RELEASE OF VASOPRESSIN DURING SUPPRESSION OF THE OESTROUS CYCLE IN THE RAT BY TAMOXIFEN AND HYPEROSMOTIC OR HYPOVOLEMIC CHALLENGES

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ABSTRACT

Previous studies have shown that surgical ovariectomy of the rat results in a fall in the plasma visopressin concentrations suggesting that ovarian steroids may influence hormone release and after surgical ovariectomy the response to hypertonicity was unaffected but that to hypovolemia was attenuated. To determine whether a similar fall is observed on suppression of the oestrous cycle, vasopressin concentrations were monitored after treatment with the antiestrogen preparation tamoxifen. Treatment with tamoxifen was found to result in a fall in circulating vasopressin concentrations, with little effect on fluid balance.

To determine whether the ovary could influence the vasopressin release in response to known stimuli, hormone concentrations were measured in tamoxifen ovarectomized rats during extracellular fluid hypertonicity produced by Intra-peritoneal (i.p.) injection of hypertonic saline and hypovolemia produced by i.p. injection of polyethylene glycol. It was found that after treatment with tamoxifen, the response to hypertonicity was unaffected but that to hypovolemia was attenuated. Hence tamoxifen is a potent anti-estrogen drug, and tamoxifen ovariectomized rats behaved in the same manner as surgically ovariectomized rats.

INTROCUCTION

There are changes in fluid balance during M. cycle (Baylis, Spruce & Bard 1985)[1] The underlying mechanisms of which are not clear, although changes in plasma vasopressin concentrations (P AVP) have been observed (Forsling, Akerlund & Stromberg, 1981)[2]. Similarly, there are variations in fluid balance and vasopressin concentration during oesterous cycle of the rat (Forsling & Peysner, 1988)[3]. Consistent with these observations is the finding that ovariectomy of rat results in reduced circulating AVP conc. (Al-Sendi Peysner & Forsling 1985)[4].

Neurones that concentrate gonadal steroids are found to be involved in regulation of vasopressin secretion (Rhodes, Morrell & Pfaff, 1981; Sar & Stumpf 1981)[5]. Very recently Pelletier, Liano, Follea & Govindan (1988)[6] showed that many cells in supraoptic neurons were labelled with a probe complementary for mRNA coding for the oestradiol receptors.

The observations on the influence of gonadal steroids are not consistent however. Recent findings suggest that ovarian steroids have relatively little effect on plasma vasopressin concentrations (Crofton, Baer, Share & Brooks, 1985)[7]

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This could reflect the pattern of steroid hormone replacement or indicate that factors other than changing steroid hormone concentrations are involved, in man, oestrogen is known to promote sodium and fluid retention although the precise mechanism is unknown (Ferris & Francisco, 1983)[8]. It has also been demonstrated that oestrogens reduces ingestive behaviour (Thomborough & Passo, 1975)[9] which could modify vasopressin secretion by changes in plasma osmolality and blood volume. There are however no detailed studies on the relationship between gonadal steroids, fluid balance and the secretion of vasopressin which allow this question to the addressed.

A study was therefore undertaken to determine the effect of ovariectomy of mature rats on vasopressin secretion and fluid balance by Forsling & Peysner 1988.3 A series of studies has therefore been performed using alternative approaches to determine the possible causes of the reduction in plasma vasopressin following ovariectomy. Vasopressin has been detected in the ovary of the rat (Wathes, 1984)[10] So that the observed fall could in part be due to removal of this source.

To determine whether this was the case, in the study conducted by us the vasopressin concentrations and fluid balance were monitored after functional oestrogen withdrawal produced by the administration of the antiestrogen preparation tamoxifen (Furr & Jordan, 1984)[11] The reduction in circulating hormone concentrations could also result from altered responsiveness to stimuli of release. Two major stimuli are hypertonicity which may be produced experimentally by intraperitoneal injection (i.P.) of hypertonic saline (Forsling, Matziari & Aziz 1988)[12] or hypovolemia which may be produced by i.p. injection of a high molecular weight polymer Polyethylene glycol' (Forsling & Peysner 1983)[13] The response to these two stimuli was therefore followed in a second study of functionally ovariectomized rats by Tamoxifen.

MATERIALS & METHODS:

The studies were performed on 50 Srague Dawlcy female virgin rats weighing 240-280 grams. The rats were selected from the Animal House of University College London. They were shown on the basis of vaginal smearing to exhibit two or more regular cycles. They' were maintained at 12 hours' light/12 hours' darkness cycle (lights on at 0.600 h) and housed under conditions of constant temperature and humidity'. Food (R + M maintenance diet, special Diet Services Ltd. Witham, Essex, U.K.) and water were available ad libitum.

The animals were divided into two groups of 30 + 20 rats. One group of 30 rats was given Tamoxifen as daily subcutaneous injection of 1 mg/kg body weight. After three days' treatment with tamoxifen the treated rats at oestrous were given either an I.P. injection of 1.5 ml 1.5 Nacl or an I.P. injection of 700 mg/100 g body weight polyethylene glycol in 0.15 M Nacl.

Fifteen minutes after the hypertonic saline injection and 60 min after the polyethylene glycol the animals were killed by decapitation and blood samples obtained for the determination of packed cell volume, plasma electrolytes osmolality, plasma vasopressin concentrations. The pituitary' glands were removed for determination of neuro-hyperphysical hormone content in the neural lobe.

The 10 control rats were given vehicle only, 0.1 ml vegetable oil daily for three days. On fourth day five of control rats given I.P. inj. of hypertonic & five rats was given I.P. injection hypovolemic challenge and the animals killed by decapitation.

Rats were decapitated rapidly by a guillotine; care being taken to avoid squeezing the thorax during execution (Hussain et. al; 1979)[14]. 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 plasma analysed for osmolality', sodium, chloride and vasopressin. Posterior pituitary lobes were removed into chilled 0.2 mol acetic acid/1 and stored at -30 C. The uteri were removed, trimmed of fat and the weight was recorded.

ANALYSES:

Plasma osmolality was determined by the method of freezing-point depression (Advanced Digimatic Osmometer Model 3D). The Sodium concentrations were measured using a flame photometer and the Chloride concentration with a chloride meter (Coming, Model 925 Essex U.K.). The packed cell volume was determined in duplicate using heparinized microhaematocrit tubes. Plasma vasopressin concentrations were determined by radioimmunoassay after prior extraction using Sap Pak for AVP extraction. The pituitary' vasopressin was determined after extraction in 0.2 mol acetic acid/Liter.

Effect of suppression of the oestrous cycle on fluid balance and vasopressin concentration.

TAMOXIFEN TREATED GROUP:

Treatment with tamoxifen suppressed the oestrous cycle. There was a significant fall in the uterine weight P< 0.001 after the experimental period. There were no significant changes in packed cell volume, plasma osmolality' or pituitary vasopressin content. Despite this fact, plasma vasopressin concentration was significantly lower in the treated group than in controls.

Effect of Hypertonicity:

The injection of hypertonic Nacl produced an increase in plasma osmolality of approximately 3% in all groups stated. On injection of hypertonic saline, the packed cell volume of 40.8+0.9% seen in the animals receiving three daily injection of tamoxifen was not significantly different from that of 37.1- + 1,09 % in the tamoxifen treated group Table 1. There was an increase in the plasma osmolality in the untreated group from 283+2.8 milliosm/kg to 301.4+3.3 milliosm/kg. The osmolality reached in tamoxifen treated animals was 295.9+1 milliosm/kg. There was also no significant difference in the plasma vasopressin concentration as shown fig. 1 achieved nor in the pituitary vasopressin.

Table 1: Effect of baseline parameters in the rat of treatment with Tamoxifen.

	Vehicle	Tamoxifen	Tamoxifen + PEG	Tamoxifen + hypertonic
Uterine wt. rng/l00g	206+10.18	142+10.34	406+22.6	364+18.6
Packed Cell Volume%	37.4+0.49	37.1+1.09	49.9+0.73	40.9+0.9
Plasma osmolality	283 + 2.8	290.6+3.5	287.2+1.04	295+1.8
Plasma vasopressin	1.15+0.16 ’	0.38+0.1	6.6+1.6	16.2+2.0
Pit vasopressin	583.4+53	568.8+24.8	440.9+39.6	390+52.4,

In the vehicle treated group the basal plasma vasopressin concentration was l. 15+0.09 micro units/ml rising to 11.68+2.3 micro units/ml after hypertonic saline injection. In the tamoxifen treated group, the stimulated values were 16.2+2 micro-units/ml.; and the pituitary hormone content was 390.6 mille-unit/gland compared with 460 mille units/gland in the vehicle treated group.

Effect of Hypovolemia:

The injection of polyethylene glycol produced a foil in the circulating blood volume of about 20% in the groups studied. After polyethylene glycol (P.E.G.), administration to rats receiving injections of vehicle. The packed cell volume increased to 49.6+0.75% and the plasma vasopressin to 17.2+3.0 micro units/ml with no significant change in the plasma osmolality.

Injecting Tamoxifen treated animals produced an increase in the packed cell volume to 49.9+0.7% with no change in plasma osmolality. Despite the similarity of the challenge vasopressin concentrations rose to only 6.6+1.6 although the pituitary content was unaffected.

DISCUSSION:

On surgical ovariectomy of the mature female rate there is a fall in circulating plasma vasopressin concentrations with little disturbance of fluid balance (earlier observations). Functional ovariectomy by Tamoxifen caused a fall in plasma vasopressin. This fall could result from altered clearance rates of the hormone, the removal of source of vasopressin or absence of circulating ovarian steroids. It is unlikely to be due to altered clearance as the rate would have to be markedly increased to produce the circulating hormone concentrations observed, Crofton et al (1986)[7] could find no significant changes in hormone clearance over the oestrous cycle. Vasopressin has been detected in the ovary' of the rat (Wathes, 1984)[10] but is present in very low concentrations. Even if the rate of turnover in the ovary were extremely high it is unlikely that an ovarian source of hormone could make a major contribution to the circulating hormone concentrations.

This study has shown that there was still a reduction in circulating concentrations on suppression of the oestrous cycle with the ovary intact. A central effect of gonadosteroids on the neurohypophysial system is therefore suggested as indicated by the observation of De Vries, Buijs and Sluiter (1984)[15] that castration results in a reduction of vasopressinengic pathways. From the existing data on the influence of ovarian steroids on vasopressin release it is hard to predict what the effect of their removal would be. While Swonsky, Swan and Smith (1979)[16] reported that high doses of oestradiol potentiated release of vasopression, Crofton et al (1985)[17] were unable to confirm the observation in ovariectomized animals. In addition, Durr, Stamoutsos and Lindheimer (1981)[18] found that oestradiol treatment did not affect plasma vasopression concentration m the intact rat. Progesterone in the ovarectomized rat, either alone are in combination with oestradiol, reduces plasma vasopressin concentrations in the short term (Crofton et al, 1985)[17] (Beale, Duffet, Forsling and Peysneli, 1986)[19], although in the longer term release was enhanced. Progesterone, however, was not found to influence plasma vasopressin in the intact rat (Durl et al, 1981)[18] Ovarian steroids may not-act directly on hypothalamic Neurones but influence vasopressin release via an effect on salt and water balance. On the basis of the above data therefore it is difficult to describe the mechanisms whereby removal of the ovary' leads to lowered plasma vasopressin concentrations. An alternative approach as adopted in our study', is to monitor the

vasopressin response to know' stimuli of hormone release in functionally ovariectomized animals. Monitoring the response to hypertonicity and hypovolemia has been performed in the pregnant animal, when the response to both has been altered (Lindheimer, Barron and Davison, 1985)[20]. In a previous study by Forsling et al, 1988 ,[12], in the surgically ovariectomized animals, the vasopressin response to hypovolemia but not to hypertonicity was reduced. This would suggest that ovarian secretions influence vasopressin release and also gives some information as to the possible site of action Tamoxifen treatment similarly suppressed the response to hypovolemia, but not to hypertonicity. This agent, although an antioestrogen, has agonist properties in some situations (Furr and Jordan,1984).[20] However in the present study administration of Tamoxifen suppressed oestrus and produced reduction in uterine weight and basal plasma vasopressin concentrations. These results suggest that oestrogens potentiate vasopressin release, acting particularly to modify the response to alteration in plasma volume. Crofton and Share (1989)[21] too found an enhanced response to hypertonic saline in the female as compared with the male rat. The influence of gonadal steroids on vasopressin release appears to be confined to stimuli associated with the fluid status of the animal, as Williams, Carter and Lightman (1985)[22] found that the vasopressin response to immobilization was unaffected by ovariectomy.

CONCLUSION:

On functional ovariectomy by tamoxifen the oestrus cycle of the rat is suppressed. There is a fall in the uterine weight and plasma vasopressin concentrations. If these rats are given hypertonic saline challenge there is significant change in the plasma vasopressin concentration, rising from 0.38+0.1 micro units per milliliter to 16.2+2 micro units/ml. The untreated rats on hypertonic challenge had the plasma vasopressin level increased from 1.15+0.09 ug/ml to 11.68+2.3 ug/ml ($P<0.001$). When the tamoxifen treated rats were given hypovolemic challenge the plasma vasopressin level rose to 6.6+1.6 ug/ml from 0.38+0.1 ug/ml. The untreated rats on hypovolemic challenge has the plasma vasopressin level increased from 1.15+0.09 ug/ml to 17.16+3 ug/ml ($P<0.001$).

REFERENCES:

1. Baylis, P.H., Spruce, BA. & Brud, T. (1985). Osmoregulation of vasopressin secretion during the menstrual cycle. In vasopressin pp.241-247. Ed. R.W. Schrier, New York: Raven Press.
2. Forsling, M. L., Akerlund, M. & Stromberg, P. (1981). Variations in the plasma concentrations of vasopressin during the menstrual cycle. "Journal of Endocrinology 89",263-266.
3. Forsling. M.L. & Peysner K.(I981). Pituitary and plasma vasopressin concentrations and fluid balance throughout the oestrous cycle of the rat. "Journal of Endocrinology 117"397^102.
4. Al-Sendi, A., Forsling, M.L., Khan, M. & Peysner, K., (1985). Fluid balance in cycling rats and ovariectomized rats treated with oestradiol benzoate "Journal of Physiology371" 189P.
5. Rhodes, C.H., Morrell.J. L & Plaff 'D. W. (1981). Distribution of oestrogen concentrating neurophysin containing magnocellular neurons in the rat hypothalamus as demonstrated by a technique combining steroid autoradiography and immunohistology in the same issue. “Neuroendocrinology 33", 18-23.
6. Pelletier, G., Liao N, Follea N & Govindan. (1988). Mapping of oestrogen receptor producing cells in the rat brain by in situ hybridization. "Neuroscience Letters 94", 23-28.
7. Crofton J.T., Ratliff LDL., Brooks, DP. & Share, L. (1986) The metabolic clearance rate and presser responses to vasopressin in male and female rats. "Endocrinology 118", 1777-1781.
8. Ferris, T.F. & Francisco, LL. (1983). Estrogen, progesterone and kidney. In "Renal Endocrinology" P462-473.

9. Thomborough, J.R. &Passo, S.S., (1975). The effects of estrogen on sodium and potassium metabolism in rats. "Endocrinology 97" 1528-1538.
10. Wathes, D.C. (1984). Possible actions of gonadal oxytocin and vasopressin. "Journal of Reproduction and Fertility 71", 315-345.
11. Furr J.A. & Jordan, V.C. (1984). The pharmacology and clinical uses of tamoxifen. "Pharmacology and Therapeutics 25 "201-205.
12. Forsling, M.L., Matziari, & Aziz, L. (1988). A comparison of the vasopressin response of rats to intraperitoneal and intravenous administration of hypertonic saline and the effect of opioid and aminergic antagonists. "Journal of Endocrinology 345" 217-224.
13. Forsling.M.L.& Peysner, K. (1983). A method for studying hormone release during hypovolemia in the rat. "Journal of Endocrinology 345 " 5P.
14. Hussain, M L.J Manger, W.M,, Rock, T.W., Weiss, R.J.& Frantz, A.G., (1979). Vasopressin release due to manual restraint in the rat, role of body compression and comparison with other stressful stimuli. "Endocrinology 104 ". P641-644.
15. De-Vries, G.J. Buijs, R.M.& Sluiter AA. (1984). Gonadal hormone actions on the morphology of the vasopressinergic innervation of the adult rat brain. “Brain Research 298",141-145.
16. Skowsky; W.R., SwanX. & Smith. (1979). Effects of sex steroid hormones on arginine vasopressin in intact and castrated male and female rats. “Endocrinology 104 "105-108.
17. Crofton, J.T. Baer, P.G. Share, Brooks, D.P. (1985). Vasopressin release in male and female rats. Effect of gonadectomy and treatment with gonadal steroid hormones. “Endocrinology 117", 1195-1200.
18. Durr J A, Stamoutsos, B. & Lindheimer, M.D. (1981). Osmoregulation during pregnancy in the rat, evidence for resetting of the threshold for vasopressin secretion during gestation. "Journal of Clinical Investigation 68",337-346.
19. Beale, K.J., Duffet, J.M., Forsling, M.L. & Peysner, K.(1986). Observations on the effect of progesterone on fluid balance and vasopressin metabolism in the rat. “Journal of Physiology 381"38P.
20. Lindheimer, M.D., Barron, W.M. & Davison, J.M. (1985). Water metabolism and vasopressin secretion in pregnancy. "Vasopressin" 229-240.
21. Crofton, J.T., & Share, L. (1989). Osmotic control of vasopressin in male and female rats. "American Journal of Physiology 257" R738-R743.
22. Williams, T.D.M., Carter, D.A.& Lightman, S.L. (1985). Sexual dimorphism in the posterior pituitary' response to stress in the rat. "Endocrinology 116", 738-740.