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FLUID BALANCE IN CYCLING RATS AND OVARIECTOMIZED RATS TREATED WITH OESTRADIOL BENZOATE IN PHYSIOLOGICAL DOSES (50 µg/RAT)

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Plasma vasopressin concentrations have been shown to vary throughout the rat oestrus cycle. In order to determine how these fluctuations in vasopressin are related to fluid retention, studies have been carried out in ovariectomized female Sprague-Dawley rats injected subcutaneously daily with either vehicle or oestradiol benzoate in physiological doses (50µg/rat) for 14 days. The animals were housed in individual metabolism cages under 12 hr light/12 hr dark regimen with free access to food and water. Urine samples, to determine volume, were obtained and food and water intake recorded at 8-9 and 17-18 hrs. in the oestradiol benzoate treated rats, food and water intake were reduced during both dark and light periods compared to the controls possibly due to the influence of elevated circulating oestradiol levels. Urine output showed a similar pattern o0f changes which appeared to be related to the vasopressin concentrations. Plasma osmolality decreased in oestradiol benzoate treated rats which was also correlated with the plasma vasopressin levels. The results of this study indicate that water retention in oestradiol benzoate treated rats and oestradiol-induced increase in vasopressin may play a role in this fluid retention.

Key words: Cycling and ovariectomized rats, Oestradiol benzoate, Physiological doses.

Introduction

It has long been recognised that fluid retention occurs in premenstrual phase and disappears with the onset of menstruation [1]. Several hormones are known to be involved in this fluid retention. However, a 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). The major function of AVP is to regulate intravascular volume and tonicity [2].

Materials and Methods

The female Sprague-Dawley rats were housed in individual metabolic cages under 12 hr light/12 hr dark regimen with free access to food and water. The food consisted of dry standard powdered diet of constant composition (2.6 % crude oil, 14.6 % crude protein, 4.3% crude fibre, 3.8 % starch and 10.4 % sugar). Urine samples, to determine the volume, were obtained and food and water intake recorded at 8-9 and 17-18 hrs.

Bilateral ovariectomy was performed and the ovariectomized rats were divided into two groups of six rats. Each group was given subcutaneous injections of oestradiol benzoate in doses of 50 µg/rat/day and the other group was given oil injection subcutaneously in doses of 0.05 ml/100 g body weight/day for 14 days. Blood samples were obtained from parallel groups of animals between 9-10 hrs. Plasma osmolality was determined by using the method of freezing point (advanced Digmatic osmometer model 3 D 2, Advanced Instrument Inc., Needham Heights, MA, USA). Vasopressin was determined by radio immunoassay as described by Aziz, *et al.* [3]. Using the first international standard of vasopressin (77/501). Plasma was extracted using a modification of method described by Skowsky *et al.* [4]. The antibody was used against AVP coupled to thyroglobulin and the label was prepared using a solid phase lactoperoxidase method as described by Karonen *et al.* [5].

Results and Discussion

In the animals treated with lower doses of oestradiol benzoate (50 µg/rat) a diurnal variation in food and water intake was observed (Fig.1). Food and water intake were reduced in drug treated rats when compared to controls except on day 11 when rats ingested more food compared to controls, but this increased food intake was not statistically significant. The decrease in food and water intake was significant on day two of treatment.

As seen in Fig.2, in oestradiol benzoate treated rats urine output was more on day one of the study than in controls. This increased urinary excretion was inversely correlated with the vasopressin concentrations seen on that day in these animals. Maximum urinary excretion was seen on day four of the experiment in the drug treated animals and this enhanced diuresis has strong correlation (r=1) with the decreased plasma vasopressin concentration on that day. On day four, the volume of urine output showed little difference in both groups of animals. The inverse correlation of circulating plasma

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vasopressin levels seen on that day and urine output on day four was again evident in both groups of animals. On the last day of experiment, urinary excretion decreased in drug treated rats and simultaneously plasma vasopressin concentrations increased. These results showed that urine flow during the light phase was inversely correlated with the circulating vasopressin levels.

Plasma osmolality was significantly low ($P<0.005$) after 14 days in oestradiol treated animals (283.5±3.1 moSm/Kg) compared to controls (289±3.9 moSm/Kg) (milli osmoles/Kg of water).

It has been suggested that changes in the levels of endogenous ovarian hormones may influence water and food intake [6]. In this study we confirmed that food and water intake were reduced in the drug treated group compared to the controls.

In the oestrus cycle of female rats, plasma oestradiol concentrations peaked early on the day of pro-oestrus and fell rapidly in the afternoon of pro-oestrus and then remained low during remaining days of cycle [7]. Maximum water retention occured in the cycling rats during the night and morning of pro-oestrus and retention decrease during afternoon. Water retention was correlated with the increased diuresis seen at that time of the cycle. These changes were inversely correlated to the circulating plasma vasopressin levels during this stage of the cycle [8]. This explanation is in agreement with our previous observation that oestradiol treatment caused increased water retention in ovariectomized rats treated with higher doses of oestradiol benzoate (100 µg/100g body weight) [9]. It was correlated with the circulation level of vasopressin in these animals.

In this study, urine excretion in both groups during the light phase was correlated with the plasma vasopressin concentrations. Plasma osmolality was significantly lower after 14 days in the oestradiol treated animals compared to the controls, suggesting greater water retention in these animals. Plasma osmolality was inversely correlated with the vasopressin concentrations. These observations indicate a role for oestradiol in vasopressin turn over and water balance.

The observation in this study are consistent with our findings that difluoromethylornithine (DFMO) treatment incycling pro-oestrus rats caused reduction of oestradiol secretion during pro-oestrus, probably by inhibiting ornithine decarboxylase (ODC) activity which affects the vasopressin

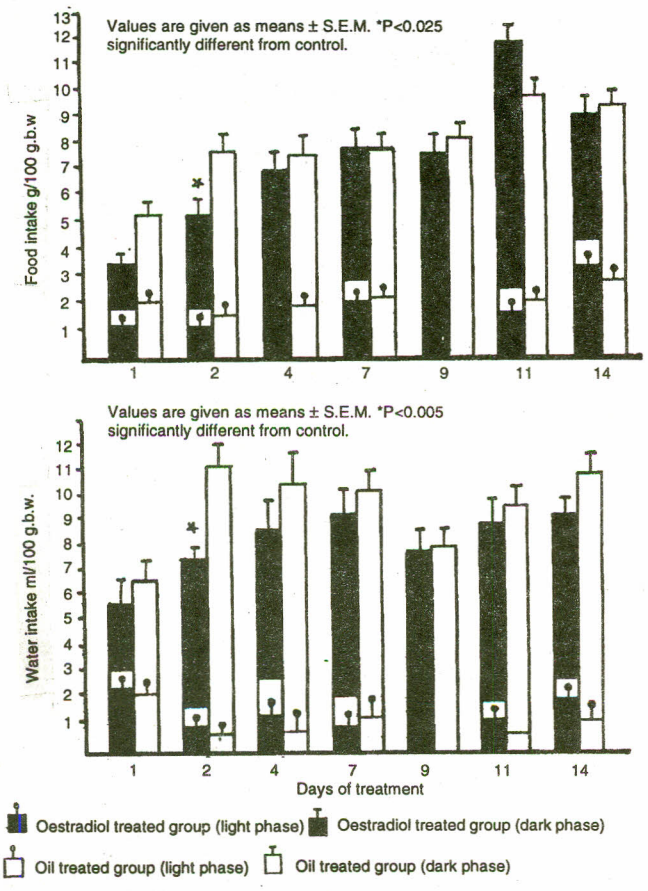

Fig. 1. Effect of oestradiol benzoate *in vivo* on food intake and water intake in ovariectomized rats.

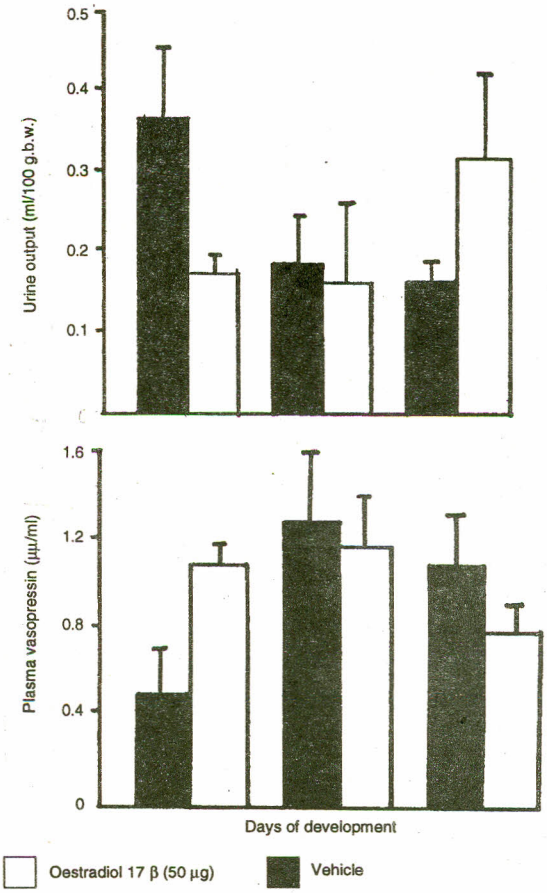

Fig. 2. Effect of oestradiol benzoate on plasma vasopressin and urine flow in ovariectomized rats during light phase. Mean ± S.E.M.

release [10]. The decreased release and secretion of this hormone may cause less fluid retention. Since increased plasma vasopressin concentration has been reported at mid-cycle (B) when fluid retention was maximum, probably the vasopressin release is oestradiol-induced, as both hormones were at their highest levels at mid-cycle.

From this study it may be concluded that the underlying cause of fluid retention in the cycling rats during pro-oestrus may be oestradiol-dependent elevation of vasopressin levels.

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