg, J. L. McNay, and E. P. Tuttle, Jr. 1964. Effects of dopamine in man: augmentation of sodium excretion, glomerular filtration rate, and renal plasma flow. *J. Clin. Invest.* **43**: 1116–1124.
7. Meyer, M. B., J. L. McNay, and L. I. Goldberg. 1967. Effects of dopamine on renal function and hemodynamics in the dog. *J. Pharmacol. Exp. Ther.* **156**: 186–192.
8. Davis, B. B., M. J. Walter, and H. V. Murdaugh. 1968. The mechanism of the increase in sodium excretion following dopamine infusion. *Proc. Soc. Exp. Biol. Med.* **129**: 210–213.
9. Yeh, B. K., J. L. McNay, and L. I. Goldberg. 1969. Attenuation of dopamine renal and mesenteric vasodilatation by haloperidol: evidence for a specific dopamine receptor. *J. Pharmacol. Exp. Ther.* **168**: 303–309.
10. Brotzu, G. 1970. Inhibition by chlorpromazine of the effects of dopamine on the dog kidney. *J. Pharm. Pharmacol.* **22**: 664–667.
11. Weil-Malherbe, H., and J. M. Van Buren. 1969. The excretion of dopamine and dopamine metabolites in Parkinson's disease and the effect of diet thereon. *J. Lab. Clin. Med.* **74**: 305–318.
12. Anton, A. H., and D. F. Sayre. 1972. Fluorometric assay of catecholamines, serotonin and their metabolites. *In* Methods in Investigative and Diagnostic Endocrinology, Part 2. S. A. Berson and I. J. Kopin, editors. North-Holland Publishing Co., Amsterdam. 398–436.
13. Gill, J. R., Jr., A. A. Carr, L. E. Fleischmann, A. G. T. Casper, and F. C. Bartter. 1967. Effects of pentolinium on sodium excretion in dogs with constriction of the vena cava. *Am. J. Physiol.* **212**: 191–196.
14. Horwitz, D., R. W. Alexander, W. Lovenberg, and H. R. Keiser. 1973. Human serum dopamine-β-hydroxylase. Relationship to hypertension and sympathetic activity. *Circ. Res.* **32**: 594–599.
15. Euler, U. S. von, and F. Lishajko. 1961. Improved technique for the fluorimetric estimation of catecholamines. *Acta Physiol. Scand.* **51**: 348–456.
16. Bonsnes, R. W., and H. H. Taussky. 1945. On the colorimetric determination of creatinine by the Jaffe reaction. *J. Biol. Chem.* **158**: 581–591.
17. Snedecor, G. W., and W. G. Cochran. 1967. The comparison of two samples. *In* Statistical Methods. The Iowa State University Press, Ames, Iowa. 6th edition. 91–119.
18. Frye, R. L., and E. Braunwald. 1960. Studies on Starling's law of the heart. I. The circulatory response to acute hypervolemia and its modification by ganglionic blockade. *J. Clin. Invest.* **39**: 1043–1050.
19. Gill, J. R., Jr. 1969. The role of the sympathetic nervous system in the regulation of sodium excretion by the kidney. *In* Frontiers in Neuroendocrinology. W. F. Ganong and L. Martini, editors. Oxford University Press, Inc., New York. **8**: 289–305.
20. Geffen, L. B., B. G. Livett, and R. A. Rush. 1969. Immunological chemical localization of chromogranins in sheep sympathetic nerves. *J. Physiol. (Lond.)*. **204**: 58P–59P.
21. Gerwitz, G. P., and I. J. Kopin. 1970. Release of dopamine-β-hydroxylase with norepinephrine during cat splenic nerve stimulation. *Nature (Lond.)*. **227**: 406–407.
22. Weishilboum, R. M., N. B. Thoa, D. G. Johnson, I. J. Kopin, and J. Axelrod. 1971. Proportional release of norepinephrine and dopamine-β-hydroxylase from sympathetic nerves. *Science (Wash. D. C.)*. **174**: 1349–1351.
23. Hoeldtke, R., B. S. Baliga, P. Issenberg, and R. J. Wurtman. 1972. Dihydroxyphenylalanine in rat food containing wheat and oats. *Science (Wash. D. C.)*. **175**: 761–762.
24. Imai, K., M. Wang, S. Yoshiue, and Z. Tamura. 1973. Determination of catecholamines in the plasma of patients with essential hypertension and of normal persons. *Clin. Chim. Acta.* **43**: 145–149.
25. Euler, U. S. von, I. Floding, and F. Lishajko. 1959. The presence of free and conjugated 3,4-dihydroxyphenylacetic acid (Dopac) in urine and blood plasma. *Acta Soc. Med. Ups.* **64**: 217–225.
26. Häggendal, J. 1963. The presence of conjugated adrenaline and noradrenaline in human blood plasma. *Acta Physiol. Scand.* **59**: 255–260.
27. Weil-Malherbe, H., and A. D. Bone. 1957. The fluorimetric estimation of adrenaline and noradrenaline in plasma. *Biochem. J.* **67**: 65–72.
28. Engelman, K., and B. Portnoy. 1970. A sensitive double-isotope derivative assay for norepinephrine and epinephrine: normal resting human plasma levels. *Circ. Res.* **26**: 53–57.
29. Goodall, McC., and H. Alton. 1968. Metabolism of 3-hydroxytyramine (dopamine) in human subjects. *Biochem. Pharmacol.* **17**: 905–914.
30. Rennick, B. R. 1968. Dopamine: renal tubular transport in the dog and plasma binding studies. *Am. J. Physiol.* **215**: 532–534.
31. Reinberg, A., J. Ghata, F. Halberg, P. Gervais, C. Abulker, J. Dupont, and C. Gaudeau. 1970. Rythmes circadiens du pouls, de la pression arteriélle, des excrétions urinaires en 17-hydroxycorticostéroïdes catécholamines et potassium chez l'homme adulte sain, actif et au repos. *Ann. Endocrinol.* **31**: 277–287.
32. Musacchio, J. M., M. Goldstein, B. Anagnoste, G. Poch, and I. J. Kopin. 1966. Inhibition of dopamine-β-hydroxylase by disulfiram *in vivo*. *J. Pharmacol. Exp. Ther.* **152**: 56–61.

33. Thoenen, H., W. Haefely, K. F. Gey, and A. Huerlimann. 1967. Quantitative aspects of the replacement of norepinephrine by dopamine as a sympathetic transmitter after inhibition of dopamine-β-hydroxylase by disulfiram. *J. Pharmacol. Exp. Ther.* **156**: 246–251.
34. Molinoff, P. B., S. Brimijoin, R. Weinshilboum, and J. Axelrod. 1970. Neurally mediated increase in dopamine-β-hydroxylase activity. *Proc. Natl. Acad. Sci. U. S. A.* **66**: 453–458.
35. Axelrod, J. 1972. Dopamine-β-hydroxylase: regulation of its synthesis and release from nerve terminals. *Pharmacol. Rev.* **24**: 233–243.
36. McNay, J. L., R. H. McDonald, Jr., and L. I. Goldberg. 1965. Direct renal vasodilatation produced by dopamine in the dog. *Circ. Res.* **16**: 510–517.
37. Martino, J. A., and L. E. Earley. 1967. Demonstration of a role of physical factors as determinants of the natriuretic response to volume expansion. *J. Clin. Invest.* **46**: 1963–1978.
38. Martino, J. A., and L. E. Earley. 1968. Relationship between intrarenal hydrostatic pressure and hemodynamically induced changes in sodium excretion. *Circ. Res.* **23**: 371–386.
39. Brenner, B. M., K. H. Falchuk, R. I. Keimowitz, and R. W. Berliner. 1969. The relationship between peritubular capillary protein concentration and fluid reabsorption by the renal proximal tubule. *J. Clin. Invest.* **48**: 1519–1531.
40. Kaloyanides, G. J., and M. Azer. 1971. Evidence for a humoral mechanism in volume expansion natriuresis. *J. Clin. Invest.* **50**: 1603–1612.
41. Sealey, J. E., J. D. Kirshman, and J. H. Laragh. 1969. Natriuretic activity in plasma and urine of salt-loaded man and sheep. *J. Clin. Invest.* **48**: 2210–2224.