

Influence of hypovolemic and hypertonic treatments on plasma vasopressin levels and fluid balance in the propylthiouracil-induced hypothyroid rat

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Abstract

This study was undertaken to investigate the effects of hypovolemic and hypertonic treatments on plasma vasopressin (AVP) levels and fluid balance in propylthiouracil (PTU)-induced hypothyroidism in the rat. The influence of hypothyroidism on AVP responsiveness to hypertonic and hypovolemic stimuli were compared. Adult male rats were divided into two groups. Groups I and II were intraperitoneally (i.p.) injected with saline (1ml/250g) and PTU (10mg/kg/day), respectively, for a period of two weeks. These groups were further divided in three subgroups each containing six rats. The first subgroup consisted of unchallenged rats. I.P. 700 mg polyethylene glycol was used for hypovolemic treatment. The third subgroup consisted of hypertonic (1.5 M NaCl; 1ml/100 g) stimulated animals. All rats were decapitated and trunk blood collected in heparinized tubes. Plasma samples were stored at -20°C until assayed. Plasma AVP, T3 and T4 levels were measured by radioimmunoassay. Hematocrit values and plasma Na concentrations were also determined.

In the PTU-induced hypothyroid rats, hypertonic treatment caused lower increase in plasma AVP levels ($p < 0.05$) compared to the respective control animals. In the hypovolemic group, decreases in AVP responses were not found to be statistically significant. In conclusion, although hypothyroidism does not statistically change basal AVP levels, it may affect AVP response to hypertonic stimulus. It is not clear whether changes in fluid-electrolyte balance cause disturbance in AVP release in hypothyroidism or vice versa. Therefore, these preliminary findings need to be confirmed by further investigations.

Introduction

Vasopressin (AVP) is released from nerve terminals of magnocellular neurones in the neurohypophysis and also from parvocellular neurones in the median eminence [1]. Its release is primarily determined by changes in plasma osmolality [2, 3]. This mechanism ensures the regulation of water balance and the maintenance of plasma solute concentration such that an increase in plasma osmolality results in an increased release of AVP from the posterior pituitary [4]. Consequently, more water is reabsorbed from the collecting ducts as a result of AVP's action on the kidney (via V2 receptors) and the plasma osmolality falls. A second pathway involved in the control of AVP secretion concerns changes in blood volume. This volumetric control mechanism is regulated by volume receptors (V1 receptors) located in the cardiovascular system [1]. AVP response to both hypertonic and hypovolemic stimuli may be modulated by some endocrine mechanisms [4, 5].

There are contradictory reports regarding the putative effects of the thyroid hormones, triiodothyronine (T3) and thyroxine (T4), on plasma osmolality and fluid balance [6, 7, 8]. Capacity of the kidney to concentrate urine does not develop normally in congenital hypothyroid rats, although their vasopressinergic axis responds [6]. Parallel increases in thyrotropin (TSH) and AVP levels in hypothyroidism have been taken to implicate that this neurohypophysial hormone may function as TSH [9, 10]. In propylthiouracil (PTU, a specific inhibitor of type-I deiodinase) induced hypothyroid rats, vasopressin mRNA levels are increased in the supraoptic and paraventricular nucleus of the hypothalamus [11]. However, it has been suggested that thyroideectomy has no effect on AVP mRNA levels in the hypothalamus [12]. Inhibitory effects of AVP on TSH have also been proposed in vitro [9].

It has been reported that thyroparathyroidectomy causes reduction in glomerular filtration, acid excretion and number of proximal tubular cells [8]. Administration of PTU resulted in significant decreases in plasma AVP levels and number of AVP receptors in the kidney and liver [13].

Although AVP response to hypertonicity in hypothyroidism has been studied, there is no report regarding the possible influence of hypovolemia. In the present study, we have investigated the effects of hypovolemic and hypertonic treatments on plasma AVP levels and fluid balance in PTU-induced hypothyroidism in the rat. The effects of hypothyroidism on AVP responsiveness to hypertonic and hypovolemic stimuli were compared.

Materials and methods

Adult male Wistar rats (Firat University Biomedical Unit, Elazig, Turkey) weighing 240–300 g were maintained under controlled temperatures ($21\pm1^{\circ}\text{C}$) and light conditions. Food and water were supplied *ad libitum*. The animals were divided into two groups each containing 18 rats. Group I was designated as a control group which received physiological saline (1ml/250 g, intraperitoneally i.p.). The animals in group II were i.p. injected with PTU (10mg/kg/day; Sigma Chemical Co., Dorset, UK) for a period of two weeks.

These two main groups were further divided in three subgroups each containing six rats. The first subgroup consisted of unchallenged rats. I.P. 700 mg polyethylene glycol (Mr 4000/kg body weight in 0.15 M NaCl/1; Sigma Chemical Co., Dorset, UK) was used for hypovolemic treatment. The third subgroup consisted of hypertonic stimulated animals with 1.5 M NaCl (1ml/100 g). All animals were decapitated following hypertonic (15 mins.) and hypovolemic (60 mins.) manipulation between 09.00-10.00 hrs in the morning. Trunk blood samples (5.5-6 ml) were collected in heparinized tubes. They were centrifuged (3,000 r.p.m.) for 10 minutes and plasma was transferred into fresh tubes. Plasma samples were stored at -20°C until assayed.

Heparinized capillary tubes were used to collect another series of blood samples for hematocrit measurement. They were centrifuged at 10,000 r.p.m. for five minutes and then values at the hematocrit scale were read. Plasma Na levels were determined by using an auto analyser (Ciba Corning Express Auto Analyser I.S.E.).

Plasma AVP levels were measured by radioimmunoassay (RIA) by using arginine AVP reagents (Diagnostic Systems Laboratories Inc, Kent, UK). The method for AVP extraction and assay has been detailed elsewhere [14]. Total T3 and T4 levels were also determined by RIA. Reagents for the T3 and T4 assays were obtained from Johnson & Johnson Clinical Diagnostics Ltd. (Amersham, UK). Details of the method have been previously reported [15].

The results were statistically analyzed by One-Way ANOVA (MINITAB, release 10 for Windows). The level of significance was set at $p<0.05$.

Results

In the PTU-induced hypothyroid rats, hypertonic treatment significantly decreased plasma AVP levels ($p<0.05$) compared to the respective control animals (Figure 1). In the unchallenged and hypovolemic groups, although plasma AVP concentrations were

reduced, they were not significantly different from the respective vehicle-treated rats (Figure 1).

Hematocrit values and plasma sodium (Na) concentrations were not significantly altered in the PTU-induced hypothyroid rats following hypovolemic and hypertonic treatments or in the unchallenged rats within the groups (Table 1). However, hematocrit values in the control and hypothyroid rats following hypovolemic stimulation were found to be significantly increased compared to respective unchallenged and hypertonic groups ($p<0.05$). Plasma Na levels were significantly increased in the control and hypothyroid animals following hypertonic stimulation compared to respective unchallenged and hypovolemic groups ($p<0.05$).

Total plasma T3 and T4 levels were significantly decreased in PTU-induced hypothyroid rats (Table 1). At the end of the experiments, body weights were determined and no significant changes were observed between the groups (Table 1).

Discussion

In this study, the changes in hematocrit values and plasma Na concentrations indicate that hypovolemic and hypertonic manipulations were effective. Efficacy of PTU treatment in inducing hypothyroidism was evaluated with the marked decreases in plasma levels of the thyroid hormones.

Hypothyroidism is known to affect fluid-homeostasis since it has been associated with abnormalities in renal water handling [16, 17]. However, the mechanisms

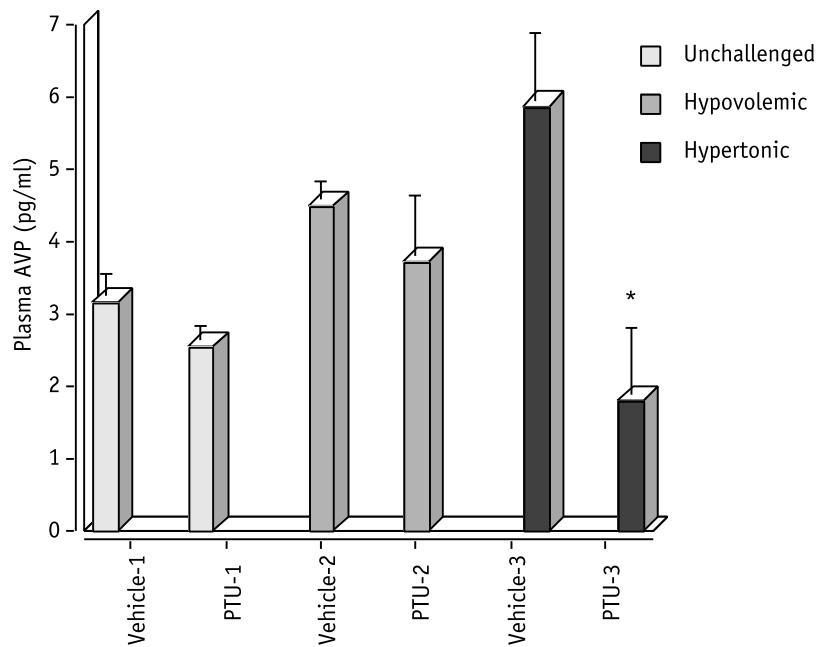


Figure 1. Plasma AVP concentrations in saline-treated and PTU-induced hypothyroid rats. Effects of hypovolemic and hypertonic treatments in these rat models were compared against to the vehicle-treated group. *: $p<0.05$ using One-Way ANOVA.

by which hypothyroidism produces such effects are currently unknown. Recently an indirect role has been attributed to AVP in this process [17].

In the present study, PTU-induced hypothyroidism did not result in a significant increase in plasma AVP levels in response to hypertonic stimulus in contrast to the previously reported findings [6]. Use of adolescent animals (aged 35 days) in Ali's and colleagues' work and adult rats in our study may account for this discrepancy. In the PTU-treated group, hypertonic manipulation caused higher increases in plasma Na concentrations compared to the responsive control animals. Moreover, plasma AVP levels of the PTU-induced hypothyroid rats were even lower than the unchallenged PTU group. These prelim-

Table 1. Plasma T3, T4, Na concentrations, hematocrit values and body weights in saline-treated and PTU-induced hypothyroid rats following hypovolemic, hypertonic stimuli and in the unchallenged group. **a:** $p<0.001$, **b:** $p<0.01$, **c:** $p<0.05$ compared to respective saline (vehicle)-treated animals; **d:** $p<0.05$ compared to respective unchallenged and hypertonic groups; **e:** $p<0.05$ compared to respective unchallenged and hypovolemic rats, using One-Way ANOVA.

	Unchallenged		Hypovolemic		Hypertonic	
	Vehicle	PTU	Vehicle	PTU	Vehicle	PTU
Body weight (gr)	230.8±4.9	235.0±7.1	225.3±2.8	233.3±2.8	229.2±5.4	236.6±1.7
Hematocrit (%)	38.0±1.6	37.3±0.6	43.8±2.4 d	42.0±1.7 d	36.6±2.7	36.0±1.2
Plasma Na (mmol/lt)	141.1±1.3	144.1±0.5	142.7±2.3	143.8±1.1	146.2±3.5 e	155.3±2.8 e
Total T3 (ng/dl)	46.1±12.1	7.9±2.6 a	43.2±11.3	7.3±3.0 a	34.9±8.3	8.5±2.8 c
Total T4 (mg/dl)	4.2±0.5	0.6±0.3 a	6.2±1.7	1.2±0.7 b	4.0±0.3	1.0±0.2 a

inary findings seem somewhat controversial since more hypertonicity would be expected to produce higher AVP release. Even though some studies have provided indirect evidence for the interaction of AVP release and the thyroid function, no conclusive work has been reported. Thyroidectomy did not alter hypothalamic AVP expression in chronically hypernatremic male rats [18]. Yonemura et al [19] have suggested that disturbance in plasma AVP regulation results from an altered glucocorticoid metabolism following thyroidectomy since hyponatremia did not suppress plasma concentrations of AVP. Similarly, the present study has shown that hypernatremia did not bring about increases in plasma AVP levels in the PTU-induced hypothyroidism. Whether glucocorticoid metabolism is altered by PTU-induced hypothyroidism remains to be investigated in a further study. Alternatively, failure of higher Na concentrations to increase plasma AVP levels may result from a change in osmotic threshold following hypothyroidism.

In the present study, hypertonicity was found to be less efficient to increase AVP levels compared to hypovolemic stimulus. This may be caused by an alteration in the plasma AVP-osmolality relationship [16].

In conclusion, although hypothyroidism does not statistically change basal AVP levels, it may affect AVP response to hypertonic stimulus. It is not clear whether changes in fluid-electrolyte balance cause disturbance in AVP release in hypothyroidism or vice versa. Therefore, these preliminary findings need to be confirmed by further investigations.

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