RELEASE OF VASOPRESSIN DURING SUPPRESSION OF OESTROUS CYCLE IN RAT BY ZOLADEX AND HYPOVOLEMIC CHALLENGE

Salma Aslam Kundi, Saleem Wazir*, Saleem Afzal, Naeema Afzal[†], Iftikhar Qayum[†], Mary L. Forsling[§]**

Departments of Physiology, *Community Medicine, **Medicine and [†]Pathology, Ayub Medical College Abbottabad & [§]Department of Physiology, St. Thomas's Hospital, London

Background: The vasopressin (AVP) response to hypovolemia has been compared in intact and chemically castrated rats. This functional ovariectomy was done to confirm the findings in surgical ovariectomy of how gonadal steroids modulate the release of AVP under hypo-volemic challenge. **Methods:** Twenty female Sprague Dawley rats were checked for oestrous over two consecutive cycles. The ten control rats were given sub-cutaneous puncture only whereas the experimental were given Zoladex implant. On the fifteenth day all the rats were given intra-peitoneal injection of poly-ehylene glycol. All the rats were de-capitated after an hour. **Results:** The uterine weight was significantly decreased in experimental group. The plasma AVP level was also significantly decreased in the experimental group. The pituitary AVP level was significantly increased in the experimental group. **Conclusion:** The chemical castration effected the AVP secretion, this proves that the sex steroids modulate the release of AVP secretion inspite of hypo-volemic challenge.

Keywords: Zoladex, Hypovolemia, Vasopressin

INTRODUCTION

Concentrated body fluids excite the supra-optic nuclei, impulses are transmitted to the posterior pituitary and Vasopressin (AVP) is secreted. This passes by way of the blood to the kidneys where it increases the permeability of the collecting ducts to water. As a result most of the water is reabsorbed from the tubular fluid, while electrolytes continue to be lost into the urine. This dilutes the extracellular fluid, the normal osmotic composition is thus regained. Hence, AVP controls the osmolality of extracellular fluid. This reduction in the plasma osmolality acts as a negative feedback signal on the hypothalamic osmoreceptors which suppresses the activity of the magnocellular neurons to decrease in AVP secretion.[1]

The endogenous opioid peptides exert a regulatory role in neurohypophysial function[1] and opiate receptors have been localized in the paraventricular and supra-optic nuclei and in the pituitary gland. Nor-adrenergic and dopaminergic pathways could modulate vasopressin release from the pituitary and even interact with the opiod pathways.[2] The supra-optic and the paraventricular nuclei are also extensively innervated by the ascending catecholamine systems. However there is little information as to an osmotic challenge.

An effect of catecholamine on the osmoregulatory response might be predicted as the degree of volume expansion or contraction influences vasopressin release to changes in the plasma osmolality. The pathway mediating the volume response appears to involve catecholaminergic projections from A1 region of the ventral medulla to the magnocellular neurons.[3] Hemodynamic information is transmitted to the VP neurons via multisynaptic pathways from the brainstem with the A1 catecholamine neurons of the ventrolateral medulla providing the final link for information about decreases in blood pressure and volume. Several neurotransmitters and neuropeptides are expressed in the A1 neurons including norepinephrine (NE), ATP, neuropeptide Y and substance P.[4] Under normal conditions, small changes in osmolality are more potent than small changes in blood volume, in affecting AVP secretion.[5] However the responsiveness of the osmoreceptor mechanism is altered by modest changes in blood volume, indicating a close inter relationship between the two variables in the control of AVP secretion.

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 fluid observed during the ovarian cycles of both humans and animals. 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.[6,7] It is possible that increased AVP secretion contributes to the premenstrual fluid retention. Large doses of progesterone produce natriuresis, probably by blocking the action of aldosterone on the kidney.

Although acute decrease in plasma volume are known to enhance the osmotically induced AVP release, it is unclear whether there is also such an interaction at the level of gene transcription. In a study[8] it was examined how acute and chronic decreases in blood volume affected the osmoregulation of AVP release & gene transcription in rats. The regression lines relating plasma to Na were decreased. The thresholds of plasma Na for AVP release were significantly decreased in the hypovolemic group. Hence it was demonstrated that acute and chronic reduction in plasma volume affected the osmoregulation of AVP release and gene transcription in different ways.

The aim of our study was to see the effect of hypovolemia on chemically castrated rats under Zoladex. We compared it to the effect of hypovolemia in intact rats where the sex steroids level was normal. Zoladex is injectable, biodegradable depot formulation of highly potent synthetic analogue of luteinizing hormone releasing hormone D-ser (But) Azygly 10-LH-RH (zoladex) (ICI 118630) which causes selective castration like effect in animals and man.

The hypothalamus is involved in the gondadotropin secretion in the female. The hypothalamic secretion is exerated by Luteinizing Hormone Releasing Hormone (LHRH) secreted into the portal hypophyseal vessels. LHRH stimulates the secretion of FSH as well as of LH. The LHRH is normally secreted in episodic bursts. These bursts appear to be essential for normal secretion of gondadotropin. If LHRH is administered by constant infusion, the LHRH receptors in the anterior pituitary down regulates and LH secretion declines to zero. The LH secretion is responsible for ovulation and the initial formation of the corpus luteum. LH stimulates progesterone secretion, the down regulation of pituitary receptors and the consequent decrease in LH secretion produced by constant elevated levels of LHRH has led to the suggestion that this hormone or some of its long acting analogs could be used as effective contraceptive agents.

Chronic administration of LHRH analogue causes a reversible chemical castration. Continuous release of the poly peptide in vivo can be measured qualitatively by the biological effect elicited in regularly cycling adult female rats. Normally these rats have an oestrous cycle of four days and the occurrence of oestrus is indicated by the presence of cornified cells in the vaginal smear. In rats given subdermal depots of Zoladex, release of the drug at an effective rate will cause fall in circulating oestrogens, which in turn leads to suppression of oestrus and absence of cornified smears. By controlling the properties of the polymers, a continuous release over 28 days can occur both invitro and invivo, these depots have been used to induce a castration like effect in rats. Chemical castration using zoladex is indistinguishable from those of surgically castrated animals.

We used zoladex implants to chemically castrate the female Sprague Dawley rats in order to study the effect of chemical castration on the release of vasopressin.

Polyethylene Glycol (PEG) is a non absorbable hydrophilic polymer which when injected intra-peritoneal results in a transudation of non-colloid water from the extra-cellular space into the peritoneal space. This leads to a rapid fall in plasma volume. There is therefore, a rise in haematocrit. The degree of haematocrit produced can be controlled by varying the concentration of the PEG employed.

MATERIAL AND METHODS

Twenty Sprague Dawley female rats weighing 240-280 gms were selected from the animal house of department of Physiology, University College, London. These rats were maintained under constant temperature conditions in a 12 hour light /12 hour dark daily cycle (light on at 6:00hours). These 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.

These rats were anesthetized with methohexital sodium (45 mg/kg body weight), the anesthetic was given as an injection intra peritoneally. The dorsal aspect of the left shoulder was shaved and cleaned. A sub-cutaneous puncture was given to the control rats in this shaved area. The experimental rats were implanted with a depot of Zoladex (1mg/kg) with a syringe piston. The rats were observed till their recovery. They were kept in clean cages and kept warm with electric lamps. The whole implantation procedure was performed under strict aseptic conditions. The rats were smeared again for two weeks. All the controls were cycling, whereas the experimental rats were noncycling and were in the dioestrous stage. The rats were brought on the fifteenth day of Zoladex implantation to the dissection room and 20 rats were decapitated. 20 rats (10 experimental and 5 controls) were injected poly ethylene glycol intraperitoneally (2 mg/100 gm body weight at a saturation of 350 mg/100 ml) of 0.15 Molar sodium chloride. All the rats were decapitated after 60 minutes.

All the rats were decapitated in the same way. The rats were rapidly decapitated by a guillotine, care being taken to avoid squeezing the

throrax during execution.[9] The blood issuing from the vessels of the trunk was collected in chilled heparinized 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 a 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 (PTAVP). The uteri of all the rats were also removed by careful dissection and weighed.

The haematocrit values were obtained by centrifugation of duplicate microsamples in a Wifug Haemicrofuge followed by measurement with a Hawksley micro-haematocrit Reader.

The plasma osmolality was measured by means of the freezing point depression apparatus (Advanced Digimatic Osmometer Model 3D2) using 200 micro litre specimen of plasma.

Plasma sodium concentrations were determined simultaneously by flame photometry (Corning Clinical Flame Photometer 410 C) Plasma chloride concentration were measured by conductivity measurement (Corning 925 Chloride Analyser).

The glass tubes containing the posterior lobes of the pituitary glands initially had 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 was performed by techniques based on the methods, involving the use of Sap Paks for AVP extraction, the use of a radio labeled I-125 AVP and an antibody raised against AVP conjugated to throglobulin. 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.

RESULTS

The intact controls and the Zoladex treated rats were both given hypo-volemic challenges .The results obtained are given in the table-1

DISCUSSION

The isotonic nature of induced plasma volume depletion is demonstrated by the close similarity of mean plasma osmolality, sodium and chloride concentration in the control and experimental groups. There was an increase in the plasma AVP level of the experimental groups compared to the controls. This difference was statistically highly significant. The plasma AVP value of both the groups is consistent with the previous result of [10] who found that no increase in plasma AVP occurs until plasma volume deficit of 6-8% are achieved but thereafter an exponential increase occurs. Another study[11] they were unable to demonstrate any effect of oestrogen administration on the release of AVP in to moderate degrees of blood volume depletion. In our study, however, he differences in plasma AVP values for intact and hypovolemic challenged group at oestrous and functionally ovarectomised rats under Zoladex was significant.

Table-1: Effect of Hypovolemia in Zoladex treated rats (Values are given as means±SD)

VARIABLE	Controls	Experimental	2-Tail Probability
Uterine weight (mg)	475.8± 20.225	169.2±4.514*	0
PCV (%)	49.6± 0.763	50,5±1.07	0.508
OSM (mosmol/kg)	287.8± 8.402	286.2 ± 3.231	0.705
Na (mosmol/kg)	135.6 ± 0.689	133.2 ± 1.631	0.215
Cl (mosmol/kg)	94.1 ± 2.801	96.2 ± 3.378	0.637
PLAVP(micro-units/milli liter)	17.1 ± 3.072	5.6 ± 0.795*	0.009
PIT AVP(milli-units/glad)	359.6 ± 43.32	467.8 ± 37.92	0.079

Control = Hypovolemic challenged controls, Experimental= Zoladex treated and hypovolemic challenged
PCV= Packed Cell Volume, OSM= Osmolality, Na= Sodium, Cl= Chloride, PLAVP= Plasma Vasopressin, PITAVP=Pituitary vasopressin
*: The difference is statistically significant

This suggests that plasma AVP release response to plasma volume depletion is enhanced by the presence of ovarian sex steroids.

The magnitude of diuresis and natriuresis produced by oxytocin in the female rat has been shown to be dependent on the stage of the oestrous cycle. A study[12] was performed in which the role of ovarian steroids in modulating the renal response to oxytocin was determined. Observations were performed on ovariectomised rats with and without steroid treatment and rats with suppressed oestrous cycles following treatment with long acting gonadotrophin-releasing hormone analogue Zoladex. The renal responses were greater following treatment with oestradiol than in the ovariectomised group. Thus ovarian steroids do influence the renal responsiveness to oxytocin with oestradiol augmenting the response.

Observations were performed on ovariectomised rats with and without steroid treatment and rats with suppressed oestrous cycles following treatment with long acting gondadotropin Ovariectomy had no significant effect on plasma oxytocin concentration although progesterone treatment produced an increase. The ovarian steroids do influence the renal responsiveness to oxytocin with long acting gondadotropin releasing hormone anolouge, Zoladex. Ovariectomy had no significant effect on oxytocin concentration,although progesterone treatment produced an increase. Thus ovarian steroids do influence the renal responsiveness to oxytocin with oestradiol augmenting the response.

The set points for vasopressin release in response to increasing plasma osmolality and hypo-volemia alter with reproductive status. Stimulated Vasopressin release following ovariectomy and oestrogen replacement[13] was studied. The neuronal activity being measured in terms of immediate early gene expression. The Vasopressin release in response to hypovolemic challenges was reduced in ovariectomised compared to intact rats and the response could be substantially restored by oestradiol replacement. This study also supports our finding of a reduced vasopressin release to hypovolemia under Zoladex castration.

REFERENCE

1. Van Leeuwen FW, Pool CW, Sluiter AA. Enkephalin immunoreactivity in synaptoid elements on glial cells in the rat neural lobe. Neuroscience 1983;8:229-41.
2. Bicknell RJ, Chapman C, Zhao BG. Opiod receptor subtypes mediating inhibition of oxytocin, vasopressin and noradrenaline release from the neurohypophysis in vitro. J Physiol 1987;388:9.
3. Swachenko P, Swanson LW. The organisation of noradrenergic pathways from the brain stem to the paraventricullar and supraoptic nuclei in the rat. Brain Research Review 1982;4:275-325.
4. Sladek CD. Vasopressin response to osmotic and hemodynamic stress: neurotransmitter involvement. Stress 2004; 7(2): 85-90.
5. Dunn FL, Brennen TJ, Nelson AE, Robertson GL. The role of blood osmolality and volume in regulating vasopressin secretion in the rat. J Clin Invest 1973;52:3212-9.
6. Butcher RL, Collins WE, Fugo NW. Plasma concentrations of LH, FSH prolactin, progesterone and oestradiol 17 B throughout the 4 day oestrous cycle of the rat. Endocrinology 1974;94:1704-7.
7. De Varies GJ, Buijis RM, Sluiter AA. Gonadal hormone action on the morphology of the vasopressingergic innervation of the adult rat brain. Brain Research 1984;298:141-5.
8. Kondo N, Arima H, Banno R, Kuwahara S, Sato I, Oiso Y. Osmoregulation of vasopressin release and gene transcription under acute and chronic hypovolemia in rats. Am J Physiol Endocrinol Metab 2004; 286(3):E 337–46.
9. Hussain MK, Manger WM, Rock TW, Weiss RJ, Frantz AG. Vasopressin release due to manual restraint in the rat: role of body compression and comparison with other with other stressful stimuli. Endocrinology 1979;104:641-4.
10. Barron WM, Stamoutson BA, Lindheimer MD. Role of volume in regulation of vasopressin secretion during pregnancy in the rat. J Clinic Invest 1984;73:923–32.
11. Barron WM, Schreiber J, Lindheimer MD. Effect of ovarian sex steroids on osmoregulation and vasopressin secretion in the rat. Endocrine Metab. Am J Physiol 1986; 250: E352–E361
12. Zhou Y, Forsling ML. Effect of reproductive status on plasma oxytocin concentrations and the renal response to oxytocin in the conscious rat. Exp Physiol 2000;85(1):73-9
13. Hartley DE, Dickson SL, Forsling ML. Plasma vasopressin concentrations and Fos protein expression in the supraoptic nucleus following osmotic stimulation or hypovolaemia in the ovariectomized rat: effect of oestradiol replacement. J Neuroendocrinol 2004; 16(3): 1917-20

Address for Correspondence:
Dr. Salma Aslam Kundi, Department of Physiology, Ayub Medical College, Abbottabad. Phone: (0992)381907 Ext: 3068
Email: salmakundi@hotmail.com