Osmotic Control of the Release of Prolactin and Thyrotropin in Euthyroid Subjects and Patients with Pituitary Tumors

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The effects of acute changes in serum osmolality on basal serum PRL and TSH levels and on responses of prolactin (PRL) and thyrotropin (TSH) to the thyrotropin-releasing hormone (TRH) analogue, N^{3im}-methyl-TRH, were studied in ten euthyroid subjects and in three patients with PRL-secreting pituitary tumors. An oral water load of 20 ml/kg had no effect on basal serum PRL or TSH levels but did result in an increased PRL response to methyl-TRH in the ten euthyroid patients. Intravenous infusion of 5% sodium chloride in the ten euthyroid subjects significantly depressed basal serum PRL levels but had no effect on the PRL response to methyl-TRH. Infusion of hypertonic saline significantly decreased the TSH response to methyl-TRH. In the three patients with pituitary tumors, oral water loading and hypertonic saline infusion had no significant effect on the basal serum PRL and TSH or the PRL and TSH responses to methyl-TRH. The patients with pituitary tumors had a higher basal serum osmolality and a proportionately higher serum concentration of arginine vasopressin than the euthyroid patients. These data suggest that changes in osmolality in euthyroid patients may have a direct effect on the anterior pituitary's PRL and TSH response to a releasing factor.

A CONSIDERABLE BODY of evidence suggests that prolactin (PRL) has played a critical role in the adaptation of teleost fish to a fresh water environment by causing the retention of salt.[1-3] Other studies have shown an effect of PRL on the mammalian kidney. Lockett and Nail reported that administration of ovine PRL decreased the urinary excretion of sodium in the rat and the cat.[4,5] Burstyn et al. and Horrobin et al. showed that administration of ovine PRL caused sodium retention in sheep and man, respectively.[6,7] The role of PRL in the physiologic regulation of osmolality and the osmolar control of PRL release in man is controversial. Buckman and co-workers reported that an oral water load suppressed serum PRL to less than 50% of baseline in normal subjects and individuals with "functional galactorrhea," but had no significant effect on PRL levels in patients with pituitary tumors.[8] Subsequently, Buckman and Peake reported that infusion of hypotonic saline markedly lowered serum PRL, while infusion of hypertonic saline markedly elevated the serum PRL levels in normal subjects.[9] In contrast, Adler et al. reported a small increase in plasma PRL after oral water loading in men but not in women, and no effect of intravenous infusion of either hypotonic or hypertonic saline

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on plasma PRL.[10] Recently, Wartofsky et al. reported that water loading had no effect on basal serum PRL and thyrotropin (TSH) or on thyrotropin-releasing hormone (TRH) stimulated plasma PRL and TSH in hypothyroid subjects.[11] In this paper we report the effects of oral water loading and hypertonic saline infusion on serum PRL and TSH before and after administration of the N^{3im}-methyl analogue of TRH (methyl-TRH)[12] in euthyroid subjects and patients with PRL-secreting pituitary tumors.

MATERIALS AND METHODS

Assays

Serum osmolality was measured on an Advanced Instruments Wide-Range Osmometer. Serum arginine vasopressin (AVP) was measured by the double antibody radioimmunoassay described by Skowsky et al.[13] The mean basal serum AVP was 1.4 ± 1.3 (SEM) μU/ml in 30 normal controls allowed water ad libitum, and the sensitivity of the assay was 0.3–0.5 μU/ml. Serum PRL was measured by minor modification of the homologous radioimmunoassay described by Sinha and co-workers.[14] The mean basal serum PRL was 5.7 ± 3 (SD) ng/ml in 35 normal male controls and 7.7 ± 4.3 ng/ml in 22 normal female controls. Serum TSH was measured by the double antibody method described by Pekary et al.[15] The mean basal TSH in 40 normal controls was 1.5 ± 1.0 (SD) μU/ml.

Oral Water Load in Euthyroid Subjects

Ten euthyroid volunteers (2 females, 8 males) ages 21–57 were fasted and fluid deprived overnight. An indwelling needle was placed in an antecubital vein at about 8:30 a.m. (at least 1 hr after the subjects awoke) and kept patent by infusion of isotonic saline at a rate of approximately 1.5 ml/min. The subjects were sitting throughout the studies except to walk to and from the toilet. Blood samples for measurement of serum osmolality, PRL, and TSH were drawn at -60 min, at -30 min, and at 0 min just prior to administration of a 100 μg i.v. bolus of methyl-TRH. Tap water was given in a dose of 20 ml/kg body weight between -60 and -30 min. Blood samples were obtained at 10, 15, 30, 45, 60, and 90 min after injection of 100 μg of methyl-TRH.

Hypertonic Saline Infusion in Euthyroid Subjects

Approximately 7 days after the oral water load study the same ten volunteers were fasted and fluid deprived overnight. Blood samples were obtained for measurement of serum osmolality, PRL, TSH, and AVP at -60 min, at -30 min, and at 0 min prior to i.v. administration of 100 μg of methyl TRH. Between -60 and -30 min 5% sodium chloride was infused (3.0 ml/kg of body weight). Blood samples were drawn at 10, 15, 30, 45, and 60 min after injection of methyl-TRH.

No Pretreatment in Euthyroid Subjects

Seven days after the saline infusion the ten euthyroid volunteers were fasted overnight. After two baseline samples of blood for measurement of PRL and TSH were drawn (-15 and 0 min), the subjects were given 100 μg of methyl-TRH. Blood samples were obtained at 10, 15, 30, 45, 60, and 90 min after injection of methyl-TRH.

Patients With PRL-secreting Tumors

Three euthyroid patients with PRL-secreting pituitary tumors (receiving no medications) were given methyl-TRH after an oral water load, hypertonic saline infusion, or no pretreatment, and measurements made in exactly the same manner as that described for the ten euthyroid subjects.

Statistical comparisons of the serum osmolality, PRL and TSH before and after an oral water load and hypertonic saline infusion were performed using a paired Student's t test.

The PRL and TSH responses to methyl-TRH after the three pretreatment conditions were compared using Dunnett's multiple comparison procedure.[16]

RESULTS

Oral Water Load and Hypertonic Saline in Euthyroid Subjects

Results in Table 1 show that the water load decreased the serum osmolality in the ten euthyroid subjects from 290.8 ± 2.0 to 283.3 ± 2.1 mOsm/kg H_2O at 2 hr ($p < 0.001$). There was no significant change in the serum PRL or the serum TSH level after the oral water load. Table 2 shows that the maximum PRL response to 100 μg methyl-TRH after an oral water load, 21.0 ± 2.2 ng/ml, was slightly greater ($p < 0.01$) than the maximum PRL response to methyl-TRH with no pretreatment, 17.1 ± 2.1 ng/ml. There was no significant difference in the max ΔTSH response to methyl-TRH after an oral water load.

Table 1 shows that the hypertonic saline infusion increased the serum osmolality in the ten euthyroid subjects from 291.0 ± 2.2 to 301.0 ± 4.3 mOsm/kg H_2O ($p < 0.001$). There was a small but significant decrease in the serum PRL level after hypertonic saline infusion—7.5 ± 0.5 to 6.2 ± 0.5 ng/ml ($p < 0.01$); however, there was no significant change in the serum TSH level. Table 2 shows that the max ΔPRL response to 100 μg methyl-TRH after pretreatment with hypertonic saline infusion was not significantly different from the max ΔPRL response to methyl-TRH after no pretreatment. The max ΔTSH response to methyl-TRH after pretreatment with an infusion of hypertonic saline (7.7 ± 1.8 μU/ml) was significantly less than the max ΔTSH response to methyl-TRH after no pretreatment (10.9 ± 2.2 μU/ml; $p < 0.05$). There was no discernible effect of methyl-TRH on the AVP levels in either the normal subjects or the patients with PRL-secreting tumors. However, following the termination of the hypertonic saline infusion, there was the expected fall in AVP toward preinfusion levels; this could mask a depression in AVP resulting from methyl-TRH administration.

Oral Water Load and Hypertonic Saline in Patients with PRL-secreting Pituitary Tumors

Results in Table 1 show that the water load decreased the serum osmolality in the three patients with PRL-secreting tumors from 297.0 ± 2.1 to 290.1 ± 1.9 mOsm/kg H_2O ($p < 0.001$). There was no significant change in PRL or TSH following an oral water load.

The hypertonic saline infusion increased the serum osmolality in the three patients with PRL-secreting tumors from 299.3 ± 1.9 to 322.7 ± 4.3 mOsm/kg H_2O. There was no significant change in the serum PRL or TSH after hypertonic saline infusion. Table 2 shows that the max ΔPRL and max ΔTSH response to methyl-TRH after pretreatment with oral water loading or hypertonic saline infusion were not significantly different from the responses to methyl-TRH after no pretreatment.

Table 1 shows that the mean serum AVP after overnight fluid deprivation was greater ($p < 0.05$) in the three patients with PRL-secreting tumors (9.3 ± 2.7 μU/ml) than in the ten euthyroid subjects (4.5 ± 0.7 μU/ml). After hyper-

Table 1. Mean (± SEM) Response of Serum Osmolality, PRL, TSH, and AVP in Ten Euthyroid Subjects and Three Patients With PRL-secreting Tumors to Administration of 100 μg Methyl-TRH at 0 Time*

	Time (min)									
	−60	−30	−15	0	+10	+15	+30	+45	+60	+90
Euthyroid subjects										
No pretreatment										
Prolactin (ng/ml)			5.9 ± 0.4	5.9 ± 0.4	21.9 ± 2.9	22.9 ± 2.3	20.4 ± 2.6	17.0 ± 2.4	14.1 ± 2.0	10.6 ± 1.3
TSH (μU/ml)			1.8 ± 0.4	1.7 ± 0.4	6.2 ± 1.4	9.4 ± 2.1	11.9 ± 2.4	12.6 ± 2.7	11.3 ± 2.4	9.6 ± 2.2
Oral water load										
Osm. (mOsm/kg H_2O)	290.8 ± 2.0	284.6 ± 2.1		283.3 ± 2.1	283.0 ± 2.2	283.0 ± 2.2	282.7 ± 2.3	283.5 ± 2.3	284.2 ± 2.3	285.1 ± 2.5
Prolactin (ng/ml)	6.5 ± 0.5			6.1 ± 0.5	23.5 ± 2.9	26.0 ± 3.0	23.0 ± 2.8	18.9 ± 2.5	15.7 ± 2.3	11.0 ± 1.1
TSH (μU/ml)	1.7 ± 0.4			1.3 ± 0.3	4.2 ± 1.0	8.5 ± 1.9	10.2 ± 2.2	11.5 ± 2.2	8.0 ± 1.8	6.6 ± 1.5
5% NaCl infusion										
Osm. (mOsm/kg H_2O)	291.0 ± 2.2	301.5 ± 5.0		301.0 ± 4.3	298.4 ± 3.3	297.3 ± 2.9	296.3 ± 2.3	294.1 ± 2.3	289.0 ± 2.1	
Prolactin (ng/ml)	7.5 ± 0.5			6.2 ± 0.5	20.5 ± 2.6	24.5 ± 2.2	21.4 ± 2.3	18.4 ± 2.0	15.7 ± 1.7	
TSH (μU/ml)	1.2 ± 0.3			1.1 ± 0.3	3.5 ± 1.0	6.4 ± 1.6	8.5 ± 1.9	7.1 ± 1.3	7.1 ± 1.7	
AVP (μU/ml)	4.5 ± 0.7	7.1 ± 0.9		7.1 ± 0.9		6.0 ± 0.6	5.2 ± 0.7	4.8 ± 0.6	4.0 ± 0.9	
PRL-tumor patients										
No pretreatment										
Prolactin (ng/ml)			252.3 ± 96	241.6 ± 96	246.8 ± 92	250.8 ± 81	253.1 ± 87	254.7 ± 79	250.8 ± 86	243.7 ± 88
TSH (μU/ml)			1.1 ± 0.5	1.0 ± 0.4	4.2 ± 2.0	6.8 ± 3.8	8.0 ± 3.9	8.0 ± 3.2	7.3 ± 3.0	5.9 ± 2.2
Oral water load										
Osm. (mOsm/kg H_2O)	297.0 ± 2.1	291.8 ± 2.1		290.1 ± 1.9	289.6 ± 2.0	290.1 ± 2.1	291.7 ± 2.4	292.6 ± 2.5	293.2 ± 2.6	293.9 ± 2.4
Prolactin (ng/ml)	230.9 ± 98			258.0 ± 96	236.3 ± 97	250.8 ± 98	246.3 ± 91	246.5 ± 93	244.5 ± 95	252.2 ± 97
TSH (μU/ml)	0.6 ± 0.2			0.5 ± 0.2	2.3 ± 1.4	3.6 ± 2.5	5.5 ± 2.5	5.3 ± 2.9	4.8 ± 2.4	3.9 ± 1.5
5% NaCl infusion										
Osm. (mOsm/kg H_2O)	299.3 ± 1.9	323.1 ± 3.9		322.7 ± 4.3	311.2 ± 4.1	307.0 ± 4.0	302.0 ± 3.5	299.1 ± 3.0	300.0 ± 3.1	
Prolactin (ng/ml)	236.4 ± 94			213.2 ± 91	211.5 ± 84	228.5 ± 81	232.0 ± 89	239.1 ± 91	236.6 ± 92	
TSH (μU/ml)	0.6 ± 0.2			0.6 ± 0.2	1.2 ± 0.7	4.0 ± 2.3	5.3 ± 2.4	6.4 ± 2.6	5.3 ± 2.0	
AVP (μU/ml)	9.3 ± 2.7	12.4 ± 3.8		13.7 ± 3.3		8.4 ± 3.4	8.4 ± 3.4	7.4 ± 3.1	6.6 ± 2.6	

*Subjects received no pretreatment, pretreatment with an oral water load, or hypertonic saline infusion.

Table 2. Mean (± SEM) Max ΔTSH and Max ΔPRL Responses in Ten Euthyroid Subjects and Three Patients With PRL-secreting Tumors After 100 μg of Methyl-TRH i.v.*

Type of Pretreatment	Euthyroid Subjects		Subjects With PRL Tumors	
	Max ΔTSH	Max ΔPRL	Max ΔTSH	Max ΔPRL
None	10.9 ± 2.2	17.1 ± 2.1	7.5 ± 3.5	20.2 ± 13.9
Oral water load	9.0 ± 2.1	21.0 ± 2.2†	5.3 ± 3.3	15.9 ± 11.7
Hypertonic saline infusion	7.7 ± 1.8†	19.0 ± 2.0	5.2 ± 2.3	24.0 ± 14.0

*Subjects received no pretreatment, pretreatment with an oral water load, or hypertonic saline infusion.

†Significant difference in the max Δ response after pretreatment with either an oral water load or hypertonic saline infusion compared with the max Δ response after no pretreatment.

tonic saline infusion the serum AVP was greater ($p < 0.01$) in the three patients (13.7 ± 3.3 μU/ml) than in the ten normal subjects (7.1 ± 0.9 μU/ml). The mean serum osmolality after overnight fluid deprivation and the increase in serum osmolality after hypertonic saline infusion were also greater ($p < 0.01$) in the three patients with PRL-secreting tumors than in the ten euthyroid patients.

DISCUSSION

Our finding that serum PRL was slightly but significantly depressed following administration of hypertonic saline in euthyroid subjects is consistent with the role of PRL in causing salt retention, which has been repeatedly demonstrated in experimental animals.[1-6] Although an oral water load had no significant effect on the basal serum PRL levels, in contrast with the findings of Buckman et al.,[8,9] there was a significantly greater PRL response to methyl-TRH following pretreatment with an oral water load. This provides evidence that osmolality may alter the anterior pituitary lactotrophs' response to a PRL-releasing factor. Our finding of a slight but significant decrease in TSH response to methyl-TRH following pretreatment with a hypertonic saline infusion was surprising, and was possibly related to an effect of osmolality on the thyrotroph responsiveness to TRH stimulation. In contrast to the findings in euthyroid subjects, oral water loading and hypertonic saline infusion did not alter basal serum PRL levels nor PRL response to methyl-TRH in the patients with PRL-secreting tumors.

The patients with PRL-secreting tumors displayed a higher serum osmolality after overnight fluid deprivation than did the euthyroid patients. The basal serum AVP was elevated appropriately for the serum osmolality in the patients with PRL-secreting tumors. The greater elevations in serum osmolality after overnight fluid deprivation in these patients possibly reflects a decrease in urinary osmolar clearance, which has previously been observed after ovine PRL infusion in man[7] and in patients with PRL-secreting tumors.[17]

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