

Normal pituitary hormone response to thyrotrophin and gonadotrophin releasing hormones in subjects exposed to elemental mercury vapour

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Abstract

Exposure to elemental mercury (Hg) vapour results in an accumulation of Hg in the pituitary, the thyroid, and the testis. In this study, basal serum concentrations of pituitary hormones (thyrotrophin (TSH), prolactin (PRL), follicle stimulating hormone (FSH), and luteinising hormone (LH)) or their response after administration of thyrotrophin and gonadotrophin releasing hormones did not differ between 11 male workers (mean urinary Hg (U Hg) concentration 26 nmol/mmol creatinine) and nine male dentists (U Hg concentration 1.3 nmol/mmol creatinine) exposed to elemental Hg vapour when compared with matched referent groups (U Hg concentration 0.6 and 0.4 nmol/mmol creatinine). Thus there was no evidence of an effect of Hg on the pituitary. Neither was there any association between exposure to Hg and serum concentrations of free thyroid hormones (S FT₃, S FT₄), testosterone, or cortisol. Increased plasma concentrations of selenium (Se) were associated with increased basal serum concentrations of TSH, decreased concentrations of basal serum cortisol, and decreased release of FSH.

Samples taken at necropsy from industrial workers exposed to elemental mercury (Hg) vapour^{1,2} and dentists³ showed high concentrations of Hg in the pituitary gland. Accumulation of Hg at this site also occurred in experimental animals after exposure to inorganic Hg.⁴ It is not known, however, whether mercury affects pituitary function. The thyroid

gland also accumulates Hg as was first shown decades ago⁵ and confirmed more recently.¹ The functional consequences of this have been studied in pigs and rats^{6,7} but not in humans. The testis accumulates Hg⁵ but it is not known if this affects testosterone secretion.

Selenium (Se) influences some hormonal functions,^{8,9} and modifies the metabolism and toxicity of both inorganic Hg salts and methyl-Hg in experimental animals.¹⁰ Further, there is probably an interaction between Hg and Se in man, as indicated by studies of concentrations of the elements in blood and urine^{11,12} and in organs, including the pituitary and thyroid glands, from necropsies carried out on workers exposed to elemental Hg vapour.¹

To investigate if exposure to elemental Hg vapour had a negative effect on the secretion of pituitary hormones and on thyroid and testicular function, industry workers and dentists exposed to Hg vapour were compared with age matched unexposed controls with respect to thyrotrophin releasing hormone (TRH) and gonadotrophin releasing hormone (GnRH) stimulation tests. Furthermore, possible associations between hormones and Se state were investigated.

Material and methods

SUBJECTS

The group of workers comprised seven male workers from a chloralkali plant, three repairmen from a fluorescent tube factory, and one worker from a Hg refinery. The mean exposure time was 4.5 (range 2-18) years. During the exposure period, blood Hg (B Hg) was determined more or less regularly in all except the refinery worker. The individual average B Hg calculated from all observations on each subject varied between 70 and 170 nmol/l. No time trends in the individual concentrations were found. For the refinery worker, who had the greatest intensity and duration of exposure, an average B Hg concentration of 275 nmol/l could be calculated only for the last eight of the total of 18 years exposure. From a few measurements made during the first 10 years, an average twice as high might be assumed during that period. For the seven chloralkali workers and the refinery worker, data on previous urinary Hg (U Hg)

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concentrations were available. Among the chloralkali workers 90% of the U Hg values in the morning spot samples were within 25–50 nmol/mmol creatinine. For the refinery worker, the average of 117 (range 34–195) nmol/mmol creatinine (seven samples), was for the past 18 months only. The mean age of the group was 33 (range 23–49). Their referent group (referents I) consisted of 10 age matched (mean 32, range 24–39 years) male municipal workers who had had no occupational exposure to Hg.

The group of dentists comprised nine men who had been practising for an average of 28 (range 25–30) years, and who were still working as dentists. In this group, no information was available concerning earlier B Hg or U Hg concentrations. The mean age of the dentist group was 57 (range 55–62). Their referent group (referents II) consisted of 11 age matched (mean age 57, range 51–62) male municipal workers.

The subjects were studied after giving informed consent, and the study was approved by the Ethical Committee of Lund University, Sweden.

STUDY PROTOCOL

The investigations were performed from a Tuesday through Saturday. From history and physical examination, all subjects were considered healthy and had blood pressure and liver and kidney function within reference limits. Body mass index did not differ between groups. None of the subjects was taking medication, and none had been heavily exposed to alcoholic beverages or to organic solvents.

Amalgam fillings (I Akesson *et al*, personal communication) and fish consumption¹³ were registered. The dentists' referent group had a significantly lower number of amalgam fillings, which was due to an increased number of dental prostheses. The mean weekly fish consumption did not differ between the groups.

For the hormone studies, all subjects were investigated in a supine position between 8:00 and 10:00 am after an overnight fast. After resting in a supine position for 15 minutes, 100 µg GnRH and 200 µg TRH were injected consecutively (0 minutes) through an indwelling needle in an antecubital vein. No difference in the pituitary hormone responses was found if GnRH and TRH were given alone or in combination.¹⁴ Blood samples for measurement of serum thyrotrophin (S TSH), follicle stimulating hormone (S FSH), luteinising hormone (S LH), and prolactin (S PRL) concentrations were collected at –15, 0, 10, 20, 30, 45, and 60 minutes. Blood samples for determination of serum cortisol (S cortisol) concentrations were collected at –15 and 0 minutes. At 0 minutes the serum samples were collected for measurement of free thyroxine (S FT₄), free triiodothyronine (S FT₃), sex hormone binding globulin (S SHBG), free testosterone (S f test), total

testosterone (S t test), bilirubin, alkaline phosphatase, λ-glutamyl transpeptidase, aspartate and alanine aminotransferases, creatine, and albumin concentrations or activities. Blood and plasma were collected for determination of Hg (erythrocyte (Ery) Hg and plasma (PHg)) and Se (P Se) concentrations.

For all pituitary hormones and cortisol, the basal hormone concentrations were calculated from the mean of the concentrations in samples collected at –15 minutes, and 0 minutes. To summarise responses at different times and adjust for basal concentration differences, incremental areas were used to measure the responses of LH and FSH to GnRH, and TSH and responses of PRL to TRH. To measure the relative increment of serum TSH, PRL, LH, and FSH after TRH and GnRH stimulation, the ratios of incremental areas and the basal hormone concentrations were measured (IA/basal hormone concentration). The S f test concentration was calculated by a direct method (radioimmunoassay) and from the ratio of S t test and SHBG (S t test/S SHBG).¹⁵

Ten dentists entered the study but one was excluded because of low total and free testosterone concentrations together with high S LH and S FSH concentrations because of a history of orchitis.

Urine was collected during an eight hour period the night before the investigation and U Hg, albumin, and creatinine concentrations were determined.

HORMONE ASSAYS

Serum samples were stored at –20°C. For each hormone, all samples from each subject were analysed in the same assay. In each assay, samples from exposed subjects and their referents were mixed in equal proportions and assayed in duplicate, except for S f test, which was assayed in triplicate.

Serum FSH, SLH and S cortisol concentrations were measured by specific double antibody assays (radioimmunoassays; Farnos Diagnostica, Turku, Finland). The intra and interassay coefficients of variation (CVs) for S FSH and S LH were ≤5.5%. The reference range was 0.8–22 IU/l for S FSH and 2–15 IU/l for S LH. For S cortisol, the intra and interassay CVs were ≤5%. Serum TSH and S PRL were analysed with a fluoroimmunoassay technique (Delfia, LKB, Finland). For S TSH and S PRL, the intra-assay CVs were 2.6% and 6%. The reference interval for S TSH was 0.3–3.8 IU/l and for S PRL 2–12 µg/l.

Serum FT₃ and S FT₄ were assayed by radioimmunoassay (Amersham International, Amersham, UK), with intra-assay CVs of 2.9% and 4.7% and interassay CVs of 3.7% and 6.9%. The reference interval for S FT₃ was 2.9–8.9 pmol/l and for S FT₄ 10–25 pmol/l. Serum SHBG concentration was

measured by radioimmunoassay¹⁶ with intra and interassay CVs of 4.1% and 7.2%. The reference interval was 0.5–4 mg/l. Serum f test was measured by radioimmunoassay (DPC, LA, USA), with an intra-assay CV of 11%. For normal adult men the reference range was 31–163 pmol/l. Serum t test was measured by radioimmunoassay with an intra-assay CV of 4%.

METAL ANALYSIS

Mercury concentrations in urine, plasma, and erythrocytes were determined by a cold vapour atomic absorption technique.^{17,18} The detection limit was 1.0 nmol/l in urine and plasma and 2.0 nmol/l in erythrocytes. Each sample was analysed in duplicate. The precision, as calculated from duplicate analyses and expressed as CVs, was for U Hg 12%, 5% and 2% in the concentration ranges 1–10 (mean = 4.8, n = 21), 10–50 (mean = 19, n = 11), and 50–750 (mean = 393, n = 11) nmol/l. For P Hg, the CVs were 11%, 3%, and 1% in the concentration ranges 1–10 (mean = 4.4, n = 27), 10–50 (mean = 23; n = 8), and 50–175 (mean = 114, n = 8) nmol/l. For Ery Hg the CVs were 7%, 3%, and 2% in the concentration ranges 6–25 (mean = 18, n = 22), 26–50 (mean = 36, n = 10) and 51–310 (mean 113; n = 11) nmol/l. The accuracy was checked by analyses of a reference sample (Seronorm, Nycomed, Oslo, Norway) with a value of 5.6 nmol/l. Our results from different runs averaged 6.5 nmol/l (SD 0.87 range 5.6–8.0, n = 6). For a lysophilised urine sample (Lanonorm, Nycomed, Oslo) with a reference value of 49 nmol/l our average result was 49 nmol/l

(SD 4.0, range 45–60, n = 12). Plasma Se concentration was determined by a fluorimetric method.^{19,20} The detection limit was about 0.03 μmol/l. The CV calculated from duplicate analyses was 4%. The accuracy was checked by analyses of a reference sample (Seronorm TM, Nycomed, Oslo) containing 1.14 (SD 0.08) μmol/l. The result obtained was 1.13 (SD 0.05) μmol/l (n = 10).

OTHER ANALYSES

Serum samples were analysed for concentration or activity of bilirubin, alkaline phosphatase, λ-glutamyl transpeptidase, aspartate and alanine aminotransferases, creatinine, and albumin, and urine samples were analysed for albumin by routine methods. Urinary creatinine was analysed according to Lustgarten & Wenk.²¹ (and P Mascon, personal communication). The precision was 5% in the concentration range of 5–39 mmol/l. Urinary Hg concentrations were corrected for creatinine concentrations and expressed as nmol Hg/mmol creatinine.

STATISTICAL METHODS

The Mann-Whitney U test was used for comparisons of Hg, Se, and hormone parameters between the different groups. Correlations between Hg concentrations and hormone parameters were tested with Spearman's rank correlation test. The associations between hormone parameters and Hg and Se concentrations were tested in a multiple regression analysis allowing for age. Age was dichotomised (0 = ≤39 years, 1 = ≥49 years) in these analyses. The term

Table 1 Concentrations of Hg and hormone parameters in workers and dentists exposed to elemental Hg vapour and matched referent groups (arithmetic mean (range))

	Referents I (n = 10)	Hg workers (n = 11)	Referents II (n = 11)	Dentists (n = 9)
P Hg (nmol/l)	5.2 (1.0–14)	93 (27–175)*	3.9 (1.5–8.7)	9.5 (5.1–21)†
Ery Hg (nmol/l)	18 (6.6–34)	110 (38–310)*	20 (9.9–33)	39 (20–79)†
U Hg (nmol/mmol creatinine)	0.6 (0.1–1.3)	26 (14–44)*	0.4 (0.1–1.1)	1.3 (0.5–3.9)†
P Se (μmol/l)	1.2 (0.9–1.4)	1.1 (0.8–1.4)	1.1 (0.9–1.3)	1.2 (1.0–1.5)
S FT _t (pmol/l)	6.7 (5.6–8.1)	6.6 (4.9–8.6)	6.1 (5–7.1)	5.9 (5.1–6.9)
S FT _f (pmol/l)	15.0 (12–21)	15.2 (11–20)	13.6 (10–20)	14.6 (10–19)
S f test (pmol/l)	74 (35–93)	73 (42–109)	61 (38–87)	54 (38–74)
S SHBG (mg/l)	1.8 (1.2–2.3)	1.7 (1.1–2.6)	2.4 (1.3–3.5)	1.9 (1.3–3.0)
S t test/S-SHBG	3.2 (1.6–4.6)	3.4 (1.3–5.0)	2.2 (1.5–3.2)	2.1 (1.0–2.8)
S cortisol (pmol/l)	460 (190–690)	440 (210–740)	393 (205–540)	394 (310–550)
S Basal PRL (μg/l)	4.3 (2.1–7.2)	6.7 (2.0–22.0)	3.4 (1.8–6.7)	3.9 (2.3–7.4)
PRL IA†	630 (400–980)	660 (210–1450)	540 (340–1370)	715 (280–1710)
PRL IA/Basal PRL	160 (95–320)	185 (20–480)	160 (100–210)	210 (90–740)
S Basal TSH (IU/l)	1.2 (0.5–2.1)	1.3 (0.9–1.7)	1.2 (0.4–2.7)	1.2 (0.4–1.6)
TSH IA	380 (220–620)	390 (150–710)	290 (90–560)	330 (130–490)
TSH IA/Basal TSH	330 (240–480)	320 (140–590)	270 (160–510)	270 (220–320)
S Basal LH (IU/l)	6.9 (5.2–10.5)	8.3 (5–13)	8.0 (5–11.8)	6.9 (3.4–11.7)
LH IA	1150 (480–3290)	1230 (390–2870)	920 (450–1660)	780 (450–2040)
LH IA/Basal LH	170 (50–420)	170 (30–500)	120 (60–180)	110 (70–180)
S Basal FSH (IU/l)	3.6 (1.1–10.2)	4.2 (2.3–5.6)	5.8 (1.9–13.5)	6.8 (1.3–27.0)
FSH IA	210 (60–940)	200 (70–490)	180 (60–350)	260 (50–1230)
FSH IA/Basal FSH	50 (20–90)	55 (14–190)	30 (20–50)	40 (10–90)

*Referents I v Hg workers, p < 0.0001.

†Referents II v dentists, p ≤ 0.006.

‡IA = Incremental area.

significant refers to $p \leq 0.05$. All tests were two tailed.

Results

The Hg exposed groups had significantly higher concentrations of P Hg, Ery Hg, and U Hg compared with their matched referent groups. The Hg concentrations in the dentists were much lower compared with the industry workers. No significant differences in P Se concentrations were found between the four groups. The exposed groups and their specific referent groups did not differ significantly in any hormone concentration, irrespective of whether basal values, IAs, or relative increments were compared (table 1).

Considering the individual values for all 41 subjects, there were no significant correlations between either P Hg or Ery Hg and any hormone concentration irrespective of basal value, IA, or relative increment. On the other hand, U Hg was positively correlated with the basal concentration of S PRL ($r = 0.33$, $p = 0.03$) (figure). When age and P Se were allowed for in a multiple regression analysis, association between U Hg and the basal concentration of S PRL persisted, although it did not reach the formal significance level ($p = 0.06$). Multiple regression analysis did not show any other associations between U Hg, P Hg, or Ery Hg concentrations and the hormonal parameters. In a multiple regression analysis allowing for age and U Hg P Se was positively associated with basal concentrations of S TSH ($p = 0.02$), and negatively associated with both the relative increment of S FSH ($p = 0.01$) and basal S cortisol concentration ($p = 0.04$).

Table 2 Age coefficients for hormone concentrations in a multiple regression analysis (41 subjects) allowing for P Se and U Hg concentrations

Hormones	Age* coefficient	p Value
S Basal PRL ($\mu\text{g/l}$)	†	0.4
PRL IA†	—	> 0.5
PRL IA/Basal PRL	—	> 0.5
S Basal TSH (IU/l)	—	> 0.5
TSH IA	-95.3	0.06
TSH IA/Basal TSH	-69.6	0.04
S Basal LH (IU/l)	—	> 0.5
LH IA	—	0.1
LH IA/Basal LH	-60.0	0.05
S Basal FSH (IU/l)	—	0.1
FSH IA	—	> 0.5
FSH IA/Basal FSH	-21.0	0.04
S FT ₃ (pmol/l)	-0.9	0.005
S FT ₄ (pmol/l)	—	0.14
S f test (pmol/l)	-15.4	0.006
S t test (ng/ml)	-0.9	0.04
S SHBG (mg/l)	0.4	0.06
S t test/SHBG	-1.0	0.003
S cortisol (pmol/l)	-96.5	0.04

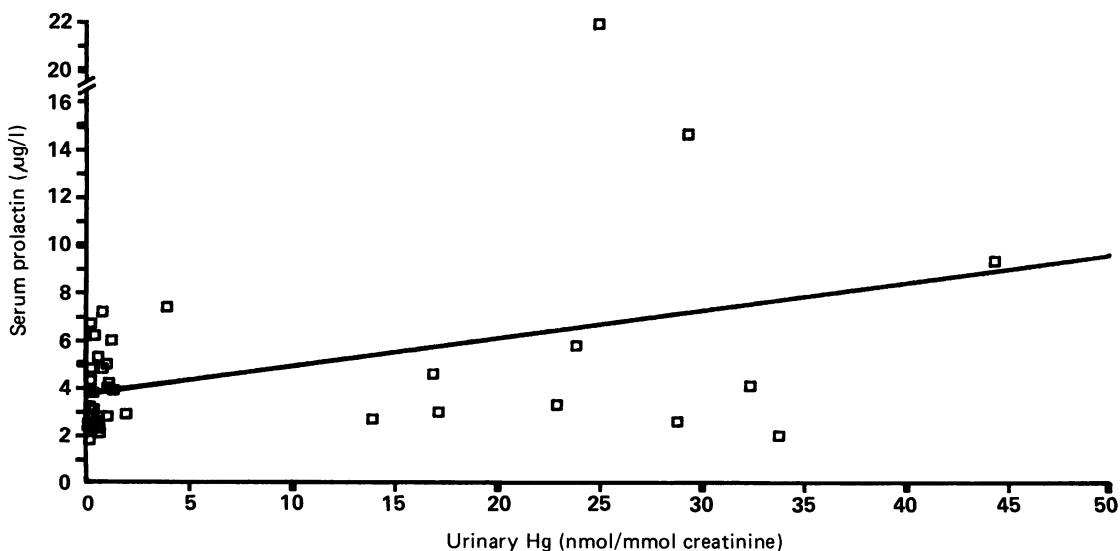
*Age was dichotomised (0 = ≤ 39 years, 1 = ≥ 49 years).

†Coefficients only mentioned when $p \leq 0.06$.

‡IA = Incremental area.

The relative increments of TSH and TSH IAs were negatively associated with age ($p = 0.04$ and $p = 0.06$), as was S FT₃ ($p = 0.005$) (table 2). Further, the relative increment of S FSH ($p = 0.04$) and S f test ($p = 0.006$), S t test ($p = 0.04$) and the ratio of S t test/S SHBG ($p = 0.003$) had significant negative correlations with age. Also, S cortisol concentration was negatively associated with age ($p = 0.04$).

The most highly exposed worker who, at the time of the stimulation tests had the highest P Hg and the



Association between U Hg and basal S PRL in 41 subjects (Spearman's rank correlation test, $r = 0.33$, $p = 0.03$).

second highest U Hg concentrations of all subjects, did not exhibit any extreme values within the ranges obtained for the different effect parameters.

Discussion

Several observations support the reliability of the hormone analysis and the validity of the study groups. Thus in agreement with an earlier study²² age was negatively correlated with the relative increment of S TSH. Furthermore, the sex hormone concentrations and their negative association with age also agreed well with earlier studies.^{23,24}

Only chloralkali workers with a known relatively high exposure level to elemental Hg vapour entered the study. The exposure level in the dentists was lower, but the lifetime exposure in the studied group must be considered substantial as they had been working continuously for at least 25 years; compared with another study in which younger dentists were investigated (I Åkesson *et al*, personal communication) they had higher P Hg and U Hg concentrations. Furthermore, the P Se concentrations in dentists, chloralkali workers, and in referents were in agreement with the concentrations in other studies of Swedes.¹³

In experimental animals⁶ and in man,^{1,5} Hg shows a high affinity for the thyroid. In pigs, the blood thyroxine concentration was reduced after administration of inorganic Hg.⁶ Mercury accumulates in the mouse testis²⁵ and exposure to ethyl-Hg can affect sperm production and reduce libido.²⁶ Decreased libido has also been reported after severe exposure to raised concentrations of Hg.²⁷ It is not known, however, if an accumulation of Hg in these organs directly affects the production or secretion of thyroid hormones and testosterone. No differences between thyroid hormones or serum testosterone concentrations could be demonstrated between exposed groups and their referents.

Fifty years ago, Stock was astonished by the high quantities of Hg in human pituitaries from subjects who had died by accident without any known exposure to Hg.³ Accordingly, Nylander observed that pituitary glands sampled from dentists after death contained excessive amounts of Hg when compared to the rest of the brain.³ In postmortem samples from Hg mine workers with a long sustained exposure, Kosta *et al*¹ showed that the pituitary and the thyroid gland contained the highest concentrations of Hg in the whole body. Furthermore, Thorlassius-Ussing *et al*⁴ noted that Hg was present in the thyrotrophs and follicular cells in rat pituitaries, which might be interesting from a functional point of view. We found no effect. The pituitary seems to be a resistant organ as it also has a high capacity to maintain and to regain normal endocrine function after surgery for a pituitary adenoma.²⁸

Mercury accumulated intracellularly in the lysosomes and granules of somatotrophs, thyrotrophs, and corticotrophs. Apart from vacuolation of lysosomes, no structural damage was seen in the cells containing Hg.²⁹ Mercury is probably sequestered in lysosomal dense bodies after binding to selenium³⁰ and more selenium is retained in subjects with high Hg concentrations in tissue.^{1,31}

Urinary Hg correlated positively with basal S PRL concentrations but Ery Hg and P Hg did not display such correlations, probably due to a poorer association with long term Hg exposure. Administration of mercury to female hamsters causes alteration in the reproductive system.³³ This alteration might be due to changes in the pituitary content of FSH and LH³³ but increased concentrations of PRL is a possible explanation, as hyperprolactinemia interferes with normal ovulation.³⁴ The secretion of PRL is regulated by dopamine, which acts as a PRL inhibitory factor.^{35,36} Dopamine is released from the median eminence of the hypothalamus, and cell bodies originating from the arcuate nucleus innervate the median eminence.³⁷ Mercury has been shown to accumulate in many of the neurons of the arcuate nucleus.³³ Thus theoretically Hg could interrupt the dopaminergic inhibition of prolactin secretion at the hypothalamic level. The significant correlation between basal S PRL and U Hg concentrations is, however, based mainly on a small group of chloralkali workers some of which had high U Hg concentrations and increased concentrations of S PRL. Thus a further study comprising a larger group of workers with high exposure to Hg is needed to obtain a firmer conclusion.

Plasma Se concentration was positively associated with basal S TSH concentration and negatively associated with the relative increment of S FSH and with basal S cortisol concentration. It is interesting that Se accumulates intracellularly in the secretory granules of the thyrotrophs, gonadotrophs, corticotrophs, and somatotrophs in rats after high exposure.³⁸ Functionally, accumulation of Se in somatotrophs might be important as rats treated with very high doses of Se had markedly reduced growth and growth hormone secretion.⁹

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