Fluid Balance Over the Oestrus Cycle of the Rat

Pages with reference to book, From 231 To 234

Muhammad Azhar Khan (Department of Physiology and Chemistry, Quaid-e-Azam Medical College, Bahawalpur.)
Misbahulain Khan (Department of Physiology and Chemistry, Islarnia University, Bahawalpur.)
Muhammad Aslam (Department of Physiology and Chemistry, Army Medical College, Rawaipindi.)

Abstract

Plasma vasopressin concentrations vary throughout the rat oestrus cycle. In order to determine the relationship of these fluctuations to fluid retention, studies were carried out in cycling female albino rats. The animals were housed in individual metabolism cages under 12 hourlight/12 hour dark regimen, with free access to food and water. Urine samples to determine volume, osmolality and eiecroiytes concentrations were obtained and food and water intake recorded between 0800-0900 and 1700-1800 hours. Plasma volume, blood volume and haematocrit were determined Food intake was significantly reduced on pro-oesirul/oestrus compared to dioestrusl and dioestrus II, possibly due to the influence of the elevated circulating oestradiol levels. Water intake also reduced.during the dark phase of pro-oestrus leading to significant decrease in urine flow and a rise in urine osmolality. During the light phase of pro-oestrus, there was a significant increase in urine flow and electrolytes excretion. This enhanced diuresis appears to be due to reduced plasma vasopressin levels during the afternoon of pro-oestrus. These results in cycling rats show that vasopressin may play a rolein fluid retention during the rat oestrus cycle (JPMA 46: 231, 1996).

Introduction

Fluid retention which occurs in the premenstrual phase and disappears with the onset of menstruation, has long been recongnized[1]. Several hormones including prolactin, angiotensin 11, aldesterone, catecholaxnines and ovarian steroids are known to cause 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 antidiuretichormone, arginine vasopressin (AVP). The major function of AVP is regulation of intravascular volume and tonicity Increased levels of AVP have been.reported in patients with dysmenorrhoea2. Plasma AVP levels change during normal menstrual cycle in healthy women, the highest levels being at the time of ovulation[3]. Changes in AVP levels may thus play a role in premenstrual oedema and dysmenorrhoea[2]. Plasma vasopressin levels vary throughout the rat oestruns cycle[4]. These variations in vasopressin levels may result from changes in ciitulating levels of ovarian steroids. It has been reported that rat ovariectomy causes a fall in plasma vasopressin levels which can be reversed by daily oestrogen therapy[5]. Butcheret al[6] reported that oestradiol levels begin to riseiate on.dioestrus I and achieve peak during afternoon of pro-oestnis. It then remains low during the days of ocstrus and early dioestrus. 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 undertaken to study the relation between fluid retention throughout the 4 days oestrus cycle and vasopressin release.

Materials and Methods

Albino rats were housed in individual metabolic cages under 12 hour light! 12 hour dark regimen, with free access to food and water. Urine samples to determine volume, osmolality and electrolytes concentrations were collected and food and water intake recorded at between 0300-0900 and 17001800 hours. Plasma volume, blood volume and haematocriit were also determined. Rats vagina was smeared by 1 5-2 ml of warm tap water followed by collection of vaginal sample The stage of cycle was identified by studying the pattern of cells seen under microscope. Dioestrus I and II were characterised by the presence of a large number of small rounded celk (leukocytes). The pro-oestrus was identified by the presence of clumps of relatively large epithelial cells, whereas the ocstrus stage showed a characteristic pattern of cornified cells with sharp edges. The osmolality of urine was determined by depression freezing point osmometer using an advanced digimatic osmometer. Urinary sodium and potassium concentration in the urine were determined by a flame photometer while urinary chloride concentration was measured by chloride meter.
On the last day of experiment, all the rats were anaesthetised and the vessels cannulated. 40 microliter of Evensblue was injected into the jugular vein. animal was decapicitated after five minutes of injection and bled into heparinized tubes. The tubes were placed in ice bath. The haernatocrit was determined immediately. The tubes were centrifuged for 15 minutes at 40 and plasma withdrawn. Plasma and blood volume were determined in ml/100 g.b.w.

Results

Food intake was significantly reduced durring the dark phase of pro-oestrus/oestrus compared to dioestrus I ($P<0.02$) and dioestrus H ($P<0.05$) (Table 1).

Table I. Light and dark variations in food intake (q/100 g.b.w) during 4 days oestrus cycle in the rat. [n=4-6 animals mean±SD±SEM *($P<0.05$) **($P<0.02$) vs values at pro-oestrus (dark phase)].

Days of the cycle	Dark phase	Light phase
Dioestrus I	6.8±0.5±0.2**	2.4±0.7±0.3
Dioestrus II	6.6±0.5±0.2*	2.0±0.2±0.1
Pro-oestrus	6.1±0.5±0.2	1.9±0.5±0.2
Oestrus	6.4±0.5±0.2	1.9±0.2±0.1

During the dark phase, water intake decreased from a mean value of 9.4±0.4 on dioestrus Ito 8.7±0.4 ml!100 g.b.w. on pro-oestnis (Table II).

Table II. Light and dark variations in water intake. Urine output and water retention (intake-output) during 4 days oestrus cycle in the rat.

Water intake (ml/100.g.b.w.) n=4-6 animals mean±SD±SEM.

Days of the cycle	Dark phase	Light phase
Dioestrus I	9.4±0.9±0.4	1.6±0.2±0.1
Dioestrus II	9.2±0.7±0.3	1.5±0.2±0.1
Pro-oestrus	8.7±0.9±0.4	1.3±0.5±0.2
Oestrus	9.2±0.7±0.3	1.5±0.2±0.1

Urine output (ml/100 g.b.w.). n=4-6 animals mean±SD±SEM •(P<0.05) ••(P<0.01) •••(P<0.001) vs values at pro-oestrus (dark phase) and ••(P<0.01) vs values at dioestrus I (light phase).

Days of the cycle	Dark phase	Light phase
Dioestrus I	3.4±0.7±0.3••	0.84±0.1±0.05
Dioestrus II	3.5±0.7±0.3•••	1.0±0.2±0.1
Pro-oestrus	2.4±0.5±0.2	1.2±0.2±0.1•
Oestrus	2.9±0.5±0.2•	1.0±0.2±0.1

Water retention (ml/100 g.b.w). n=4-6 animals mean±SD±SEM •(P<0.02) vs values at Dioestrus I (light phase).

Days of the cycle	Dark phase	Light phase
Dioestrus I	6.0±0.7±0.3	0.76±0.5±0.2
Dioestrus II	5.7±0.5±0.2	0.5±0.2±0.1
Pro-oestrus	6.3±0.7±0.3	0.1±0.5±0.2•
Oestrus	6.3±0.5±0.2	0.5±0.4±0.2

Urine flow was significantly reduced during the dark phase of pro-oestrus as compared to dioestrus I (P<0.0l), dioestrus II (P The change in urine output (Table II) paralleled that of vasopressinconcentrations

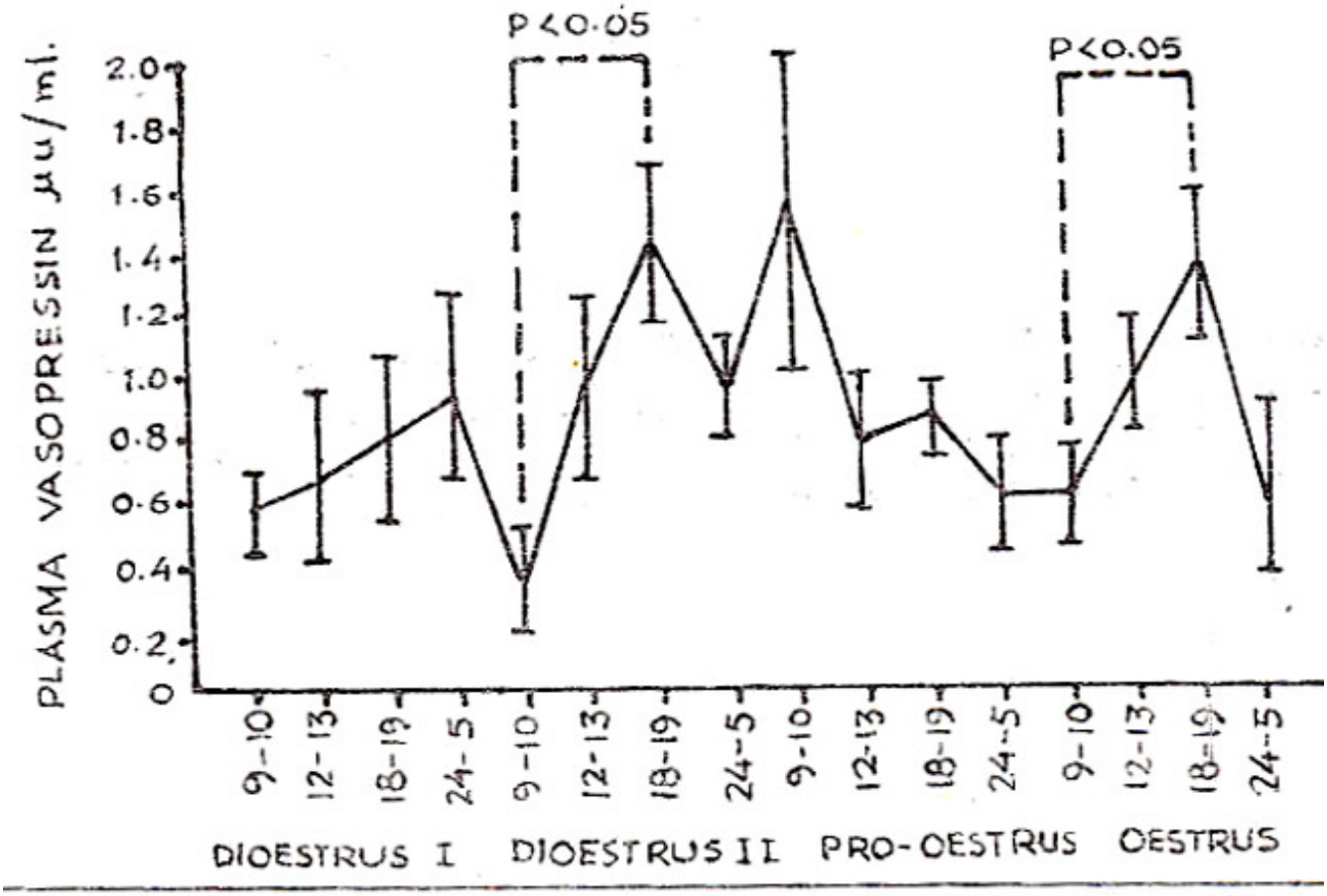

Figure. Variation in plasma vasopressin levels during the oestrus cycle.

(Figure)whichwere higherduring the light phase than the dark phase except on the day of pro-aestrus. During the light phase of prn-oestms there was a significant increase in urine flow ($P<0.05$) (Table II). This enhanced diuresis appears to be due to reduced plasma vasopressin levels during the afternoon of pro-oestrus; on the day of pro-oestrus plasma vasopressin concentrations were high in the morningbetween 9.00-10.00 h (1.53±0.51. mu/ml) and low in the afternoon between 1800-1900 h (0.87±0.11 mu/ml)/ Increased levels of progesterone may be involved in 'this suppression of vasopressin as elevation of progesterone occurrred between noon and mid-night of pro-oestrus when plasma vasopressin has fallen. Water retention was much higher during the dark phase than during the light phase (Table III).

Table III. Light and dark variations in urine osmolality (moSm/kg.b.w) during 4 days oestrus cycle in the rat.

n=4-6 animals mean±SD±SEM *($P<0.05$) **($P<0.01$) vs values at pro-oestrus (dark phase).

Days of the cycle	Dark phase	Light phase
Dioestrus I	1527±116±52**	1520±176±80
Dioestrus II	1573±188±84*	1460±198±90
Pro-oestrus	1889±288±125	1390±220±100
Oestrus	1725±157±70	1650±220±100

Water retention increased from a value of 5.7±0.2 on dioestrus 1110 6.3±0.3 ml/100 g.b.w. on pro-oestrus. Although this difference is not statistically significant, it may be related to the fall in urine output observed during the datk phase of pro-oestrus. During the light phase there was a significant reduction in water retention on pro-oestrus as compared to dioestrus I (P<0.02) which paralleled on increase in urine out-flow. Dark phase sodium intake at pro- oestrus was less than at dioestrus I (P<0.01) and dioestrus II (P<0.01) (Table IV).

Table IV. Light and dark variations in sodium intake, sodium output and sodium retention (intake-output) during 4 days oestrus cycle in the rat.

Sodium intake (m.mole/100 g.b.w.). n=4-6 animals mean±SD±SEM
*(P<0.01) vs values at pro-oestrus (dark phase).

Days of the cycle	Dark phase	Light phase
Dioestrus I	0.6±0.02±0.01*	0.16±0.02±0.01
Dioestrus II	0.6±0.02±0.01*	0.15±0.02±0.01
Pro-oestrus	0.5±0.02±0.01	0.15±0.02±0.01
Oestrus	0.55±0.04±0.02	0.13±0.02±0.01

Sodium output (m.mole/100 g.b.w.). n=4-6 animals mean±SD±SEM
*(P<0.001) vs values at dioestrus I and Dioestrus II (dark phase).

Days of the cycle	Dark phase	Light phase
Dioestrus I	0.4±0.05±0.02	0.12±0.02±0.01
Dioestrus II	0.4±0.05±0.02	0.11±0.02±0.01
Pro-oestrus	0.3±)0.05±0.02*	0.13±0.02±0.01
Oestrus	0.35±0.02±0.01	0.12±0.05±0.02

Sodium retention (m.mole/g.b.w.). n=4-6 animals mean±SD±SEM.

Days of the cycle	Dark phase	Light phase
Dioestrus I	0.2±0.05±0.03	0.04±0.02±0.01
Dioestrus II	0.2±0.02±0.01	0.04±0.05±0.02
Pro-oestrus	0.2±0.05±0.02	0.02±0.05±0.02
Oestrus	0.2±0.05±0.02	0.01±0.05±0.02

The fall in sodium ingestion at pro-oestrus was reflected in a significant decrease in sodium excretion at dark phase of pro-oestrus (P<0.0O1). Sodium excretion showed a tendency to rise at light phase of pro-oestrus. Sodium retention waS higher during the dark phase of the day than during the light phase. Dark phase potassium intake at pro-oestrus was less than at dioestrus I (P<0.05) (Table V).

Table V. Light and dark variations in potassium intake. Potassium output and potassium retention (Intake-Output) during 4 days oestrus cycle in the rat.

Potassium intake (m.mole/100 g.b.w.). n=4-6 animals mean±SD±SEM
*(P<0.05) vs values at pro-oestrus (dark phase).

Days of the cycle	Dark phase	Light phase
Dioestrus I	0.96±0.05±0.02*	0.32±0.1±0.04
Dioestrus II	0.95±0.07±0.03	0.31±0.05±0.02
Pro-oestrus	0.84±0.09±0.04	0.23±0.1±0.04
Oestrus	0.92±0.07±0.03	0.27±0.05±0.02

Potassium output (m.mole/100 g.b.w.). n=4-6 animals mean±SD±SEM
*(P<0.02) **(P<0.01) vs values at pro-oestrus (dark phase).

Days of the cycle	Dark phase	Light phase
Dioestrus I	0.74±0.09±0.04**	0.2±0.02±0.01
Dioestrus II	0.75±0.09±0.04*	0.21±0.05±0.02
Pro-oestrus	0.6±0.09±0.04	0.22±0.05±0.02
Oestrus	0.7±0.05±0.02	0.21±0.05±0.02

Potassium retention (m.mole/g.b.w.). n=4-6 animals mean±SD±SEM
*(P<0.01) vs values as pro-oestrus (light phase).

Days of the cycle	Dark phase	Light phase
Dioestrus I	0.22±0.09±0.04	0.12±0.02±0.01*
Dioestrus II	0.2±0.07±0.03	0.1±0.05±0.02*
Pro-oestrus	0.24±0.07±0.03	0.01±0.05±0.02
Oestrus	0.22+0.07+0.03	0.06+0.05+0.02

Potassium excretion showed an increase at light phase of pro-ocstrus. This is accompanied by significant increase in urine flow at light phase of pro-oestrus. Potassium excretion showed similar trend as sodium excretion. Dark phase potassium balance (Table V) showed no statistically significant cyclical change in potassium retention. However, light phase values demonstrate a significant decrease in the retention of potassium at pro-oestrus when compared to diocstrus I (P<0.001) and dioestrusH (P

Table VI. Light and dark variations in chloride intake, chloride output and chloride retention (Intake-Output) during 4 days oestrus cycle in the rat.

Chloride Intake (m.mole/100 g.b.w.). n=4-6 animals mean±SD±SEM
*(P<0.05) **(P<0.001) vs values at pro-oestrus (dark phase).

Days of the cycle	Dark phase	Light phase
Dioestrus I	1.32±0.07±0.03**	0.4±0.05±0.02
Dioestrus II	1.3±0.09±0.04*	0.4±0.07±0.03
Pro-oestrus	1.2±0.09±0.03	0.36±0.1±0.04
Oestrus	1.24±0.09±0.04	0.4±0.02±0.01

Chloride output (m.mole/100 g.b.w.). n=4-6 animals mean±SD±SEM
*(P<0.02) **(P<0.001) vs values at pro-oestrus (dark phase).

Days of the cycle	Dark phase	Light phase
Dioestrus I	0.55±0.05±0.02**	0.2±0.05±0.02
Dioestrus II	0.53±0.07±0.03*	0.2±0.02±0.01
Pro-oestrus	0.4±0.05±0.02	0.2±0.05±0.02
Oestrus	0.53±0.02±0.01**	0.21±0.05±0.02

Chloride retention (m.mole/g.b.w.). n=4-6 animals mean±SD±SEM.

Days of the cycle	Dark phase	Light phase
Dioestrus I	0.77±0.12±0.05	0.2±0.07±0.03
Dioestrus II	0.77±0.09±0.04	0.2±0.02±0.01
Pro-oestrus	0.8±0.09±0.04	0.16±0.1±0.04
Oestrus	0.71±0.12±0.05	0.19±0.05±0.02

showed the trendsimilarto sodium and potassium intakes. Light phase values of urinaiy chloride output showed no statistical difference throughout the cycle whereas dark phase values followed the same pattern of changes as observed in sodium and potassium outputs. Urinary chloride excretion showed a significant reduction on thc dark phase of pro-oestrus when compared to those of dioestrus I (P<0.001), dioestrus II (P<0.02) and oestrus (P<0.001). Plasma volume and blood volume increased during the pro-oestrus phase of the cycle while the hacrnatocrit decreased as compared to the other stages of the cycle in the cvclinc rats. The data indicate water retention during pro-oestrus compared to the other days of the cycle in the cycling female rats.

Discussion

It has been suggested that changes in the levels of endogenous ovarian hormones may influence water and food intake[7]. In the cycling female rats, plasma oestradioi concentration peaks early on the day of pro-oestrus and then fall rapidly in the afternoon of pro-oestrus, It remains low during the days of oestrus and early dioestrus[6]. Food intake was significantly reduced at pro- ocstrus/ocstrus as compared to dioestrus I and dioestrus IT ($P<0.02$). It is possible that the suppression of food and water intakes observed on the day of pro-oestrus may be due to an inhibitory effect exerted by the elevated circulating oestradiol concentrations seen at that time of the cycle. This explanation is in agreement with our observation that oestradiol treatment reduces food and water intake in ovariectomized rats[8]. Water intake was reduced during dark phase of pro-oestrus leading to significant decrease in urine (P ($P<0.001$). The urine flow correlated with plasma vasopressin concentrations. The low urine flow seen during the light phase of the days, except the day of pro-oestnis, was associated with an increased plasma vasopressin levels and increase in urine osmolality. High urine flow observed during the light phase of
pro-oestrus was associated with reduced vas resm and low urine osmolalitv. This enhanced diuresis resulted significant reduction in waler retention at that time of cycle. Similar changes were observed in sodium, potassium and chloride balance. The fall in unnarv electrolytes exercise seen during the dark phase of pro-oestrus was accompanied by fall in urine flow and an increase in water and electrolyte tes retention. Plasma and blood volume increased while the haem tocrit decreased during the dark phase of pro-oestrus The study indicates water retention during the pro-oestrus compared to the other days of the cycle. These changes correlate with circulating plasma vasopressin levels in the cycling rats during this stage of the cycle.

Anti diuretic hormone, vasopressin may play a role in fluid retention during the 4 days ocstrus cycle in the rat The precise cellular mechanism how oestrugen affects the release and secretionofvasopressin is not known. However, it may be concluded that the underlying cause of fluid retention in tie cycling rats during the pro-oestrus may be the ocstradioldependent elevation of vasopressin levels.

Acknowledgements

We wish to thank Prof. Dr. Iqbal Ahmad Khan. Principal, Quaid-i- Azam Medical College, Bahawalpur and Prof. Dr. M. KhanBabar, Head of Pharmacology Department. Q.A.M.C. Bahawalpur for helpful discussion. We also thank Mr. :Matloob Hussain Akhtar, Research Officer and Mr Qasser Iftikhar Sheikh, Lecturer Biochemistry Department. Islarnia University, Bahawalpur for assistance with expcn-mental work. Typing service rendered by Mr. Muhammad Boota Shaheen, Stenotypist of PMRC Research Centre. Q.A.M.C. Bahawalpur are also gratefully acknowledged.

References

1. Greene. R and Dalton. K The premenstrual syndrome Br 1953;1 1007-1014
2. Akerlund, M., Stromberg. P. and Forsling. ML Pnmarv dysmer: :~h:.ea are vasopressin Br J. Obstet Gynaccol. 1979;86:484-487
3. Forsling. ML, Akeriund, M and Stromberg. P Vanations in plasma concentrations of vasopressin during the menstrual cycle Endocrni 1981,89:263-266.
4. Forsling ML and Peysner, K. Diurnal variation in plasma and vasopressin in the rat, 3. Physiol., 1984;358 04.
5. Skowsky; WE.. Swan. L., Smith, P. Effect of sex stcrod horm es vasopressin in intact and castrated male and female rat. Endocrinology, 1979;104:105-108

6. Butcher, EL., Collins, WE: and Fugo, N.W Plasma concentrations of LH, FSH, Prolactin, Progesterone and Estradiol- 1 713 throughout the 4 day oesteus cyclle of the rat. Endocrinology, 1974.94:1704-1708.
7. Wade, G.N. (1976)Sex hormones regulatory behaviour and body weight Ad'. Stud. Behav., 1976,6:201.279.
8. Khan, MA. Aslam, M. and Babar NI K Fluid balance n cycling and ovariectomized rats treated with oestradiol benzoate Pak. Anned Forces Med 3, 1994;44:7-14.