EFFECT OF TAMOXIFEN ON PLASMA ARGININE VASOPRESSIN LEVELS

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Ovariectomy of the mature female rat results in a fall in the plasma vasopressin concentration although the ovarian content of the hormone is low. To investigate this further, functional ovariectomy was done in the rats by administration of an antioestrogen acting drug (Tamoxifen).

Studies performed on female rats indicated significant reductions in uterine weight and plasma vasopressin levels.

The uterine weights decreased from 206.9 mg 1100 gm body weight in control group to 142.2 mg /100 gm body weight in experimental group.

Plasma vasopressin levels decreased from 1.15 microunits /ml in the control group to 0.38 microunits /ml in the experimental group.

The study confirms that like surgically ovariectomized rats, Junctional ovariectomy also causes decreases in uterine weights and plasma vasopressin levels.

INTRODUCTION

Arginine vasopressin is a nonapeptide of the posterior pituitary that causes anti-diuresis in man and most other mammals.

There has been considerable interest expressed over the past decade 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 fluids observed during the ovarian cycles of both animals and humans.

Ovarian sex steroids (oestrogens) have many actions on the body. One of it is some degree of salt and water retention as noticed just before menstruation [2]. It is believed that oestrogens mediate these effects by a direct action on the renal tubules and probably also by the release of the hormone vasopressin [3, 4] which is involved in the regulation of intravascular volume and composition. It is possible that increased AVP secretion contributes to the premenstrual fluid retention.

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The plasma AVP (p1 AVP) concentration varies during the menstrual cycle [5], being highest at the time of ovulation and lowest immediately before menstruation. It has been known that ovariectomy results in a fall in the circulating concentration of vasopressin [6].

The aim of this study was to find out the ovarian contribution to the release of AVP by suppressing ovarian function by drugs which cause functional castration. An anti-oestrogens preparation, Tamoxifen, was used for this purpose.

Tamoxifen (Nolvadex, property of Imperial Chemical Industries Ltd.) was introduced in early 1970. This non-steroidal anti-oestrogenic agent is thought to counter a hyper-oestrogenized state by peripheral receptor blockade[7].It was used as a subcutaneous injection in our study.

hi the rat, tamoxifen is a partial oestrogen agonist with anti-oestrogenic properties. The two major metabolites of tamoxifen are also anti-oestrogenic [8] in rats. Tamoxifen inhibits binding of estradiol to the rat uterine oestrogen receptors in vitro. Several studies have shown that tamoxifen binds directly to the cytoplasmic oestrogen receptors of the rat uteri and rat mammary tumor cells. Administration of tamoxifen results in translocation of cytoplasmic oestrogen receptors to the nuclear component. Probably as a result of

this event, cytoplasmic progesterone receptor synthesis is initiated. Thus rather than diminishing activity of oestrogen, tamoxifen might increase the level of total estradiol. However, since this may be accompanied by an increase in sex hormone binding

globulin, the result could be diminution in the amount of biologically available estradiol. Peripheral competition for oestrogen receptors leads to an abrogation of the effects of increased levels of estradiol on target tissues producing anti-oestrogenic effects.

MATERIALS & METHODS

Twenty Sprague-Dawley female virgin rats weighing 240-280 gm 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-hour 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 ocstrous cycles by daily microscopic examination of vaginal smears over a period of fourteen days, representing two consecutive four-days cycles.

Twenty of the regularly cycling rats were separated for the Tamoxifen study. Ten were experimental and ten were controls. The ten experimental rats were injected with tamoxifen daily subcutaneously for three days in a dose of 1.0 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 twenty rats were decapitated. Decapitation was done rapidly by guillotine, care being taken to avoid squeezing of the thorax during execution (Hussain et al, 1979). The blood issuing initially from the vessels of the trunk was immediately collected into capillary tubes for microhaematocrit determination. The remaining blood was centrifuged at 2500 rpm for fifteen minutes at 4°C, in a refrigerated centrifuge (MSE Coldspin). The plasma was separated and osmolality and electrolyte concentrations determined. The remaining plasma was stored at -20°C for subsequent extraction and assays of plasma vasopressin.

The pituitary gland of each rat was also removed by careful dissection immediately after decapitation of the rat and homogenized in a test tube containing 1.0 ml of

0.2 M acetic acid and stored at -20°C. It was stored for subsequent extraction and analysis of pituitary vasopressin (pI AVP).

The uteri of all the rats were also removed by careful dissection and weighed.

RESULTS & CONCLUSIONS

Results are shown in Table 1 and graphs depicted in Figure 1.

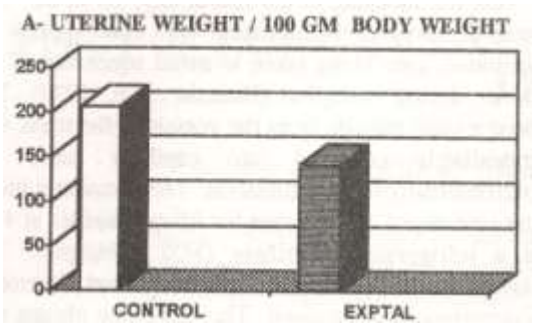
TABLE 1: TAMOXIFEN TREATED EXPERIMENTAL RATS VS CONTROL GROUP

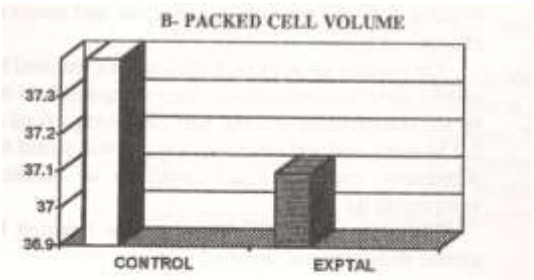
TAMOXIFEN ONLY					STATISTICAL ANALYSIS	
VARIABLE		NUMBER OF CASES	MEAN VALUES	STANDARD ERROR	POOLEP VARIANCEJESTI.MAI.E DEGREES OF FREEDOM	POOLEP VARIANCEJESTI.MAI.E 2- TAILED PROBABILITY
UTERINE WEIGHT*	CONTROL	10	206.9	10.18	15	<0.001
	EXPTAL	10	142.2	10.34		
PCV	CONTROL	10	37.4	0.49	15	0.98
	EXPTAL	10	37.1	1.09		
OSM	CONTROL	10	283	2.8	15	0.15
	EXPTAL	10	290.6	3.5		
Na	CONTROL	10	138.2	0.69	15	0.55
	EXPTAL	10	137.5	0.87		
Cl	CONTROL	10	108.2	1.57	15	0.45
	EXPTAL	10	108.4	1.84		

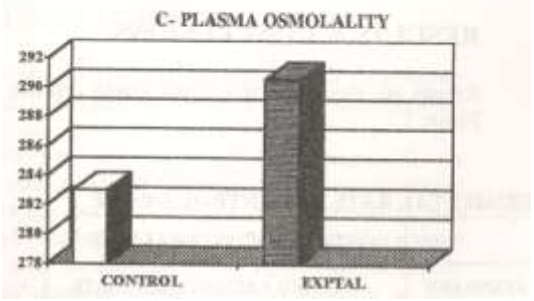
PI AVP	**CONTROL**	**10**	**1.15**	**0.16**	**12**	**<0.001**
	EXPTAL	**10**	**0.38**	0.1		
Pit AVP	**CONTROL**	**10**	**583.4**	**52.97**	**12**	**0.85**
	EXPTAL	**10**	**568.7**	**24.8**		

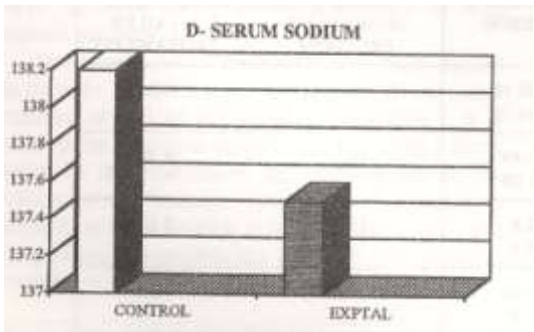
***=Wt (mg)/100 gm body weight; CONTROL=intact unchallenged untreated rats; EXPTAL=tamoxifen treated rats; PCV = Haematocrit; OSM = milliOsm/L; Cl=milliOsm/L; PI A VP=micro units/ml; Pit AVP=milliunits/gland.**

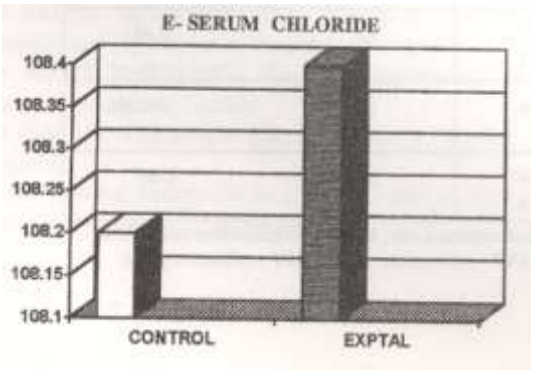
FIGURE 1: CONTROL RATS COMPARED TO TAMOXIFEN TREATED RATS.
(Comparison of Uterine Weights, PCV, Osmolality, Sodium, Chloride, Plasma & Pituitary Arginine Vasopressin)

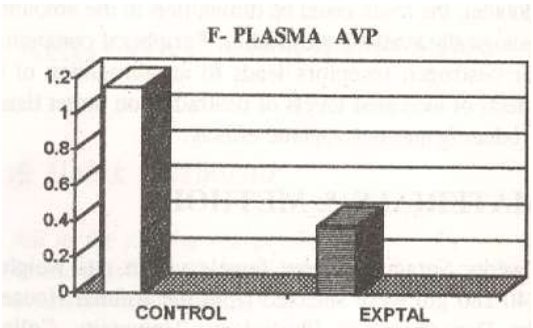


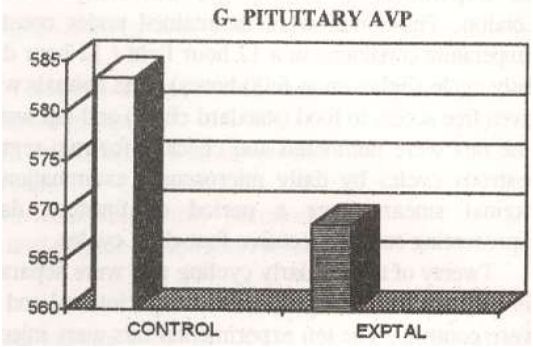












Uterine weights were significantly decreased due to tamoxifen treatment. This reduction is statistically highly significant ($p < .001$) by student's t-test.

Plasma AVP (pi AVP) in the Tamoxifen treated group was significantly less than in the control group ($p < 0.001$).

Other tested parameters (PCV, osmolality, sodium & chloride levels & pituitary vasopressin concentration) showed no significant differences between the control and experimental groups.

It is concluded from these results that chemical castration by injecting tamoxifen results in a significant reduction in the uterine weight/100 gm body weight, thus confirming that tamoxifen suppresses the uterine cycle and prevents the action of oestrogens on the uterus as the mechanism of uterine weight reduction.

Reduction of plasma vasopressin levels also confirms the observation that plasma AVP levels are raised in the presence of oestrogens. This supports the study by Forsling et al, 1982, that ovarian steroids modulate the vasopressin response.

DISCUSSION

The uterine weight is significantly reduced under tamoxifen treatment. This supports the findings in surgically ovariectomized rats where the uterine weight was decreased by 60-70% 9. In chemical castration by tamoxifen, effects of surgical ovariectomy were mimicked. Tamoxifen affects energy balance via oestrogen receptors rather than anti-oestrogen binding sites[1].

Administration of estradiol/progesterone to ovariectomized rats increased their uterine weights. There is a pronounced increase of uterine weight with administration of exogenous oestrogen, though the effect of oestrogen on uterine weight varies between mice of different strains [11].

Administration of tamoxifen in rats also decreased the plasma level of vasopressin by three folds. Skowsky et al [12] reported that rat ovariectomy caused a fall in vasopressin levels and this can be reversed by daily oestrogen therapy.

Plasma vasopressin levels have been shown to vary throughout the oestrous cycle and following ovariectomy. Khan MA et al [13,14] reported that oestradiol-induced increase in vasopressin plays a role in fluid retention. Increased plasma concentration of vasopressin and increased oestradiol concentration at mid-cycle was associated with maximum fluid retention.

Tamoxifen treatment prevents increase in uterine weight in intact female rats and decrease in uterine weight in ovariectomized rats [15]. But in our study there was a decrease in uterine weight; this may be because of the higher doses of tamoxifen used which caused chemical castration. The effects of tamoxifen as oestrogen agonist and antagonist are dose related. The dose response of tamoxifen in our study clearly shows it to be an oestrogen antagonist as depicted by the decrease in uterine weight and plasma vasopressin levels.

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