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VASOPRESSIN HORMONE RECEPTOR DENSITY AND GENE EXPRESSION IN LITHIUM INDUCED POLYURIA RATS

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ABSTRACT : Lithium, a drug frequently used for treatment of affective disorders, is known to cause a vasopressin resistant state, leading to polyuria and polydipsia. It has been suggested that lithium interacts with the renal V_2-vasopressin receptor and makes the receptor dysfunctional. Detailed studies on the influence of lithium on the AVP receptor, however, have so far been difficult due to the lack of a suitable radioligand with high specific activity and high affinity. In this study, the effect of Lithium carbonate (20mg/kg i.p) on vasopressin receptor binding in the kidney and brain and the effect on mRNA expression was determined. The results of this study suggest that there was significant change in the receptor binding and gene expression in tissues of polyuria rats as compared to the control rats.

Keywords: Vasopressin hormone receptor, Gene expression, Lithium, polyuria rats

INTRODUCTION

Arginine vasopressin (AVP) a neuro-hyphophysial hormone is synthesized in the supraoptic and paraventricular nuclei of the hypothalalmus. AVP is the major physiological regulator of renal water excretion. AVP acts on vasopressin V_2 receptors in the kidney and increases the water permeability of the renal distal tubules and collecting ducts, thereby accelerating water absorption. However, during some physiological and clinical circumstances, the renal ability to concentrate urine in response to AVP is decreased.

In humans and animals, sustained administration of AVP and water results in water retention with progressive hyponatremia for several days, which is then followed by variable degrees of escape from the vasopressin-induced antidiuresis. Vasopressin V_2 receptors belong to the seven-transmembrane domain, G protein-coupled receptor superfamily and are mainly present in the renal distal tubules and collecting ducts (5). Activation of vasopressin V_2 receptor leads to an increase in intracellular cAMP by stimulating adenylate cyclase activity through Gs (1,2). AVP regulates the water permeability of renal collecting tubule cells in two ways. Short term regulation is achieved by shuttling of aquaporin-$_2$ water channels from intracellular vesicles into the apical plasma membrane.

On the other hand, Lithium, a drug used for affective disorders like the manic depressive psychosis induces a state of polyuria and polydypsia. It has been suggested that the vasopressin V_2 receptor in the kidney and also the brain might become dysfunctional. Marples *et al* (3) have demonstrated an effect of lithium in the collecting duct of the rat to decrease the density of apical water channels (aquaporin-2), possibly explaining at least some of the lithium-induced nephrogenic diabetes insipidus. This however, does not exclude a role of vasopressin receptors. A direct effect of lithium on the renal V_2-receptor has only recently addressed by binding studies (4). These studies, however, have been hampered by the lack of a radioligand with high specific activity and high affinity for the renal AVP-receptor. However we used a specific radioligand to perform our studies. This study was hitherto conducted to ascertain the effect of Lithium carbonate treatment on the vasopressin receptor density and messenger RNA levels in the kidney and brain of Wistar albino rats.

MATERIALS AND METHODS

ANIMALS

Experiments were performed on Wistar albino rats of either sex weighing, between 180-220 g obtained from experimental animal center of Christian medical College, Vellore, India. Animals were housed in groups of 5-6 animals in polypropylene plastic cages under hygienic conditions, lined with paddy-husk bedding. Animals were housed in a colony room once the experiments completed under controlled temperature (25+/- degree C), relative humidity of (60+/- 2%) and were exposed to a 12 hour light :12 hour dark cycle, with food and water available *ad libitum*. Control group of animals received an injection of the vehicle (0.2ml) only. Test group of animals were treated with Lithium carbonate (20mg/kg i.p) for a duration of 11 days.

Receptor binding of vasopressin :

^{125}I AVP binding to membranes isolated from kidney and brains of untreated and Lithium carbonate treated Wistar rats was performed. Membranes (20 µg protein) were incubated in a total volume of 100µl for 1 hour at room temperature (25 ^{0}C) in 50 mM Tris.Cl (pH 7.4), 150 mM NaCl, 0.1 mM EDTA. 5mM $MgCl_2$, 0.1% BSA, 1µM Aprotinin amd 1mM PMSF. Binding was determined in the presence of increasing concentration of ^{125}I AVP. Non specific binding was estimated by including 10µM unlabelled AVP. To reduce non specific binding the filters were presoaked in polyethyleneimine for 1 hour. The incubations were terminated by the addition of 1 ml of ice cold wash buffer (50 mM Tris, 150mM NaCl pH 7.4 at 4 C) containing 0.2 % BSA. Bound ^{125}I AVP was separated from free ^{125}I AVP by rapid filteration through GF/C filter papers followed by three washes with 6ml wash buffer. Bound radioactivity was determined in a Gamma counter (Perkin Elmer, Model 1470, USA).

Extraction of RNA

Tissue samples were placed in 0.9 ml RNAzol (Qiagen, USA) and homogenized by repeated trituration with a 21-gauge needle and syringe. RNA was extracted by the addition of 0.2 ml chloroform for 15 minutes on ice and centrifuged for 6 minutes at 11500 x g at 4^{0} C. The aqueous layer transferred to a new tube, and chloroform extraction was again done. RNA was precipitated by the addition of 1 volume isopropranol for 15 hours at -20^{0} C, followed by centrifugation at 11500 x g for 30 minutes.

Estimation of AVP messenger RNA

The localization and optical density of AVP mRNA levels of kidney and brain of control and lithium treated animals was conducted. Total RNA was extracted and were then hybridized (overnight at 45°C) with a random primed 35S-dATP-labeled vasopressin cDNA-probe (American Type Culture Collection, Rockville, MD) in an final volume of 40µl. These were washed, dried, and subsequently exposed to Kodak Biomax X-ray film (Eastman- Kodak, Rochester, NY). Quantification of mRNA density in the kidney and brain was performed using a computer image analysis system (NIH Image).

Dot Blot hybridization

RNA samples were incubated at 72 ^{0}C for 5 minutes, chilled quickly and fractionated on a 1% agarose- 3% formaldehyde gel. After electrophoresis, gels were stained in 100 mM ammonium acetate containing 1 µg/ml ethidium bromide and destained in 25 mM sodium phosphate, pH 6.5. RNA was visualized and photographed under UV light. RNA were transferred to a nylon membraine overnight by capillary action and immobilized by UV crosslinking. Hybridization was performed at 50^{0}C for 16 hour in the same buffer with the addition of 100µl ^{32}P- labeled probe. Autoradiograms of hybridized blots were scanned (Bio-Rad Laboratories, USA) and the arbitrary densitometry unit measure of each band minus background for the film was used in calculating the relative abundance of mRNAs.

Statistical analysis

All results are expressed as the mean ± 6 SEM, and differences between groups were analyzed statistically using one-way ANOVA followed by *post-hoc* comparisons via the Student-Newman-Keuls test.

RESULTS

The results of receptor binding studies conducted in kidney and brain are shown in Table 1 and Table 2. As depicted in the two tables the results indicate a loss of AVP receptor density in both kidneys and brain of the lithium carbonate treated animals thus siginifying the possible reason for polyuria. The BMax values of the lithium treated animals were significantly lower ($P<0.05$) than the control group of animals.While there was less difference in the Kd values of the control and lithium group kidneys the brain Kd values were appreciably different. The control values of the kidney and brain mRNA was 44.3 pg/µg and 56.5 pg/µg of the total RNA respectively. The dot blot hybridization suggest that there was 23.5 % and 51.2 % increase in AVP mRNA quantity of the kidney and brain of lithium carbonate treated animals as compared to the control group of animals. These results are depicted in Figure 1 and Figure 2.

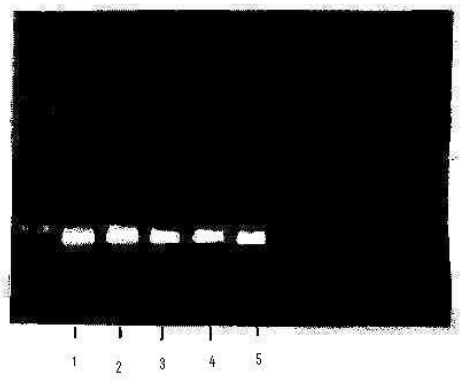

Figure 1 : Figure 1 shows the AVP mRNA in the kidney and brain of control and Lithium induced polyuria rats as detected by . Lane 1 : Kidney (Lithium), Lane 2 : Brain (Lithium), Lane 3 : Kidney (Control), Lane 4 : Brain (Control), Lane 5 : Standard (β-Actin)

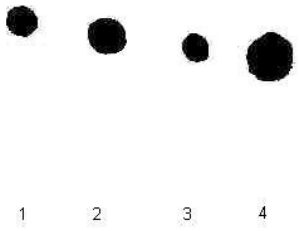

Figure 2: Dot blot hybridization of AVP mRNA isolated from the kidney and brain of control and lithium treated rats. No.1: Brain (Control), No.2: Brain (Lithium), No. 3: Kidney (Control), No.4 : Kidney (Lithium).

Table 1: Individual Kd and BMax values of vasopressin receptors of untreated and treated rat kidney membranes after Lithium injection of 20mg/kg i.p for 11 days.

Treatment	Kd (nmol/l)	BMax (nmol/kg)
Lithium		
1	0.97	119
2	0.73	97
3	0.67	99
4	1.23	134
5	0.78	91
6	0.87	105
Control		
1	0.52	201
2	0.55	193
3	0.47	132
4	0.64	113
5	1.11	113
6	0.93	122

Table 2 : Individual Kd and BMax values of vasopressin receptors of treated and untreated rat brain membranes after Lithium injection of 20mg/kg for 11 days.

Treatment	Kd (nmol/l)	BMax (nmol/kg)
Lithium		
1	0.77	107
2	0.79	103
3	0.68	99
4	0.68	96
5	0.72	111
6	0.74	101
Control		
1	0.81	126
2	0.87	129
3	0.84	136
4	0.90	134
5	0.92	138
6	0.95	147

DISCUSSION

The results of our study clearly demonstrate that vasopressin receptor density gets altered by lithium carbonate treatment and the expression of the AVP mRNA also get enhanced. These results are in tandem with earlier reports (5). It is well known that the majority of renal papillary vasopressin receptors are V_2-vasopressin-receptors from collecting ducts, and only few V_1 vasopressin receptors are present. Mutations in the vasopressin receptor can affect receptor binding to the vasopressin ligand (6) Taken together our study suggests that V_2-vasopressin receptors are reduced on basolateral membranes of renal papillary collecting duct cells. Similar changes might occur on cortical collecting tubules as well.

Although we did not measure cAMP generation in such tissue it is possible that it might be reduced to a comparable extent as was the density of AVP receptors and that this then might contribute to the lithium induced nephrogenic diabetes insipidus resulting in polyuria. The opposite, i.e. a concomitant increase in AVP receptor density and cAMP production was recently demonstrated after chlorpropamide treatment (7).

An imbalance between V_1 and V_2-vasopressin receptors might, however, also be of some importance. For instance, prostaglandins, stimulated by the AVP-vasopressin- receptor action of AVP, are well known to reduce the V_2-induced production of cAMP in collecting duct cells and thus their AVP-dependent increase in water permeability. Rather than just a diminishment in the number of AVP receptors, an imbalance between both receptor subtypes could also be involved in the lithium induced concentrating defect. It would thus be interesting to study the binding of both V_1 and V_2-vasopressin receptor ligands in the same membrane preparation. Lithium probably causes reciprocal changes in the secretion and action of vasopressin by diminishing the renal sensitivity to AVP, while increasing the sensitivity of the vasopressin secretory response to osmotic stimulation and it appears a similar action is seen in the brain. In our study, plasma vasopressin and plasma osmolality were not significantly increased after lithium.

In the present study, we developed a binding assay for the highly specific activity of the vasopressin V_2 receptor function. Under the assay conditions employed, only 20–25 µg membrane protein/ sample was needed for vasopressin V_2 receptor analysis. Thus, only one or two rats are required per saturation curve using [^{125}I]V_2RA compared with three to five rats using a tritiated ligand. Under these assay conditions, the binding of [^{125}I]V_2RA to rat kidney and brain membranes was shown to be rapid, saturable, dependent on the membrane protein concentration, and less than 20% of the total radiolabeled ligand concentration was bound (8). Thus, radioligand binding assay conditions were developed that meet the criteria necessary for determination of BMax and Kd using the Scatchard analysis.

In the genetic model of polyuria i.e the Brattleboro rat the mRNA levels were markedly increased in the hypothalamus (9,10). It has been shown that chronic hyper-osmolality causes an increase in AVP mRNA levels by two fold (11). The results of the studies conducted on the mRNA levels reveal that there was upregulation of the AVP gene in the kidney and brain after treatment of the animals with Lithium carbonate for a duration of 11 days. This may be due to inducement of chronic stress and possible role of corticotrophic hormones is not excluded (12). These results are in accordance with the earlier studies where there was upregulation of the AVP mRNA after lithium treatment in the hypothalamus of the rats (13). According to a study conducted by Arima *et al* neuronal activity was important for the regulation of vasopressin gene expression in the suprachiasmatic nucleus and this may be true for the brain (14). It is quite likely that renal nerves criss-crossing the kidney may contribute to the expression of the AVP mRNA in the kidney and a co-relation of the AVP action on adenylate cyclase in water deprived animals has been shown by this author in a earlier published work (15).

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