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ORIGINAL ARTICLE

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

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Abstract

OBJECTIVES: This study was undertaken to investigate the effects of hypovolemic and hypertonic treatments on plasma vasopressin (AVP) levels and fluid balance in thyroidectomy-induced hypothyroidism in the rat. The influence of hypothyroidism on AVP responsiveness to hypertonic and hypovolemic stimuli were compared.

MATERIALS & METHODS: Adult male rats were divided into two groups. The rats were surgically thyroidectomized (hypothyroid) or sham-operated (euthyroid). Two weeks later these groups were further divided in three subgroups each containing six rats. The first subgroup consisted of unchallenged rats. The second group underwent hypovolemic treatment by using I.P. 700 mg polyethylene glycol. The third subgroup consisted of hypertonic (1.5 M NaCl; 1 ml/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 and K concentrations were also determined.

RESULTS: In the hypothyroid rats, hypovolemic treatment significantly reduced the expected increases in plasma AVP levels ($p<0.05$) compared to the respective intact animals. In the hypertonic group, similar increases occurred in plasma AVP levels of hypothyroid and euthyroid rats. Hematocrit values and plasma Na concentrations were not significantly different in the hypothyroid rats compared to euthyroid rats.

CONCLUSION: In conclusion, thyroidectomy-induced hypothyroidism may affect AVP response to hypovolemic stimulus although it has no important effect on basal AVP levels nor AVP response to hypertonic stimulus.

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].

Even though it is suggested that hyper- and hypothyroid rats exhibit hyper and hypo-responsiveness of AVP secretion to osmotic stimuli, respectively [6], there are contradictory reports regarding the putative effects of the thyroid hormones, triiodothyronine (T3) and thyroxine (T4), on plasma osmolality and fluid balance [7, 8, 9]. Capacity of the kidney to concentrate urine does not develop normally in congenital hypothyroid rats, although their vasopressinergic axis responds [7]. Parallel increases in thyrotropin (TSH) and AVP levels in hypothyroidism have been taken to implicate that this neurohypophyseal hormone may function as TSH [10, 11]. In propylthiuracil (PTU, a specific inhibitor of type-I deiodinase) induced hypothyroid rats, vasopression mRNA levels are increased in the suprooptic and paraventricular nucleus of the hypothalamus [12]. However, it has been suggested that thyroidectomy has no effect on AVP mRNA levels in the hypothalamus [13]. Inhibitory effects of AVP on TSH have also been proposed in vitro [10].

It has been reported that thyroparathyroidectomy causes reduction in glomerular filtration, acid excretion and number of proximal tubular cells [9]. Administration of PTU resulted in significant decreases in plasma AVP levels and number of AVP receptors in the kidney and liver [14].

In our previous study [15], hypertonic treatment significantly reduced the expected increases in plasma AVP levels compared to the respective control animals in the PTU-induced hypothyroid rats. In the unchallenged and hypovolemic groups, decreases in plasma AVP levels were not found to be statistically significant. So, in that study, it was concluded that PTU-induced hypothyroidism may affect AVP response to hypertonic stimulus rather than hypovolemic stimulus.

In the present study, we have investigated the effects of hypovolemic and hypertonic treatments on plasma AVP levels and fluid balance in surgically thyroidectomy-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 temperature (21±1°C) and light conditions (lights on between 07.00 h and 19.00 h). Food and water were supplied *ad libitum*. The animals were divided into two groups each containing 18 rats. Group I was sham-operated. The animals in group II were surgically thyroidectomized and the parathyroids were implanted into the sternocleidomastoid muscle. After a period of two weeks, both 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 (1 ml/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 (3000 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 and K 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 [16]. RIA also determined total triiodotronin (T3) and tyroxin (T4) levels. 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 [17].

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 thyroidectomy-induced hypothyroid rats, hypovolemic treatment significantly reduced the expected plasma AVP levels ($p<0.001$) compared to the respective euthyroid animals (Fig. 1). In the hypertonic group, there was no significant difference between plasma AVP concentrations of hypothyroid and euthyroid rats (Fig. 1). In the unchallenged group, plasma AVP levels were similar in hypothyroid and euthyroid rats.

Hematocrit values and plasma sodium (Na) concentrations were significantly altered in the hypothyroid rats following hypovolemic and hypertonic treatments, respectively compared to the unchallenged group (Table 1). Although plasma potassium (K) concentrations were not significantly altered in the unchallenged group, they were significantly reduced in the hypovolemic and hypertonic subgroups (Table 1).

Total plasma T3 and T4 levels were significantly decreased ($P<0.001$) in thyroidectomy-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).

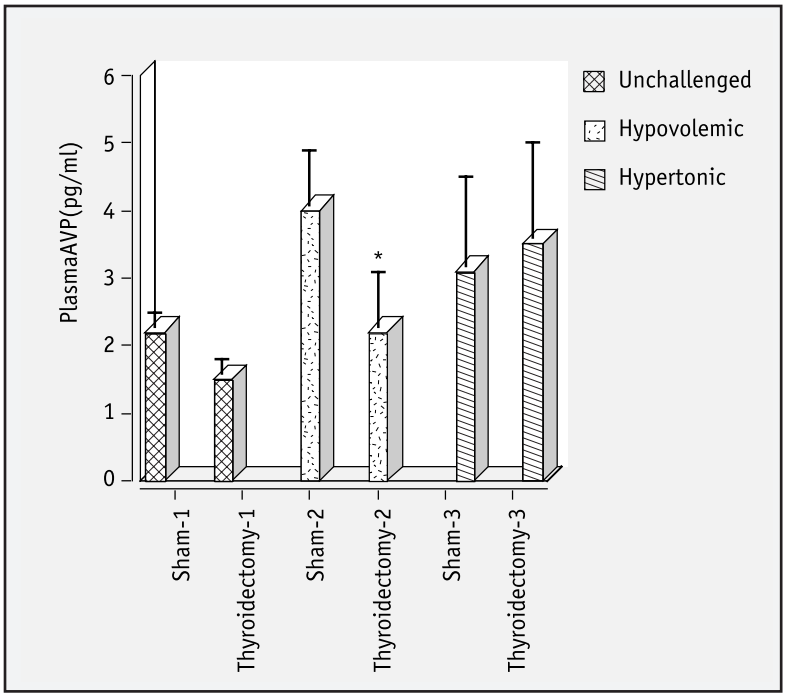

Fig. 1. Plasma AVP levels (Mean+SEM) in sham-operated and surgically thyroidectomized rats following hypovolemic, hypertonic stimuli and in the unchallenged group. * $p<0.001$ compared to sham-operated group.

Discussion

In this study, the changes in hematocrit values and plasma Na concentrations show that hypovolemic and hypertonic manipulations were effective. The fact that there are not any significant changes in hematocrit and plasma sodium levels between the groups shows a similar decrease in blood volume and increase in hypertonicity in the challenged groups. Efficacy of thyroidectomy 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 [18, 19]. However, the mechanisms by which hypothyroidism produces such effects are currently unknown. It may be caused by an alteration in the plasma AVP-osmolality relationship [18]. Recently an indirect role has been attributed to AVP in this process [19].

	Unchallenged		Hypovolemic		Hypertonic	
	Sham-Tx	Tx	Sham-Tx	Tx	Sham-Tx	Tx
Body weight (gr)	230.0±4.1	232.5±4.1	230.0±4.7	226.7±3.8	232.5±4.4	221.7±5.6
Hematocrit (%)	37.7±0.8	33.7±1.7	50.8±0.9	48.3±1.5	39.2±0.7	38.2±1.2
Plasma Na (mmol/lt)	139.7±2.5	137.2±3.8	138.0±3.9	145.8±3.7	146.1±4.4	150.3±5.8
Plasma K (mmol/lt)	6.5±0.9	6.0±0.3	7.2±0.3	5.4±0.2 [a]	5.8±0.2	4.5±0.3 [b]
Total T3 (ng/dl)	40.7±10.3	10.6±1.9[c]	32.7±1.6	13.04±1.94 [b]	29.8±7.5	9.7±1.5 [b]
Total T4 (mg/dl)	2.06±0.6	0.9±0.4[c]	3.4±0.4	1.14±0.37 [c]	2.1±0.9	0.7±0.3 [c]

Table 1. Plasma T3, T4, Na, K concentrations, hematocrit values and body weights in sham-operated and thyroidectomy-induced hypothyroid rats following hypovolemic, hypertonic stimuli and in the unchallenged group. a: $p<0.0001$, b: $p<0.001$, c: $p<0.05$ compared to respective sham-operated-animals using One-Way ANOVA.

In the present study, as a new finding, thyroidectomy-induced hypothyroidism not only suppressed an increase in plasma AVP levels in response to hypovolemic stimulus but also prevented completely the response to hypovolemic stimulus in contrast to the previously reported findings [15]. PTU-induced-hypothyroidism did not bring about a significant increase in plasma levels in response to hypertonic stimulus whereas it produced a significant increase in plasma AVP levels in response to hypovolemic stimulus. The different response in AVP secretion to hypovolemic and hypertonic stimuli in PTU or thyroidectomy-induced rats is to be determined. It can be suggested that PTU may itself cause a decrease centrally or peripherally in the sensitivity of the neuroendocrine reflex controlling AVP secretion osmotically whereas thyroid hormones may have an effect only in the regulation of volume control. This hypothesis needs to be confirmed by further studies.

Our finding that thyroidectomy did not alter AVP response in response to hypertonic stimulus is consistent with the results of a previous study [20] where thyroidectomy did not alter hypothalamic AVP expression in chronically hypernatremic males although significant increases in paraventricular (PVN) AVP mRNA were found in hypothyroid rats receiving hypertonic stimulus.

Even though some studies have provided indirect evidence for the interaction of AVP release and the thyroid function, no conclusive work has been reported. Yonemura et al. [21] 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, and hypernatremia did not increase plasma concentration of vasopressin. Contrarily, the present study has shown that hypernatremia brought about increases in plasma AVP levels in the thyroidectomy-induced hypothyroidism. Because in our study hyponatremia following thyroidectomy did not occur, any alteration in glucocorticoid metabolism, which prevented AVP response to hypertonic stimulus, can be ruled out.

In conclusion, although thyroidectomy does not statistically change basal AVP levels, it may affect AVP response to hypovolemic stimulus. The mechanism by which thyroidectomy manipulates AVP response to hypovolemia is unclear. Also, the importance of this result needs further studies.

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