EFFECT OF TAMOXIFEN ON PITUITARY VASOPRESSIN LEVEL

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Background: Estrogens modulate the release of Arginine Vasopressin (AVP). There is an increased level of AVP in plasma and pituitary under osterogen hormones. **Methods:** Ten rats were experimental and ten were controls. The ten experimental rats were injected tamoxifen daily subcutaneously for three days in does of 1 mg/kg body weight in 0.1 ml vegetable oil base. The ten control rats were given 0.1 ml of vegetable oil vehicle only subcutaneously. On the morning of the fourth day all the twenty rats were decapitated. Blood was centrifuged at 2500 rev/min for 15 min at 4^0C. Plasma was stored at –20^oC for assay of AVP. Pituitary gland was removed and homogenized in a test tube containing 1 ml of 0.2 molar acetic acid & stored at –20^oC for analysis of pituitary AVP. Uteri of all rats were removed by careful dissection & weighed. **Results:** Uterine weight and plasma AVP levels were decreased but the pituitary AVP level was unaffected. **Conclusion:**Under Tamoxifen (anti-estrogen) effect the plasma vasopressin decreased but the level in the posterior was not affected. The effect of osterogen on Hypothalamo Pituitary axis (HPA is measurable in the hypothalamus therefore probably anti-oestrogenic effect would operate more at hypothalamic level rather than posterior pituitary. This needs further investigation at the Hypothalamus level.

Key Words: Vasopressin, Tamoxifen, Pituitary

INTRODUCTION

There has been considerable interest expressed over the past two decades in the relationship between steroid hormones and the secretion of vasopressin in both animals and humans. Ovarian sex steroids play a major role in the mechanisms underlying the changes in body fluid observed during the ovarian cycles of both humans and animals.

Arginine Vasopressin (AVP) is a neuroendocrine hormone synthesized in the hypothalamus, and is stored and secretedby the posterior pituitary gland in response to stimuli such as plasma hypertonicity and hypotension. It causes antidiuresisin man and most other mammals. Secrection of the hormone is affected by changes associated with the menstrual cycle in women and oestrous cycle in rats.

In primates the sexual cycle is menstrual, its most conspicuous feature is the periodic vaginal bleeding that occurs with the shedding of the uterine mucosa (menstruation).

In mammals the sexual activity of the male is more or less continuous, but in most species the sexual activity and several different cells are involved at different points in the menstrual cycle. Most of the time the female avoids the male and repulses his sexual advances. Periodically however, there is an abrupt change in behaviour and the female seeks out the male, attempting to mate. These short episodes of heat or oestrous are so characteristic that the sexual cycles in mammalian species that do not menstruate is named the estrous cycle. This change in female sexual behaviour is brought on by a rise in the circulating blood estrogen level. In spontaneous ovulating species with estrous cycle, such as the rat, there is no episodic vaginal bleeding but the underlying endocrine events are the same as those in the menstrual cycle.

Two main steroid hormones produced are oestradiol (oestrogen) and progesterone which has progestational activity. The naturally occurring oestrogens are steroids that are secreted by the

theca interna and granulosa cells of the ovarian follicles, by the corpus luteum, by the feto-placental unit, and in small amounts by the adrenal cortex and the testis. The biosynthetic pathway involves their formation from androgens. They are also formed by aromatization of androstenedione in the circulation. Almost all the oestradiol comes from ovary, oestrogens have many actions on the body, one of it is some degree of salt and water retention as noticed just before menstruation. It mediates these effects by direct action on the renal tubules and probably also by the release of the hormone vasopressin which is involved in the regulation of intravascular volume and composition [1,2] . It is possible that increased AVP secretion contributes to the premenstrual fluid retention.

Progesterone is a C21 steroid secreted by the corpus luteum and the placenta. It is an important intermediate in steroid biosynthesis in all tissues that secrete steroid hormones, and small amounts apparently enter the circulation from the testis and adrenal cortex. Progesterone has an anti oestrogenic effect on myometrial cells, decreasing their excitability, their sensitivity to oxytocin, and their spontaneous electrical activity, while increasing their action potential. It decreases the number of oestrogen receptors in the endometrium and increases the rate of conversion of 17β oestradiol to less active oestrogens. Large doses of progesterone inhibit LH secretion and potentiates the inhibitory effects of estrogens. Large doses of progesterone produce natriuresis, probably by blocking the action of aldosterone on the kidney.

Some investigators have failed to observe any significant effect of these gonadal steroids on osmoregulation or AVP secretion.[3] In contrast others have documented changes in plasama AVP concentration. During the rat oestrous cycle or alterations in rat pituitary content of AVP in response to exogenous oestradiol and progesterone[4]. Skowsky and colleagues[5] demonstrated that large doses of oestradiol increased basal serum AVP concentration and decreased serum osmolity in rats, suggesting that osmoregulation was influenced by gonadal steroid hormones.

Changes in the AVP concentration due to alterations in wather balance were also seen over the estrous cycle of rat. Increased neurosecretory activity in supra-optic and para ventricular nuclei has been observed during the pro-oestrous and oestrous in cyclic animals [6]. Variations in AVP concentration during oestrous cycle reflects the ovarian steroid concentrations in plasma, being highest when oestradiol concentration are highest and lowest when progesterone concentrations are increased [1].

Little is known of the manner in which ovarian steroids may influence AVP release but the study of Barron[7] suggests that oestrogens alters the slope of the plot of plasma AVP against plasma osmolality. Oestradiol receptors are shown in the hypothalamic nuclei and neurohypophysis [8] and gonadal steroids have also been shown to influence AVP pathways [2]. A combination of audio radiogrpah and immunohistochemistry technique has indicated that oestradiol can concentrate in magnocellular neurons [8,9] and have a stimulatory effect on activity of neurons in paraventricular nucleus [10].

Oestradiol recepters have also been shown to be located in cells of arcuate nucleus and the tuberoinfundibular dopaminergic neurons originating from this nucleus innervates the megnocellular nuclei[11]furthermore estrogen have been shown to modify catecholamine content in paraventricular nucleus[4] and to concentrate in the nuclei of noradrenergic neurons that project as paraventicular nuclei [12]. The influence of eastrodiol on vasopressin release is not however established. While some[5], described a stimulatory effect others groups have found no effect. We set out to examine the effect of chemical castration due to tamoxifen and its effect on vasopressin release from the posterior pituitary.

Tamoxifen (Nolvodex, property of imperial chemical industries limited) was introduced in early 1970. This nonsteroidal anti estrogen, was used as a subcutaneous injection in our study.

MATERIAL AND METHODS

Twenty Sragues Dawley femal virgin rats weighing 240-280 grams were selected from the Animal House of the department of physiology, University College, London. These rats were maintained under constant temperature conditions in a 12 hours light/12 hour dark daily cycle (lights on at 6:00 Hours). The animals were given free access to food (standard chow) and tap water. The rats were numbered and checked for two regular oestrous cycles by daily microscopic examination of vaginal smears over a period of fourteen days, representing two consecutive four days cycle.

Ten rats were experimental and ten were controls. The ten experimental rats were injected tamoxifen daily subcutaneously for three days in does of 1 mg/kg body weight in 0.1 ml vegetable oil base. The ten control rats were given 0.1 ml of vegetable oil vechicle only subcutaneously. On the morning of the fourth day all the twenty rats were decapitated.

The rats were rapidly decapitated by a guillotine, care being taken to avoid squeezing the thorax during execution [13]. The blood issuing from the vessels of the trunk was collected in chilled heparinised tubes. Small aliquots were drawn immediately into capillary tubes for microhematocrit determination.

The remaining blood was centrifuged at 2500 revolutions/minute for fifteen minutes at 4° C, in refrigerated centrifuge (MSE, coldspin). The plasma was separated and osmolality and, electrolyte concentration determined. The remaining plasma was stored at - 20° C for subsequent extraction and assay of plasma vasopressin.

The pituitary gland of each rat was also removed immediately after death of the rat by careful dissection and homogenised in a test tube containing I ml of 0.2 Molar acetic acid and stored at - 20° C. it was stored for subsequent extraction and analysis of Pituitary vasopressin (Pt AVP). The uteri of all the rats were also removed by careful dissection and weighed.

The glass tubes containing the posterior lobes of the pituitary glands initially had 1 ml of 0.21 M acetic acid. The gland was homogenised in it with a glass rod. 1 ml acetic acid was added again and further homogenised. Finally 0.5 ml acetic acid was added and final homogenization took place to make up the final amount of 2.5 ml and boiled for five minutes. This mixture was centrifuged at 2100 revolutions/minute for 20 minutes. The supernatant was removed into another tube and stored in a deep freezer at -20 C until assay.

The plasma was also stored for AVP assay in the deep freezer at -20 C until assay. Assay on both pituitary AVP levels and plasma AVP levels was to be done at a later date when all the samples were collected.

The radioimmunoassay for AVP were performed by Dr. Mary Forsling, using techniques the use of Sap Paks for AVP extraction, the use of a radio labeled 125 I-AVP and an antibody raised against AVP conjugated to thyroglobulin. The techniques employed were basically the same for the plasma and pituitary assays, except that a more sensitive antibody is needed for the former because of the much lower AVP concentrations involved.

The procedure for the pituitary AVP sampling involved the dilution of 50 micro litre extract with a 5.95 ml of buffer (TRIS+human serum albumin) from which duplicate 100 micro litre neat and double diluted samples were prepared. To each of these was added 100 micro litre of standardized labeled AVP solution and 25 micro litre of anti body solution, the sample tubes being vortexed after each addition. After incubation at 4 C over night, the unbound AVP was removed by addition of charcoal, the remaining solution then being removed for radioactive counting by suction pipette following centrifugation.

The same procedure was followed with a standard AVPsolution to produce 12 samples of known concentrationeach being half the strength of the other. The radioactive counts obtained with these references samples (LKB Wallac Mine Gamma Counter Model 1275) were then plotted as a semi log graph of percentage bound 125 I AVP against the amount of AVP/sample.

This standard curve was then used to construct parallel curves throught the experiment points obtained with the pituitary AVP samples and to determine the amount of AVP present (in units U defined by the First International Standard for

AVP, 77-501). The plasma concentration of AVP is presented in the text in micro U / ml of plasma and the pituitary AVP content in UM/gland (milli units/gland).

The significance of the differences between the means of groups were determined by means of students t test for paired or unpaired samples, as appropriate. Most of the results were analyzed by computer (Commodore CBM Model 8096) and the probabilities read from tables. Probability values <0.05 are taken to be significant and values between 0.05 and 0.10 possibly significant.

RESULTS

1. Uterine Weight:

The uterine weight was significantly decreased due to tamoxifen treatement. This reductionis statistically highly significant $P < 0.001$ by student's t-test.

2. Plasma AVP (PAVP)

The PAVP concentration in the unchallenged and untreated control rat was 1.147 + 0.16 micro units/ml (S.E.M) where as in the tamoxifen treated rat it was 0.38 + 0.1 micro units/ml (S.E.M). this reduction under tamoxifen influence is highly significant statistically ($P < 0.001$) by a student's t-test.

3. PIT-AVP:

The tamoxifen treated group compared to the unchallenged, untreated control rats show no significant statistical difference.

DISCUSSION

The Decrease in plasma vasopression (AVP) after ovariectomy did not appear to be due to the removal of ovarian source of vasopressin since although ovarian vasopressin has been reported to have rapid turnover [14] the content is only 0.004% of that in the pituitary. There was no decreased in the pituitary vasopressin to account for the reduced plasma vasopressin concentration. Pituitary AVP content was also shown [15] to be similar in sham operated and ovariectomized rats, confirming that removal of gonadal steroids has no significant effect on AVP storage in posterior pituitary. Neither did the decrease in Plasma AVP concentration appear to be due to factors normally assumed to suppress vasopressin release, namely a blood volume expansion or decrease in plasma osmolality. Although one cannot rule out subtle modulation.

A fall in the plasma AVP could result from removal of the effect of ovarian steroids on the pathways controlling release.

Decarboxylase (ODC) activity as a result of oestradiol stimulation can cause an increase release of AVP from neurohypophysis [16].

Table-1: Comparison of the control and experimental groups

	Control	Experimental	Pooled Variance Estimate		
			T-Value	Degree of Freedom	2-Tail Probability
Uterine* Weight	206.9+10.18	142.2+10.34	4.45	15	<0.001
PLAVP	1.15+0.16	0.38+0.1	4.065	12	<0.001

PITAVP	583.4+52.97	568.7+24.8	0.25	12	0.85

* Wt/100 grm body weight.

Uterine weight in millgrams/100 grm body weight. PLAVP=micro units/milli liters. PITAVP=milli units/gland

Area postrema the most caudal circum-ventricular organ located on dorsal surface of medulla is also involved in the regulation of AVP. The AVP synthesis & release are tonically stimulated by Area postrema in basal state and regulated in the stimulated states.[17]

The action of sex steroids on the hypothalamus-pituitary-adrenal (HPA) axis is measurable in the hypothalamus.[18] Sheep treated with tamoxifen were compared to controls. Estrogen significantly increased hypothalamic corticotropin releasing factor and AVP concentration. The result demonstrate that the effect of estrogen on HPA axis is measurable in the hypothalamus, and is therefore not primarily at the pituitary.

Our studies support their results and we need to do further investigation at the hypothalamic level as well. The set points for vasopressin release in response to increasing plasma osmolality and hypovolaemia alter with reproductive status, in a study[23] vasopressin release following ovariectomy and oestrogen replacement was done. It was found that vasopressin release in response to both stimuli was reduced in ovariectomized rats and the response was restored by oestradiol replacement. We need to do more work involving the Hypothalmo Pituitary axis (HPA) and the level of AVP under anti-estrogen effects.

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