FLUID REGULATION AND ADH IN RAT OESTROUS: THE EFFECTS OF OESTRADIOL

Muhammad Azhar Khan and Muhammad Aslam

To determine how fluctuations in vasopressin are related to fluid ha la nee, studies were carried out in ovariectomized Sprague-Dawley rats injected daily with either vehicle or oestradiol benzoate (50, µg/rat) for 14 days. The animals were housed individually under 12 h light/12 h dark regimen with free access to food and water. Inputs & outputs were recorded and urine samples obtained to determine volume and osmolality. Plasma vasopressin levels were determined by radioimmunoassay. In the oestradiol benzoate-treated rats, food and water intakes were reduced during both dark and light periods compared with vehicle-treated rats. Urine output showed a similar pattern of changes and appeared to be related to the vasopressin concentrations. Urine osmolality also correlated with the plasma vasopressin levels. These results indicate water retention in oestradiol benzoate-treated rats, and that oestradiol-induced increase in vasopressin may play a role in this fluid retention.

INTRODUCTION

Premenstrual fluid retention which disappears with the onset of menstruation has been recognised for a long time [1] Several hormones including prolactin, angiotensin II, aldosterone, catecholamines and ovarian steroids are known to cause this fluid retention. But the precise mechanism responsible for premenstrual fluid accumulation is still unknown. Another hormone which may play a role in causing fluid balance changes during ovarian cycle is the antidiuretic hormone, arginine vasopressin (AVP).

Akerlund et al [2] have reported increased levels of AVP in patients with dysmenorrhea. Forsling et al [3] reported that plasma AVP levels changed during normal menstrual cycles in healthy women with highest levels occurring at the time of ovulation. So changes in AVP levels may play a role in premenstrual oedema and dysmenorrhea.

Forsling et al [4] have shown that plasma vasopressin levels vary throughout the rat oestrus cycle; these fluctuations in vasopressin levels may result from changes in circulating levels of ovarian steroids.

DR MUHAMMAD AZHAR KHAN. Associate Professor of Medicine, Quaid-i-Azam Medical College, Bahawalpur.
PROF Dr MUHAMMAD ASLAM, Head, Department of Physiology, Army Medical College, Rawalpindi.
Corresponding Author: PROF Dr MUHAMMAD ASLAM

Khan et al [5] reported that ovariectomy causes a fall in plasma vasopressin levels and this can be reversed by daily oestrogen therapy.

Butcher et al[6] reported that oestradiol levels began to rise late in dioestrus I, and reached a peak level at the afternoon of pro-oestrus, remaining low during the days of oestrus and early dioestrus.

Forsling et al [7] had reported that oestradiol stimulates AVP release when given to postmenopausal women while progesterone inhibits it. So sex steroids modulate AVP release.

Premenstrual tension is the commonest of the minor endocrine disorders. A large number of women suffer from a variety of symptoms. Water retention may be one of the causes of intermenstrual and premenstrual or early menstrual migraine. Anti-diuretic hormone, Arginine Vasopressin (AVP) may be involved in this fluid retention. The present study in rats was therefore undertaken to establish if there is any relation between fluid retention and vasopressin release. Various treatments aimed at either dehydration or a correction of disturbed hormonal ratio have proved partially effective.

MATERIALS AND METHODS

The animals, Sprague-Dawley rats, were housed in individual metabolic cages under 12 h light/12 h dark regimen, with tree access to food and water. Urine samples to determine volume and osmolality were obtained and food intake recorded at 8:00-9:00 and 17:00-18:00 h.

Bilateral ovariectomy was performed and the ovariectomized rats were divided into two groups; one group of six rats was given subcutaneous injections of oestradiol benzoate in doses of 50 ug/rat and the other group of six rats was given oil vehicle injections subcutaneously daily in doses of 0.05 ml/100 gm body weight for 14 days.

The osmolality of urine was determined using depression freezing point osmometer. Plasma vasopressin concentrations were determined by radioimmunoassay.

The data are presented as mean ± S.E.M., statistical analysis was performed using student's t-test. Levels of significance are indicated by symbols.

(P<**.05** = slightly sig.), **(P<.025** = slightly sig), **(P < .02 =** sig), **(P < .01 =** sig), **(P <.005=** highly sig), (P <.001 = very highly sig).

RESULTS

In the animals treated with oestradiol benzoate, food and water intake were reduced, except on day eleven, when the rats ingested more food compared to the controls, but this increase in food intake was not statistically significant. The decrease in food and water intake was significant on day two (P <0.025 = slightly sig) and (<0.00 = highly sig) respectively (Tables 1, 2). The body weight also reduced in drug treated animal; weight losses occurred on days seven, eleven and fourteen (P < 0.01 = sig), (P <0.01 = sig) and (PC0.01 = sig) respectively (Table 1).

TABLE 1: DATA SHOWING L'RINE OSMOLALITY, FOOD INTAKE AND BODY WEIGHT IN OESTRADIOL BENZOATE (50/ µg/rat) TREATED RATS DURING LIGHT AND DARK PERIODS OF THE DAYS

Phase of the cycle	Treatment	DAY 1	DAY 2	DAY 4	DAY 7	DAY 11	DAY 14
D	OIL	1524 ± 235	1851 ± 185	1607 ± 205	1622±178	1635±164	1705±162
(urine)	O.B.	1053 ± 278	1872± 137*	1675±182	2049±160**	1755±175	2054±144**
L	OIL	1674±133	1361±283	1270 ± 226	1210±194	1506±179	1270 ± 424
(urine)	O.B.	1326±156	1511 ± 100	1490±130	1356±165	1603 ± 211	1490±130
D	OIL	5.3 ± 0.7	7.6 ± 0.7	7.5 ± 0.3	7.8 ± 0.2	9.9 ± 0.6	9.5 ± 0.4
(intake)	O.B.	5.3 ± 0.6	5.2±0.5*	7.0 ± 0.4	7.8 ± 0.6	11.8±0.7	9.0 ± 0.4
L	OIL	2.0 ± 0.1	1.5 ± 0.1	2.0±0.2	2.2 ± 0.3	1.9 ± 0.4	2.6 ± 0.3
(intake)	O.B.	1.2 ± 0.4	1.1±0.3	1.8±0.3	2.1±0.4	1.6±0.4	3.4 ± 0.4
L	OIL	XX XX	205 ± 6	217 ± 6	227 ± 6	239 ± 7	249 ± 8
(body weight)	O.B.	210 ± 6	199 ± 5	205 ± 5	205 ± 4*	212 ± 5*	216 ± 5*

Urine: N = 4-6 animals; mean ±SEM, *pc0.025, **pc0.02, vs values on day 1 in drug treated animals Intake: N =4-6 animals; mean ± SEM. *p<0.025, vs values at control Body Weight: N=4-6 animals: mean ± SEM, *p <0.01, vs values at control.

The results are in agreement with the observations recorded in the cycling rats [9] as well as in the study carried out with higher doses of oestradiol benzoate (100 Mg/100 gm body weight)[5].

Urine excretion was reduced on most of the days except on day four in oestradiol benzoate treated rats. Urine osmolality increased in these animals in both phases of the cycle. There was a significant elevation of urine osmolality on days two, seven and fourteen as compared to day one in dark phase of the day in these animal,

(P< 0.05 = slightly sig), (P<0.02 = sig) and (P<0.02 = sig) respectively (Tables 1, 2).

As seen in Table 2 a trend of gradual increase in water retention was observed in oestradiol benzoate treated rats from the day following ovariectomy onward, while there was a progressively decreased water retention in the controls from day two onwards.

TABLE 2: DATA SHOWING W ATER INTAKE, OUTPUT AND RETENTION IN OESTRADIOL BENZOATE (50/ µg/rat) TREATED AM) VEHICLE TREATED RATS DURING LIGHT AND DARK PERIODS OF THE DAYS.

Phase of the cycle	Treatment	DAY 1	DAY 2	DAY 4	DAY 7	DAY II	DAY 14
D	OIL	6.6 ± 0.6	11.3 ± 0.9	10.6±1.3	10.2 ± 0.7	9.4 ± 0.7	10.7 ± 0.7
(intake)	O.B.	5.5 ± 1.3	7.6 ± 0.4*	8.5 ± 0.3	9.1 ± 0.7	8.7 ± 0.8	9.5 ± 0.9
L	OIL	2.0 ± 0.5	0.5±0.1	0.6 ±0.2	1.5 ± 0.4	0.6 ± 0.3	1.0 ± 0.1
(intake)	O.B.	2.3 ± 0.4	0.8 ± 0.2	1.2 ± 0.5	0.8 ± 0.3	0.8±0.2	1.8±0.1
D	OIL	2.6 ± 0.4	2.8 ± 0.4	3.2 ± 0.7	3.0 ± 0.5	3.3 ± 0.6	3.3 ± 0.4
(output)	O.B.	3.4 ± 0.8	2.3 ± 0.3	7.8 ± 0.5*	2.3 ± 0.3	3.2 ± 0.4	2.6 ± 0.3
L	OIL	0.5 ± 0.1	0.8±0.3	1.3 ± 0.3	0.9±0.3	0.9 ± 0.2	1.1±0.2
(output)	O.B.	1.1± 0.04	0.4±0.1	1.2 ± 0.2	1.1 ±0.2	1.1±0.2	0.8±0.1
I)	OIL	4.0±0.9	8.5 ± 0.8	7.4 ± 0.9	7.2 ± 0.4	6.7 ± 0.2	7.4±0.4
(retention)	O.B.	2.1±0.8	5.3 ± 0.5*	5.7 ± 0.3*	6.7 ± 0.4***	5.6±0.5*	7.0 ± 0.7**
L	OIL	1.5 ± 0.5	-0.4 ± 0.1	0.6 ± 0.2	0.6±0.2	0.3 ± 0.01	0.01±0.003
(retention)	O.B.	1.2 ± 0.4	0.5 ± 0.2	1.2 ± 0.03	-0.3 ± 0.04	0.3 ± 0.03	0.9 ± 0.02

Intake: N =4 - 6 animals: mean ±SEM; *p<0.005, vs values at control
Output: N = 4 - 6 animals; mean ±SEM; *p<0.02, vs values at control.
Retention: N=4 - 6 animals; mean ±SEM; *p<0.01, **p< 0.005; ***p <0.001, vs values on day 1 in drug treated animals

Significant increased water retention occurred on days two, four, seven, eleven and fourteen, as compared to day one of the experiment (P < 0.01 = stg), (P<0.01 = sig), (P< 0.001 = very highly sig), (P < 0.01 = sig) and (P< 0.005 = highly sig) respectively. Maximum water retention was observed on day seven, whereas in controls, opposite pattern of changes was observed. In these animal's retention of water was maximum on day two of the study, then a progressive decline was reported.

This data of water retention is consistent with the data obtained in rats treated with higher doses

TABLE 3: EFFECTS OF OESTRADIOL BENZOATE ON PLASMA VASOPRESSIN CONCENTRATIONS IN OVARIECTOMIZED RATS.
Oestradiol benzoate (50/wµ/rat) was injected for 14 days. Controls were given equal volume of oil vehicle

Phase of the Cycle	Treatment	DAY 1	DAY 2	DAY 14
I)	**OIL**	0.36 ± 0.08	0.18 ± 0.06	0.16 ± 0.03
	O.B.	0.17± 0.02	0.16± 0.10	0.32± 0.11

N=8-12 animals; mean ± SEM

Alter fourteen days' treatment with oestradiol. benzoate the vasopressin concentrations had risen to 0.32 + 0.11 µU/ml, whereas vasopressin concentration had fallen to 0.16+0.03 µ/U/ml over this period in the vehicle treated rats (Table 3). The data showed that oestradiol caused elevation of vasopressin levels in ovariectomized rats.

DISCUSSION

It has been suggested that changes in the levels of endogenous ovarian hormones may influence water and food intake [8]. In the cycling female rats, plasma oestradiol concentrations peak early on the day of pro-oestrous and then fall rapidly in the afternoon of pro-oestrus, remaining low during the days of oestrus and early dioestrus[6]. Oestradiol treatment reduces food and water intake in ovariectomized rats treated with oestradiol benzoate (50 µg/rat). This observation is in agreement with our finding in the cycling rats [9] and in the rats treated with higher doses of oestradiol benzoate (100 Mg/100 gm body weight)[5]. It is possible that suppression of food and water intakes observed on the day of pro-oestrus in the cycling rats and also in rats treated with both the high and physiological doses of oestradiol benzoate may be due to an inhibitory effect exerted by the elevated circulating plasma oestradiol concentrations at that time. The

oestradiol-induced decrease in food intake is also reflected in decreased body weight in these animals.

The increased urinary excretion in the ovariectomized rats on day tour was related to the decreased vasopressin concentration seen on that day. The results are consistent with the observations[9] seen in cycling rats and in the ovariectomized rats treated with higher doses of oestradiol benzoate '. Changes in urine osmolality also correlated with the plasma vasopressin levels. Increased levels of vasopressin on day fourteen of the experiment correlated with the increased urine osmolality and decreased urine output seen on that day. Same pattern of changes was seen in the cycling rats[9] and in the rats treated with higher doses of oestradiol benzoate [5].

The data indicates progressively increased water retention in the drug treated animals, whereas in the controls opposite pattern of changes were observed. In the controls the retention was maximum on day two, then a progressive decline was observed. This may be related to the progressively decreasing plasma vasopressin concentrations. The results are consistent with the observations seen in the study carried out on the cycling rats[9] and rats treated with higher doses of oestradiol benzoate[5].

The variation in vasopressin levels are also consistent with the cycling rats[9] and rats treated with higher doses of oestradiol benzoate[5]. The data showed that oestradiol treatment caused elevation of vasopressin in ovariectomized rats and the underlying cause of fluid retention may be the oestradiol induced elevation of vasopressin release.

From this study it can be concluded that the possible cause of fluid retention in oestradiol benzoate treated ovariectomized rats and the cycling rats during the pro-oestrus may be the oestradiol-induced elevation of vasopressin levels.

REFERENCES

1. Greene R and Dalton K. The premenstrual syndrome. Br Med J, 1953; 1007-1014.
2. Akerlund M, Stromberg P and Forsling ML. Primary Dysmenorrhoea and Vasopressin. Br J Obstet and Gynaecol, 1979; 86: 484-87.
3. Forsling ML, Akerlund M and Stromberg P. Variations in plasma concentrations of vasopressin during the menstrual cycle. J Endocrinal, 1981; 80: 263-66.
4. Forsling ML and Pevsner K. Diurnal variation in plasma and pituitary- vasopressin in the rat. J Physiol, 1984; 358: 104.
5. Khan MA, Aslam M and Babar MK. Fluid balance in cycling and ovariectomized rats treated with oestradiol benzoate. Pak Armed Forces Med J, 1994; 44(1): 7-14.
6. Butcher RL, Collins WE and Fugo NW. Plasma concentrations of LH, FSH, Prolactin, Progesterone and Oestradiol- 17p throughout the 4 day oestrus cycle of the rat. Endocrinal, 1974; 94: 1704-8.
7. Forsling ML. Stromberg P and Akerlund M. Effects of ovarian steroids on vasopressin secretion. Endocrine, 1982; 95: 147-51.
8. Wade GN. Sex hormones regulatory' behaviour and body weight. Adv Stud of Behav, 1976; 6: 201-79.
9. Sendi AA, Forsling ML, Khan MA and Peysner K. Fluid balance over the oestrous cycle in the rat. Br Neuroendocr Gr, 1985; p 35.