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# PLASMA ANTIDIURETIC ACTIVITY AND FREE WATER CLEARANCE FOLLOWING OSMORECEPTOR AND NEUROHYPOPHYSEAL STIMULATION IN HUMAN SUBJECTS

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Since the description of antidiuretic hormone (1) and the factors which influence its release from the neurohypophysis (2), renal investigators have been interested in correlating physiological and clinical situations to the plasma levels of this hormone. However, the accurate measurement of antidiuretic activity in human plasma has proved difficult because of the lack of a sensitive method of bioassay and the extremely low concentrations of antidiuretic hormone (ADH) which are present in peripheral blood.

Further interest in measuring antidiuretic activity in human plasma was prompted by two factors: 1) the recent development of an improved method of bioassay of antidiuretic hormone in mammalian plasma (3, 4), and 2) an opportunity to obtain blood from the superior vena cava where dilution and inactivation of antidiuretic hormone were considered to be minimal.

The purpose of this investigation was to employ procedures calculated to stimulate the secretion of ADH in normal human subjects and to correlate plasma levels of antidiuretic activity with free water clearance. Three patients with diabetes insipidus were also studied.

## METHODS

The assay method for plasma antidiuretic activity has been previously described (4) and utilizes rats anesthetized with ethanol, under constant water load; a minor modification which has been introduced involves the injection into each assay animal of fixed amounts of standard vasopressin solutions, the antidiuretic response (instead of the dose of standard vasopressin solution administered) now being related to the rat's body weight.

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Plasma samples and standard solutions of aqueous vasopressin (Pitressin, Parke, Davis & Co., Lot no. 3-161-10W 107GA) were administered intravenously and in each instance the volume of plasma or standard injected was 0.7 ml; 0.1 ml of heparin saline solution (1:100) was used to wash in the test material. In all cases the human plasma was injected into the rat within 12 minutes after blood withdrawal. The same rats were used for measurement of plasma antidiuretic activity and standard vasopressin solutions except in rare instances in which an animal developed hematuria or oliguria.

The rats' urine was collected at 10-minute intervals and the degree of antidiuresis was expressed as an antidiuretic index per 100 g of rat body weight; i.e., the percentage change in urine specific gravity calculated from the average of two 10-minute samples preceding injection and the average of two 10-minute samples immediately following injection. The antidiuretic activity of the plasma samples was determined by relating the antidiuretic index obtained with plasma to the indices obtained with known concentration of vasopressin. The reported individual values of plasma antidiuretic activity are an average of the antidiuretic response obtained from all assay animals receiving an injection of the particular plasma sample.

It was recognized that measurement of the rats' free water clearance would offer a more accurate assessment of antidiuretic activity than would changes in urine density. However, such measurements were found to be impractical because of considerable lowering of plasma and urine freezing points produced by the alcohol used for anesthesia. Although the material responsible for the antidiuretic activity of human plasma is not conclusively known, the previous observations of Macfarlane and Robinson (5) suggest that it is possibly antidiuretic hormone. Using a similar bioassay procedure in rats, these workers compared the antidiuretic activity of human plasma with standard vasopressin solutions: parallel dose response curves and almost identical time flow curves were obtained, and it was also found that the antidiuretic activity of both the plasma and the vasopressin solutions could be inactivated by thioglycollic acid.

The 6 normal subjects were volunteer male medical students, and of the 3 patients, 2 had diabetes insipidus due to intracranial lesions and a third had renal diabetes insipidus resulting from gross bilateral hydro-

TABLE I  
*Renal response and plasma antidiuretic activity following osmoreceptor  
 and neurohypophyseal stimulation in normal subjects*

Subject Age Height Weight	Time	Urine		Plasma (sup. vena cava)		Clearances		
		Flow	Osmolality	Osmolality	Antidiuretic activity	C <sub>osm</sub>	C <sub>H<sub>2</sub>O</sub>	
		<i>ml/min</i>	<i>mOsm/kg</i>	<i>mOsm/kg</i>	<i>μU equiv. vasopressin/ml</i>	<i>ml/min</i>		
R.N. 23 yrs 177 cm 74 kg	-205-0	0.47	1,134	283	<30*	1.88	- 1.41	
	0-80	Hydration: 2.5 L H <sub>2</sub> O orally						
	80-105	11.2	49	284	<30†	1.93	9.27	
	125-145	18.3	50			3.21	15.09	
	145-148	Infuse 3 mg nicotine bitartrate, 1 mg/min						
	145-165	24.0	53	286	<30‡	4.45	19.55	
	165-185	19.0	51			3.39	15.61	
	205-225	15.5	50			2.71	12.79	
	264			288				
	265-275	Infuse 450 ml 20% mannitol, 45 ml/min						
	265-285	20.8	388	293	110‡	27.54	- 6.74	
	285-305	15.4	401			21.29	- 5.89	
305-325	11.5	500			19.96	- 8.46		
W.W. 24 yrs 185 cm 86 kg	-178-0	0.70	797	284	<30‡	1.96	- 1.26	
	0-40	Hydration: 2.0 L H <sub>2</sub> O orally						
	60-80	15.8	48	275	<30‡	2.76	13.04	
	110-113	Infuse 3 mg nicotine bitartrate, 1 mg/min						
	100-120	13.8	58	285	<30‡	2.81	10.99	
	120-160	3.0	310			3.26	- 0.26	
	160-180	12.3	100			4.32	7.98	
	180-200	17.8	51			3.19	14.61	
	R.R. 23 yrs 188 cm 82 kg	-185-0	0.40	1,319	288	<30‡	1.83	- 1.43
		0-70	Hydration: 2.5 L H <sub>2</sub> O orally					
80-100		9.2	76	278	<30‡	2.51	6.69	
140-160		10.2	60			2.20	8.00	
180-183		Infuse 3 mg nicotine bitartrate, 1 mg/min						
160-190		12.7	55	285	<30*	2.45	10.25	
190-205		4.6	201			3.24	1.36	
205-225		8.0	81			2.27	5.73	
225-245		13.3	57	286		2.65	10.65	
250-260		Infuse 350 ml 20% mannitol, 35 ml/min						
245-273		13.6	207	299	<30*	9.41	4.19	
273-297		8.4	415			11.74	- 3.34	
D.F. 23 yrs 178 cm 76 kg	-120-0	0.43	949	287	<30‡	1.42	- 0.99	
	0-45	Hydration: 2.0 L H <sub>2</sub> O orally						
	60-80	11.9	62	275	<30‡	2.68	9.22	
	100-120	12.0	61			2.66	9.34	
	132-135	Infuse 3 mg nicotine bitartrate, 1 mg/min						
	120-140	11.5	64	286	>200*	2.57	8.93	
	140-160	1.2	533			2.24	- 1.04	
	160-180	5.5	173			3.33	2.17	
	180-200	14.2	67			3.33	10.87	
	249			287	<30‡			
	250-262	Infuse 240 ml 20% mannitol, 20 ml/min						
	240-260	18.5	103			6.44	12.06	
	260-280	6.9	447	296	<30*	10.42	- 3.52	
	280-300	5.0	525			8.99	- 3.99	

\* Average of response in three rats.

† Average of response in four rats.

‡ Average of response in two rats.

§ Subject unable to void between first morning urine specimen (-210 min) and 100 minutes after oral administration of water; this second urine sample (320 ml) was discarded.

TABLE I—(Continued)

Subject Age Height Weight	Time	Urine		Plasma (sup. vena cava)		Clearances			
		Flow	Osmolality	Osmolality	Antidiuretic activity	C <sub>osm</sub>	C <sub>H<sub>2</sub>O</sub>		
		ml/min	mOsm/kg	mOsm/kg	μU equin. vasopressin/ml	ml/min			
D.T. 22 yrs 170 cm 68 kg	-210	Fluid deprivation, 14 hrs 1,040							
	0	§		287	40.0*				
	0-60	Hydration: 3.5 L H <sub>2</sub> O orally							
	90			277	<30‡				
	100-120	18.3	117			7.73	10.57		
	120-140	14.3	52			2.69	11.61		
	143-146	Infuse 3 mg nicotine bitartrate, 1 mg/min							
	140-160	15.7	49	282	>200*	2.73	12.97		
	160-180	14.5	53			2.72	11.78		
	200-220	16.6	76			4.43	12.17		
	240-260	22.6	73			5.77	16.83		
	265			286					
	266-276	Infuse 400 ml 20% mannitol, 40 ml/min							
	260-280	27.1	149			13.60	13.50		
	280-300	19.2	233	297	<30*	15.06	4.14		
300-320	24.7	166			14.14	10.56			
G.H. 24 yrs 175 cm 67 kg	-195-0	0.46	1,115	288	<30‡	1.78	- 1.32		
	0-70	Hydration: 3.0 L H <sub>2</sub> O orally							
	80-100	10.5	87	275	<30*	3.32	7.18		
	140-160	12.7	62			2.86	9.84		
	167-170	Infuse 3 mg nicotine bitartrate, 1 mg/min							
	173-175	Infuse 2 mg nicotine bitartrate, 1 mg/min							
	160-180	16.7	60			3.58	13.12		
	180-200	17.8	64	282	<30*	4.04	13.76		
	220-240	20.9	61	286		4.46	16.44		
	250-270	Infuse 250 ml 20% mannitol, 12.5 ml/min							
	240-265	18.1	110			6.73	11.37		
	265-280	10.4	445	296	<30*	15.64	- 5.24		
	280-300	6.2	434			9.28	- 3.08		

nephrosis. The superior vena cava (SVC) was catheterized using a no. 6 Courmand cardiac catheter, the position of the catheter tip being checked fluoroscopically. Because of the possibility of anomalous venous drainage of the posterior pituitary gland, it was decided to bypass the internal jugular veins for the certainty of obtaining blood from the neurohypophysis. Blood samples for the determination of plasma osmolality and antidiuretic activity were taken from the SVC. Throughout each experiment urine collections were obtained at frequent intervals (usually 20 minutes) by requesting the subjects to void normally. In the patients with diabetes insipidus urine samples were obtained by means of an indwelling bladder catheter.

Plasma and urine osmolality were determined using a Fiske osmometer. Osmolal clearance and free water clearance were calculated as follows. Osmolal clearance in milliliters per minute, C<sub>osm</sub> = U<sub>osm</sub>V/P<sub>osm</sub> (U<sub>osm</sub> = urine osmolality, mOsm per kg; P<sub>osm</sub> = plasma osmolality, mOsm per kg; V = urine flow, milliliters per minute); free water clearance in milliliters per minute, C<sub>H<sub>2</sub>O</sub> = V - C<sub>osm</sub>.

The subjects and patients refrained from food and liquid

for at least 12 hours prior to reporting for the experiment. The degree of dehydration was assessed by determining the loss in body weight from the evening before to the morning of the experiment.

The first blood sample was taken after a period of 13 to 14 hours' dehydration; following this the subject or patient was encouraged to drink from 2 to 3 L of water and the second blood sample was withdrawn during water diuresis. After the initial water loading and until the end of the experiment, a constant degree of hydration was maintained by the oral administration of water or orange juice in quantities equivalent to the urine volume.

Approximately 1 hour following the removal of the second blood sample, nicotine bitartrate (3 to 5 mg) was infused into a peripheral vein and 5 to 10 minutes later the third blood sample was obtained. The last blood sample for bioassay was withdrawn 5 to 10 minutes after 240 to 450 ml of 20 per cent mannitol had been infused into a peripheral vein.

In the two patients with diabetes insipidus due to intracranial lesions, an intravenous infusion of vasopressin (50 mU per hour) was given at the end of the investigation.

## RESULTS

*Normal subjects.* The results are listed in Table I. The weight lost during the 13 to 14 hour dehydration period varied from 1 to 1.5 kg. In five of the six subjects the plasma levels of antidiuretic activity in the dehydrated state were too low to demonstrate any difference from those in the hydrated state. At the end of the dehydration period, the free water clearance had a negative value ( $-0.99$  to  $-1.41$  ml per minute), while clearances of up to 13 ml per minute were recorded at the time of blood withdrawal in the hydrated state. In one subject (D.T.) in whom a measure of free water clearance was not obtained during dehydration, the ratio of urine to plasma osmolality was 3.62. This latter subject, who was unable to void at the end of the dehydration period, had the greatest weight loss during fluid deprivation, and was the only subject in whom plasma antidiuretic activity could be demonstrated ( $40 \mu\text{U}$  equivalents per ml) during the dehydrated state.

The response to the intravenous infusion of nicotine was variable. Two of the six subjects (R.N. and G. H.) had no side effects after nicotine bitartrate (3 to 5 mg) and there was no antidiuretic response nor detectable increase in plasma antidiuretic activity. W.W. and R.R. both experienced side effects following 3 mg of nicotine bitartrate, consisting of paresthesia, slight nausea and dizziness and exhibited a marked decrease in urine flow and free water clearance; however, a significant change in plasma antidiuretic activity could not be detected in either subject. D.F., who also experienced paresthesia and dizziness, had an antidiuresis associated with a large increase in plasma antidiuretic activity. A similar increase in plasma activity occurring in D.T. was associated with similar side effects but there was only a minimal reduction in urine flow and free water clearance.

Following the infusion of 20 per cent mannitol a marked decrease in free water clearance was observed in all subjects, but in only one (R.N.) was there a demonstrable increase in plasma antidiuretic activity.

*Patients with diabetes insipidus.* The results are shown in Table II. The weight lost during the 12 to 13.5 hours of dehydration was consider-

ably greater than in the normal subjects, being 2.3 kg in M.J., 2.1 kg in W.K. and 4.6 kg in J.N. The patient with renal diabetes insipidus (W.K.) had high levels of plasma antidiuretic activity which disappeared following hydration, whereas in the two patients with disease of the pituitary-hypothalamic type (M.J. and J.N.), values of less than  $30 \mu\text{U}$  equivalents of vasopressin per ml plasma were obtained in both the dehydrated and hydrated states. After 12 hours' dehydration in M.J., the urine osmolality rose slightly above that of the plasma, giving a free water clearance of  $-0.14$  ml per minute. Simultaneous creatinine clearances were measured in this case; these showed no significant difference between the periods of dehydration and hydration, being 85 and 82 ml per minute, respectively (the cause of the slight impairment of renal function was not ascertained). The administration of nicotine in M.J. caused mild side effects and, although followed by a demonstrable increase in plasma antidiuretic activity, there was no significant decrease in free water clearance. Vasopressin administration in M.J. and J.N. produced negative values for free water clearance; this did not occur in the patient with renal diabetes insipidus (further details of this case including the results of vasopressin infusions will be published in a separate report).

## DISCUSSION

The very low levels of plasma antidiuretic activity in normal subjects at the end of a 13 to 14 hour dehydration period agree well with the values previously reported by Bisset (6) and Heller (7) on internal jugular venous blood from human subjects. In nine patients, at surgical operation, Bisset recorded a detectable antidiuretic activity in only two cases, the values being 12 and  $20 \mu\text{U}$  per ml blood, while in three subjects (not under general anesthesia), Heller obtained values of less than  $20 \mu\text{U}$  per ml in two and less than  $11 \mu\text{U}$  per ml in the third subject.

There remains little doubt that levels of plasma antidiuretic activity of less than  $30 \mu\text{U}$  equivalents of vasopressin per ml are capable of producing the degree of antidiuresis obtained in our dehydrated subjects. Theobald (8) has demonstrated that a single intravenous injection of 5 to 10 mU of vasopressin will produce almost maximal antidiu-

TABLE II

*Renal response and plasma antidiuretic activity following osmoreceptor and neurohypophyseal stimulation in patients with diabetes insipidus*

Patient Diagnosis Age, Sex Height Weight	Time	Urine		Plasma (sup. vena cava)		Clearances	
		Flow	Osmolality	Osmolality	Antidiuretic activity	$C_{osm}$	$C_{H_2O}$
		ml/min	mOsm/kg	mOsm/kg	$\mu U$ equiv. vasopressin/ml	ml/min	
M.J. Diabetes insipidus following hemorrhage from eosinophilic adenoma of pituitary. 42 yrs, ♀ 160 cm 63 kg	-30-0	1.7	339	313	<30*	1.84	- 0.14
	0-75	Hydration: 2.5 L H <sub>2</sub> O orally					
	75-90	4.9	170			2.80	2.10
	90-105	4.7	160	295	<30†	2.55	2.15
	105-120	5.2	164			2.90	2.30
	161-164	Infuse 3 mg nicotine bitartrate, 1 mg/min					
	165-180	5.7	166	292	41.0†	3.24	2.46
	180-195	5.5	173			3.26	2.24
	210-225	5.1	185			3.23	1.87
	225-285	Infuse vasopressin 50 mU/hr					
	225-240	2.8	193			1.86	0.94
	240-255	2.5	300			2.58	- 0.08
	255-270	1.3	420	291		1.88	- 0.58
	270-285	2.0	513			3.53	- 1.53
W.K. Renal diabetes insipidus due to hydro- nephrosis 29 yrs, ♂ 161 cm 55 kg	-25-0	2.5	134	301	>200†	1.11	1.39
	0-75	Hydration: 2.0 L H <sub>2</sub> O orally					
	75-90	4.7	122			1.96	2.74
	90-105	5.7	110	291	<30†	2.14	3.56
	105-120	5.3	110			2.00	3.30
J.N. Diabetes insipidus following head injury 16 yrs, ♂ 183 cm 99 kg	-160-0	2.7	236	298	<30*	2.14	0.56
	0-95	Hydration: 3.5 L H <sub>2</sub> O orally					
	100-120	14.3	58	294	<30*	2.82	11.48
	160-180	11.8	58			2.37	9.43
	215			288			
	236-239	Infuse 3 mg nicotine bitartrate, 1 mg/min					
	220-240	12.0	59			2.48	9.52
	240-265	11.1	62	281	<30*	2.45	8.65
	265-290	10.6	63			2.38	8.22
	295-305	Infuse 400 ml 20% mannitol, 40 ml/min					
	305-380	Infuse 450 ml 20% mannitol, 6 ml/min					
	290-320	28.8	165	302	<30‡	15.74	13.06
	320-340	31.5	200			20.93	10.57
	340-360	31.5	205	300		21.52	9.98
	360-380	29.5	209			20.55	8.95
380-440	Infuse vasopressin 50 mU/hr and 180 ml 20% mannitol, 3 ml/min						
380-400	25.0	242			20.37	4.63	
400-420	17.0	291	295		16.77	0.23	
420-440	16.2	296			16.31	- 0.11	

\* Average response in three rats.

† Average response in two rats.

‡ Average response in five rats.

resis in man; assuming a distribution of the hormone which is limited to the plasma volume, such doses would result in plasma concentrations of approximately 2 to 4  $\mu$ U per ml. Further work in human subjects (9-11) has shown that maximal antidiuresis can be achieved by the intravenous administration at rates of 50 mU or less per hour.

The individual variation encountered after nicotine administration in human subjects is well documented (12-14) and receives further confirmation from the results of this study. R.N. (non-smoker) and G.H. (smoker) had no side effects, decrease in  $C_{H_2O}$  or increase in plasma antidiuretic levels following nicotine infusion. This lack of response is consistent with the view that these two subjects were insensitive to the dose of nicotine administered, and no appreciable quantities of ADH were liberated from the neurohypophysis. Both W.W. and R.R. had significant side effects and an antidiuretic response, but there was no detectable increase in plasma antidiuretic activity. While there is little doubt that ADH release was stimulated in both of these subjects, the concentrations in the superior vena caval blood at the time of sampling were too low to permit detection. D.F. showed a marked increase in plasma antidiuretic activity which was associated with a renal response. The other normal subject showing a well marked increase in plasma antidiuretic activity (D.T.) experienced paresthesia and lightheadedness but there was only a minimal reduction in free water clearance. A similar anomalous result following nicotine occurred in one of the patients with diabetes insipidus (M.J.). In these two instances the possibility that the plasma produced antidiuresis in the rats by means of a non-ADH mechanism cannot be entirely excluded since glomerular filtration and solute excretion rates were not measured in these animals. The possibility that the nicotine infused into the human subject had any direct effect upon the liberation of ADH in the rats used for assay can be excluded with reasonable certainty. Burn, Truelove and Burn (13) have demonstrated that extremely large doses of nicotine must be injected into a rat before any antidiuretic effect is noted. This dose would have to be at least 1,000 times greater than the amount of nicotine the rats actually received in our assays.

The intravenous infusion of 20 per cent man-

nitol was associated in each normal subject with a rise in plasma osmolality and a pronounced decrease in free water clearance. The latter finding was attributed to ADH release from osmoreceptor stimulation and did not occur in J.N., the patient with diabetes insipidus, in whom a mannitol infusion was performed. Of the two subjects (R.N. and G.H.) showing the greatest reduction in  $C_{H_2O}$ , only R.N. had a detectable increase in plasma antidiuretic activity. However, due to infusion difficulties in G.H., the mannitol was delivered over a 20 minute period instead of the usual 10 minutes. This slow rate of mannitol infusion may have initiated a slow continuous stimulation of the osmoreceptors and thus a steady but small release of ADH which was not detectable by the assay method. In fact, this type of slow continuous release may indeed be the reason for our inability to detect any appreciable change in plasma antidiuretic levels following the mannitol infusion in any of the other subjects. The reports of Theobald (8) and others (9-11) demonstrate that very small quantities of vasopressin which could not be measured by our assay method may produce the degree of renal response noted.

The bioassay method used in this investigation for the measurement of plasma antidiuretic activity has several advantages over those previously described in that it utilizes animals with their endogenous ADH secretion inhibited by ethanol, a constant water load, intravenous injections of plasma samples into rats within 12 minutes of blood withdrawal from the superior vena cava, and the very sensitive criterion of potency of change in urine specific gravity. However, it is evident from the results obtained that small amounts of antidiuretic hormone (less than 30  $\mu$ U equivalents of vasopressin per ml of plasma), which are sufficient to produce a marked renal response, cannot be readily detected. We consider that further progress in the measurement of antidiuretic hormone in plasma is now dependent upon the development of a satisfactory method of concentrating the hormone before injection into the assay animal.

#### SUMMARY

Plasma antidiuretic activity has been measured in six healthy male subjects, using an improved rat bioassay method which included intravenous in-

jections and change in urine specific gravity as the sensitive criterion of potency. Studies were also made in three patients with diabetes insipidus, one of whom had nephrogenic disease. Blood for assay was withdrawn from the superior vena cava to minimize dilution and inactivation of antidiuretic hormone. Observations were made during dehydration and water diuresis and following intravenous infusions of nicotine and mannitol. The plasma levels of antidiuretic activity were compared with changes in free water clearance.

In five of the six normal subjects the plasma levels of antidiuretic activity in the dehydrated state were too low ( $30 \mu\text{U}$  equivalents of vasopressin per ml) to allow differentiation from those obtained after water loading. One patient with renal diabetes insipidus was shown to have high levels of plasma antidiuretic activity which disappeared following hydration, whereas two other patients, with intracranial lesions, had less than  $30 \mu\text{U}$  equivalents of vasopressin per ml of plasma in both the dehydrated and hydrated states.

The response to nicotine was extremely variable and ranged from high levels of antidiuretic activity and negative values of free water clearance to no detectable response in either of these parameters. The infusion of mannitol in the normal subjects resulted in a pronounced decrease in free water clearance, but in only one instance was there a demonstrable increase in plasma antidiuretic activity.

Despite the use of optimal methods, employing a sensitive bioassay technique in conjunction with blood sampling from the superior vena cava, the concentrations of antidiuretic hormone in plasma are too low in the majority of physiological and clinical situations to be accurately measured. It is suggested that further progress in the measurement of antidiuretic hormone in human plasma lies in the development of a satisfactory method of concentrating the hormone before injection into the assay animal.

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