NATIONAL AERONAUTICS AND SPACE ADMINISTRATION

PROGRESS REPORT

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Title: The Effect of Changing Gravity and Weightlessness of Vasopressin Control Systems

A. TUMOR IMMUNOASSAY

Seven extracts were sent to Dr. Myron Miller's laboratory in Syracuse for immunoassay. These extracts came from three patients known to have the inappropriate ADH syndrome secondary to pulmonary oat cell carcinoma. These extracts had previously been subject to bioassay in the rat and we now wished to see if their vasopressin content could be confirmed immunologically. The following results were obtained from the immunoassay.

TABLE I

IMMUNOASSAY OF SELECTED EXTRACTS FROM PATIENTS WITH INAPPROPRIATE ADH SYNDROME

#		ADH (MU/ML)
3100	POOLED PLASMA - GM	1.4
3101	" " - CN	0.98
3102	CHROMATOGRAPHIC PEAK, POOLED PLASMA - GM	0.11
3103	" " , " " - WC	0.48
3104	" " , LIVER METASTASES - CN	GT 10
3105	" " , LUNG TUMOR - CN	GT 10
3106	" " , LUNG TUMOR - CN	0.72

GT = GREATER THAN

These results compaired favorably with the values obtained by biological assay. It appears that the active agent in these cases has the chromatographic mobility of arginine vasopressin and also its biologic and immunochemical properties and therefore is probably identical to arginine vasopressin.

B. DEVELOPMENT OF ANTIBODIES TO OXYTOCIN AND VASOPRESSIN

Four rabbits are being immunized with "coupled" albumin-oxytocin complex mixed in Freunds adjuvant. Six rabbits are just beginning a course of similar treatment using an albumin-arginine vasopressin complex. Serum is being collected at monthly intervals and will be checked for antibody development shortly.

C. URINE VASOPRESSIN EXCRETION

Since 1962, aliquots of urine have been collected, frozen and stored in our laboratory from patients undergoing studies of their plasma ADH levels. Recently, a program of determining the ADH content of the samples was undertaken to ascertain the range of urinary ADH levels that might be encountered in pathological states and to see if any relationship exists between urinary ADH excretion and plasma ADH levels. There were no normal subjects in this group.

The urinary assay of ADH consisted of a modification of the plasma ADH bioassay precedure currently used in this laboratory. The urine samples which had been frozen without the addition of any preservatives were rapidly thawed and extracted with 2 volumes of ether. The washed urine was then adjusted to a pH of 4.2 and added to a column of CG-50 resin. From this point on the precedure was identical to the plasma bioassay. The extracts of urine produced antidiuretic responses that were similar to those produced by the arginine vasopressin reference standards. The high levels in some of these samples indicated that very little, if any, ADH had been lost during prolonged storage.

At present, 90 urine samples have been assayed, 57 of which had plasma ADH levels so timed that ADH clearances could be calculated.

Five patients (table II) might be classified as having diabetes insipidus (DI). DI-1 and DI-2 had pulmonary carcinoma with metastases to the brain. DI-3 had carcinoma of the thyroid with cerebral metastises as a cause of her DI. DI-4 was a boy with DI and DI-5 was a diabetic with severe retinopathy who was undergoing a pituitary stalk section. The urinary ADH levels were very low in this group ranging from not detectable to 9.4 μU/ml. Plasma samples were infrequently taken because of the difficulty in detecting ADH levels in DI. The high plasma ADH level in DI-2 occurred following a dose of vasopressin and the high plasma levels in DI-5 occurred during the surgical procedure. The mean urinary ADH excretion rate in DI without an open loop stimulus was $9.93 \pm .1$ ($N = 12$) μU/min. The mean urinary ADH excretion when injected pitressin had achieved a clinically satisfactory response (DI-2) was $11.6 \pm .9$ ($N = 7$) μU/min.

Two patients had samples taken while they were undergoing surgical procedures (table III). SURG-1 had a partial gastrectomy. SURG-2 had a partial colectomy. Their mean urinary ADH excretions were 339 ± 58 ($N = 6$) and 241 ± 29 ($N = 6$) μU/min., respectively.

Four patients had the Inappropriate Antidiuretic Hormone Secretory Syndrome (table IV). IADHS-1 had severe pulmonary emphysema as a cause of his inappropriately high ADH levels. While his urinary excretion rate (8.6 ± 3 ($N = 4$) μU/min.) and plasma levels ($2.6 \pm .4$ ($N = 4$ μU/ml.) might be considered to be within the normal range, they were difinitely too high for his plasma osmolality of 240 mOsm/Kg. and were unresponsive

to water loading. IADHS-2, IADHS-3, and IADHS-4 had oat cell pulmonary carcinomas. Their urinary ADH excretion rates were quite high (411, 50.1, and 224 μU/min.) and were unresponsive to water loading.

The average values for urinary ADH excretion, plasma ADH concentration and the renal clearance of ADH are listed in Table V. The ADH clearance data of the DI patients was insufficient to warrant any conclusion. The ADH clearance values of all of the other patients, except IADHS-2 varied between 3.7 and 11.5 ml/min. but were not really significantly different. The 43.5 ml/min. clearance of IADHS-2 cannot be explained at present.

Careful examination of the data in tables II, III and IV revealed that small changes in urinary flow in patients with low urinary outputs were associated with small changes in urinary ADH excretion that were directly proportional and were probably related to small changes in renal clearance. If the urinary output was high and not completely of osmotic nature the urinary flow was inversely proportional to ADH excretion (DI-2 and DI-5).

A plot of all 57 urinary ADH excretion rate - plasma ADH concentration value pairs (figure 1) demonstrated a fairly linear relationship between them. Five of the points from SURG-1 fell quite far above the line and represented a period when both renal and hepatic clearance could have been reduced because of events surrounding the surgical procedure. The other point of this patient fell on this line and represented the highest urinary ADH excretion rate observed (616 μU/min.). The points from IADHS-2 fell below this line as mentioned earlier.

In general, the urinary ADH excretion rates followed plasma ADH concentrations giving an average renal clearance of ADH of about 10 ml/min.

TABLE II - DIABETES INSIPIDUS

	URINE			PLASMA		
DURATION (MIN)	RATE (ML/MIN) V	[ADH] (UU/ML) U	ADH (UU/MIN) UV	[ADH] (UU/ML) P	CADH (ML/MIN) UV/P
DI-1						
720	0.67	1.4	1.0	0.0	----
720	1.39	1.2	1.6	----	----
DI-2						
60	18.92	0.6	11.0	----	----
60	10.83	0.3	3.2	6.3	0.5
60	6.33	1.1	7.1	----	----
60	4.25	3.3	14.2	----	----
60	3.00	4.6	13.6	----	----
60	2.67	4.0	10.6	----	----
60	2.33	5.5	12.8	----	----
60	1.65	7.3	12.0	----	----
DI-3						
60	0.93	0.6	0.6	----	----
15	1.53	0.3	0.5	----	----
15	1.40	0.3	0.3	0.7	0.5
15	2.67	N.D.		----	----
15	4.33	N.D.		----	----
15	4.80	N.D.		----	----
15	4.80	N.D.		----	----
15	5.40	N.D.		----	----
15	4.67	N.D.		----	----
DI-4						
60	1.50	1.4	2.1	----	----
60	1.80	0.3	0.6	----	----
60	1.08	0.7	0.8	----	----
60	0.50	1.4	0.7	----	----
60	0.83	1.2	1.0	----	----
60	1.25	0.9	1.1	----	----
60	1.00	0.8	0.8	----	----
DI-5						
240	0.73	1.9	1.4	12.8	0.1
120	0.82	9.4	7.7	6.8	1.1
120	0.53	7.5	4.0	----	----
120	0.53	3.8	2.0	3.1	0.7
360	0.90	5.3	4.8	2.7	1.8
360	3.32	0.8	2.8	----	----

* N.D. = NOT DETECTABLE

TABLE III - SURGICAL PROCEDURE

	URINE				PLASMA	
DURATION (MIN)	RATE (ML/MIN) V	[ADH] (UU/ML) U	ADH (UU/MIN) UV	[ADH] (UU/ML) P	CADH (ML/MIN) UV/P	
SURG-1						
30	1.30	474.	616.	41.2	15.0	
30	2.47	118.	291.	44.2	6.6	
30	4.17	81.3	339.	41.0	8.3	
30	1.90	117.	222.	54.2	4.1	
30	1.83	135.	248.	54.5	4.5	
30	1.30	243.	315.	45.7	6.9	
SURG-2						
30	1.83	149.	273.	21.8	12.5	
30	5.03	59.0	297.	24.7	12.0	
30	8.63	29.1	251.	17.5	14.4	
30	7.83	29.2	229.	23.7	9.7	
30	6.23	47.0	293.	20.8	14.1	
30	----	-----	-----	----	----	
30	1.12	96.9	108.	16.7	6.5	

TABLE IV - INAPPROPRIATE ADH SYNDROME

	URINE				PLASMA	
DURATION (MIN)	RATE (ML/MIN) V	[ADH] (UU/ML) U	ADH (UU/MIN) UV	[ADH] (UU/ML) P	CADH (ML/MIN) UV/P
IADHS-1						
1440	0.44	17.5	7.6	1.5	5.0
1440	0.29	48.9	14.3	2.4	6.0
1440	0.34	33.3	11.3	3.6	3.2
1440	0.10	12.5	1.3	2.7	0.5
IADHS-2						
720	0.93	359.	334.	11.7	28.5
720	0.85	340.	288.	9.7	29.7
720	1.21	445.	537.	9.1	58.9
720	1.46	341.	497.	13.8	36.1
720	1.85	283.	522.	8.5	61.8
720	1.95	245.	475.	----	----
720	1.31	339.	442.	9.9	44.7
720	1.33	342.	455.	----	----
720	0.95	295.	278.	6.3	44.8
720	0.93	307.	285.	----	----
IADHS-3						
720	0.57	73.5	41.8	4.7	8.9
720	1.04	58.4	60.4	5.8	10.5
720	0.74	71.0	52.3	5.5	9.6
720	1.00	45.4	45.4	6.5	7.0
720	0.61	70.2	42.9	5.9	7.3
720	0.88	46.7	41.2	4.2	9.8
720	0.73	63.6	46.4	5.0	9.3
720	1.18	44.5	52.5	5.9	8.9
720	0.46	56.4	25.8	4.7	5.5
720	1.15	54.6	62.9	5.1	12.3
720	0.57	58.4	33.2	6.3	5.3
720	1.13	47.7	53.6	5.3	10.2
720	1.29	39.7	51.2	6.9	7.4
720	1.25	43.6	54.4	8.7	6.3
720	2.08	30.3	63.0	8.3	7.6
720	1.46	51.0	74.4	9.7	7.7
720	0.82	68.6	56.2	6.5	8.7
720	0.96	64.3	61.6	6.5	9.5
720	0.46	104.	47.6	7.0	6.8
720	0.54	64.3	34.8	----	----

TABLE IV - INAPPROPRIATE ADH SYNDROME (CONT.)

	URINE				PLASMA	
DURATION (MIN)	RATE (ML/MIN) V	[ADH] (UU/ML) U	ADH (UU/MIN) UV	[ADH] (UU/ML) P	CADH (ML/MIN) UV/P	
ADHS-4						
720	0.80	230.	184.	13.7	13.4	
720	1.29	177.	228.	16.1	14.2	
720	1.04	181.	189.	20.1	9.4	
720	1.53	174.	265.	17.3	15.4	
720	2.64	125.	329.	18.7	17.7	
720	3.11	116.	361.	----	----	
720	0.78	231.	180.	21.0	8.6	
720	1.10	226.	248.	----	----	
720	0.61	198.	119.	21.1	5.7	
720	0.83	257.	214.	----	----	
720	0.60	244.	146.	21.2	6.9	
720	0.78	283.	220.	----	----	

TABLE V - AVERAGES FROM TABLES I, II, III, AND IV

NAME	URINE ADH (UU/MIN) UV	PLASMA ADH (UU/ML) P	ADH CLEARANCE (ML/MIN) UV/P
DI-1	1.3 + .3(N=2)	0.0	----
DI-2	12.6 + .63(N=5)	6.3	0.5
DI-3	0.5 + .1(N=3)	0.7	0.5
DI-4	1.0 + .2(N=7)	----	----
DI-5	3.8 + .9(N=6)	6.4 + 2.(N=4)	0.9 + .6(N=4)
SURG-1	339. + 58(N=6)	46.8 + 2.5(N=6)	7.6 + 1.6(N=6)
SURG-2	241. + 29(N=6)	20.9 + 1.3(N=6)	11.5 + 1.2(N=6)
IADHS-1	8.6 + 3.(N=4)	2.6 + .4(N=4)	3.7 + 1.2(N=4)
IADHS-2	411. + 33(N=10)	9.9 + .9(N=7)	43.5 + 5.0(N=7)
IADHS-3	50.1 + 2.6(N=20)	6.2 + .3(N=19)	8.4 + .4(N=19)
IADHS-4	224. + 20(N=12)	18.7 + .97(N=8)	11.4 + 1.5(N=8)

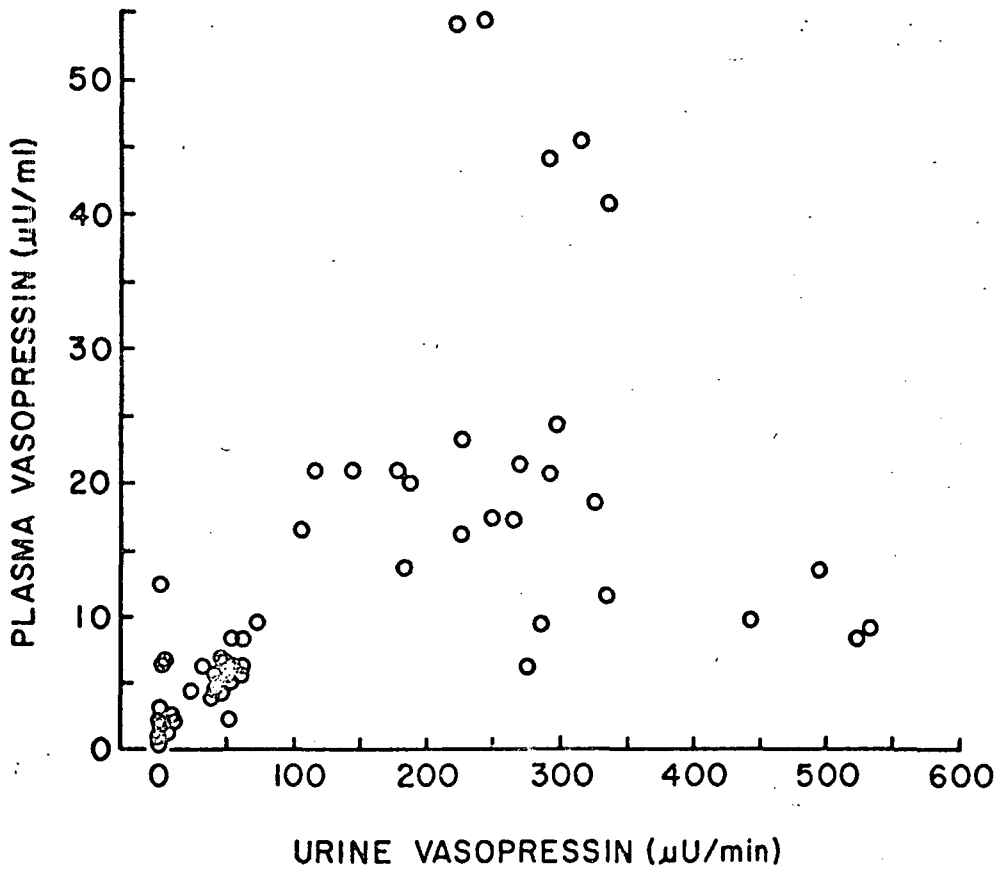
PLASMA VASOPRESSIN (μU/ml)
0
10
20
30
40
50
0
100
200
300
400
500
600
URINE VASOPRESSIN (μU/min)

figure 1

D. PUBLICATIONS:

1. Moran, W. H., Jr.: CPPB and Vasopressin Secretion, Editorial Views, J. of Anesthesiology, 34:501-504 (1971).

EXPERIMENTS SUPPORTED IN PART BY NGR-001-019
THROUGH 10/20/71

E 19	ADH STABILITY I, EFFECT OF TEMPERATURE
E 20	EFFECT OF BLEED ON ADH AND CRF
E 21	ADH STABILITY II, REPEATED FREEZE-THAW PLASMA
E 22	ADH STABILITY III, REPEATED FREEZE -THAW STANDARD SOLUTION
E 23	ADH STABILITY IV, EFFECT OF TEMPERATURE
E 31	ANTIDIURETIC ACTIVITY OF ANGIOTENSIN
E 32	ANGIOTENSIN RECOVERY ON CG-50 RESIN COLUMNS
E 33	ADH STABILITY V, NA OXALATE AND EDTA AS INHIBITORS
E 34	ADH RECOVERY I, NO ETHER WASH
E 39	ADH STABILITY VI, NA OXALATE AND EDTA AS INHIBITORS
E 40	CRF ISOLATION I, TO AMES
E 41	CRF ISOLATION II, TO AMES
E 42	ADH RECOVERY II, NO ETHER WASH
E 43	ADH RECOVERY III, PH 4.0 RESIN COLUMN
E 44	ADH RECOVERY IV, EE VS NEE
E 45	ADH RECOVERY V, EE VS NEE
E 46	ADH RECOVERY VI, EE VS NEE
E 47	CRF ISOLATION III, TO AMES
E 49	VEITNAM SAMPLES, CRF TO AMES
E 50	ADH ISOLATION I, DIURETIC PEAK
E 53	ADH ISOLATION II, NAOX AND EDTA ON DIURETIC PEAK
E 54	ADH RECOVERY VII, PLASMA
E 55	ADH ISOLATION III, NA ION IN EXTRACT
E 57	ADH ISOLATION IV, THIOGLYCOLATE CORD BLOOD
E 58	ADH ISOLATION V, THIOGLYCOLATE CORD BLOOD
E 61	ADH ISLOATION VI, PITRESSIN VS. USP POST PIT REF IN RAT
E 63	ADH STABILITY VII, STORAGE OF DILUTED EXTRACT INJECT SOL
E 64	ADH CHROMATOGRAPHY I, PITRESSIN
E 65	ADH CHROMATOGRAPHY II, ELUTION FROM PAPER
E 66	ADH CHROMATOGRAPHY III, USP PIT REF
E 67	ADH CHROMATOGRAPHY IV, RESIN + PAPER WITH USP REF
E 68	ANGIOTENSIN CHROMATOGRAPHY I,
E 69	OXYTOCIN CHROMATOGRAPHY I,
E 70	ADH CHROMATOGRAPHY VIII, PLASMA-LUNG CA POOL: GM
E 71	CRF ISOLATION IV, TO AMES
E 72	ADH CHROMATOGRAPHY V, CORD BLOOD
E 73	ADH RECOVERY VIII, RESIN PLUS PAPER CHROMATOGRAM
E 74	ADH CHROMATOGRAPHY VI, USP REF STD - DIST COEF
E 75	ADH CHROMATOGRAPHY VII, CG-50 ION EXCHANGE COL
D 7000	BIOLOGICAL HALF-LIFE OF ADH I, DOG
D 7001	BIOLOGICAL HALF-LIFE OF ADH II, DOG
D 8000	EFFECT OF TILT ON ADH SECRETION
E 76	ADH CHROMATOGRAPHY IX, PLASMA-LUNG CA POOL: CN
E 77	ADH CHROMATOGRAPHY X, PLASMA-LUNG CA POOL: WC
E 78	ADH CHROMATOGRAPHY XI, TISSUE-NORMAL LUNG: CN

EXPERIMENTS SUPPORTED IN PART BY NGR-001-019
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E 79 ADH CHROMATOGRAPHY XII, TISSUE-LUNG CA: CN
E 80 ADH CHROMATOGRAPHY XIII, TISSUE-LIVER METAST.: CN
H191933 ADH CHROMATOGRAPHY XIV, PLASMA-HEAD TRAUMA: FJ
E 81 ADH RESPONSE I, URATE, CHOH, COSM VS. DOSE
E 82 APOLLO 9 PLASMA SAMPLES
E 83 ADH IMMUNIOASSAY-BIOASSAY COMPARISON I
E 84 ADH IMMUNIOASSAY-BIOASSAY COMPARISON II
D 7002 BIOLOGICAL HALF-LIFE OF ADH III, DOG
E 85 ADH IMMUNIOASSAY-BIOASSAY COMPARISON III
E 86 SHOCK PLASMA ULTRAFILTRATE I
D 7003 BIOLOGICAL HALF-LIFE OF ADH IV, DOG
E 87 ADH PLASMA, DIABETES INSIPIDUS
E 88 ADH IMMUNIOASSAY-BIOASSAY COMPARISON IV
E 89 ADH PLASMA, INAPPROPRIATE SECRETION SYNDROME
E 90 ADH PLASMA, HEAD TRAUMA I
D 7004 BIOLOGICAL HALF-LIFE OF ADH V, DOG
E 91 DIABETES INSIPIDUS I, RAT URINE
E 92 ADH REFERENCE STANDARDIZATION I, G VS V STASTICAL STUDY
E 93 ADH REFERENCE STANDARDIZATION II, G VS V STASTICAL STUDY
E 94 ADH INFUSION STANDARD, DOG
D 7005 BIOLOGICAL HALF-LIFE OF ADH VI, DOG
H230636 DIABETES INSIPIDUS II, HUMAN URINE
E 95 ADH REFERENCE STANDARDIZATION III, G VS V STASTICAL STUDY
E 96 ADH REFERENCE STANDARDIZATION IV, G VS V STASTIAL STUDY
H235623 ADH PLASMA, IADH SYNDROME
E 97 ADH PLASMA, IADH SYNDROME-HEAD INJURY
E 98 ADH IMMUNIOASSAY-BIOASSAY COMPARISON V
E 99 ADH REFERENCE STANDARIZATION V
E 100 ADH REFERENCE STANDARDIZATION-SYNTHETIC ARGININE VASOPRESSIN
D 7007 EFFECT OF ADH INFUSION ON RENAL BLOOD FLOW
D 7008 EFFECT OF ADH INFUSION ON RENAL BLOOD FLOW
E 102 COLUMN RECOVERY EXPERIMENT
E 103 COLUMN RECOVERY EXPERIMENT
E 104 COLUMN RECOVERY EXPERIMENT
E 105 SAMPLES FROM TUMOR TISSUE OF PATIENTS WITH IADH SYNDROME SENT FOR IMMUNOASSAY
H177087 URINE ADH, IADHS
H167058 URINE ADH, IADHS
H174493 URINE ADH, IADHS
H115554 URINE ADH, PARTIAL GASTRECTOMY
H116819 URINE ADH, HYPOPHSECTOMY
H104559 URINE ADH, LUNG CA
H147018 URINE ADH, DIVERTICULITIS
H129107 URINE ADH, DI
H183493 URINE ADH,

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H179091 URINE ADH,
H158561 URINE ADH, DI
E 107 SIZEMORE STDS
H165291 URINE ADH, DI
H127365 URINE ADH, IADHS