Endocrine function in mercury exposed chloralkali workers

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Abstract

***Objective*—The aim was to study whether functional impairment of the pituitary, thyroid, testes, and adrenal glands of humans occupationally exposed to mercury (Hg) vapour can be shown as a result of accumulation of Hg in these glands.**

***Methods*—Basal concentrations of thyrotrophin (TSH), prolactin, free thyroxine (free T4), free 3,5,3'-triiodothyronine (free T3), antibodies against thyroperoxidase, and testosterone in serum, as well as cortisol in morning urine were measured in 41 chloralkali workers exposed (10 years on average) to Hg vapour, and in 41 age matched occupationally unexposed referents. The chloralkali workers had a mean urinary Hg concentration (U-Hg) of 15 nmol/mmol (27 μg/g) creatinine, and a mean blood Hg concentration (B-Hg) of 46 nmol/l. For the reference group U-Hg and B-Hg were 1·9 nmol/mmol (3·3 μg/g) creatinine and 17 nmol/l respectively.**

***Results*—The serum free T4 concentration and the ratio free T4/free T3 were slightly, but significantly, higher in the subgroups with the highest exposure, and the serum free T3 was inversely associated with cumulative Hg exposure. This indicates a possible inhibitory effect of mercury on 5'-deiodinases, which are responsible for the conversion of T4 to the active hormone T3. Serum total testosterone, but not free testosterone, was positively correlated with cumulative Hg exposure. Prolactin, TSH and urinary cortisol concentrations were not significantly associated to exposure.**

***Conclusion*—Apart from inhibition of the deiodination of T4 to T3, the endocrine functions studied seem not to be affected by exposure to Hg vapour at the exposure levels of the present study. Growth hormone secretion was not studied.**

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Inorganic mercury (Hg) accumulates in the human anterior pituitary and the thyroid gland.[1 2] Animal studies indicate accumulation also in the testes and adrenal glands.[3 4] In studies of subjects occupationally exposed to Hg vapour, no effects were seen on basal or stimulated serum concentrations of gonadotrophins, thyrotrophin (TSH), or prolactin,[5–7] or on serum free thyroid hormone or testosterone concentrations.[5 7] In one of the studies,[5] however, there was a positive correlation between urinary Hg excretion and basal prolactin concentrations.

Animal studies have shown that treatment with inorganic Hg inhibited enzymes involved in the biosynthesis of iodothyronines, resulting in decreased concentrations of serum thyroxine (T4). Mercurials may also affect the deiodination of T4 to 3,5,3'-triiodothyronine (T3).[8 9] In animals, changes in morphology and function of the adrenals and testes have also been reported.[10–11]

Subjects and methods

We examined, simultaneously in age matched pairs, 41 male chloralkali workers exposed to mercury vapour and 41 occupationally unexposed deferents from the same company. Table 1 shows their ages and duration of Hg exposure. Typical air-Hg concentrations at ordinary work were 20-50 μg/m³, but during maintenance the values were higher.[12] There were 18 smokers in the exposed group and 20 among the referents. Alcohol consumption was moderate, and there was no difference between exposed subjects and referents.

EXPOSURE ASSESSMENT

Venous blood samples were obtained between 9 00 am and 2 00 pm in metal free heparinised Venoject tubes. Morning urine samples were collected in Hg free polyethylene bottles. After separation of plasma, the samples were stored at −25°C. Mercury in whole blood, plasma, and urine was analysed by cold vapour atomic absorption spectrophotometry.[13 14] Lack of precision (coefficient of variation (CV)) as calculated from duplicate analyses was about 5%.[15] Accuracy, initially tested by comparisons with other laboratories,[15] was further checked by reanalysis in 1990 of 18 replicate plasma and urine samples together with external reference samples. The reanalysis of samples stored at −25°C for four years showed only slightly lower (mean 91 (SD 17)%) concentrations than the initial results. The simultaneous analysis of reference samples (batches 904 and 905, Seronorm, Nycomed, Oslo, and Control Blood for Metals 2, lot number 620403, Behring Institute, Marburg) showed good agreement (mean 98 (SD 8·6)%) compared with recommended values. For the exposed group, we calculated a cumulative exposure index for each subject by adding their yearly mean B-Hg values.

Table 1 Age, Hg and hormone concentrations, and exposure in exposed workers and referents

Variables	Referents (n = 41)			Exposed (n = 41)		
	Mean	Median	Range	Mean	Median	Range
Age (y)	36	37	18–61	36	34	19–65
B-Hg (nmol/l)	17	15	8–60	46	35	8–160
P-Hg (nmol/l)	6·9	6·3	2–12	37	30	14–119
U-Hg (nmol/mmol creatinine)	1·9	1·9	0·5–5·0	15	12	4·5–53
Exposure time (y)	—	—	—	9·5	10	0–31
Cum. exposure index*	—	—	—	800	520	15–4300
Serum:						
Free T4 (pmol/l)	17·0	17·1	12·4–22·3	18·1	18·2	11·5–25·8
Free T3 (pmol/l)	6·1	6·1	4·1–7·8	6·1	6·1	3·8–7·8
Free T4/free T3	2·8	2·8	2·0–3·5	3·0	3·0	1·8–4·7
TSH (mU/l)†	1·7	1·5	0·6–5·1	1·6	1·3	0·4–4·9
Prolactin (mU/l)	223	225	51–518	236	201	79–710
Testosterone (nmol/l)	18·3	17·5	8·6–30	18·1	16	7·2–60
Free testosterone(pmol/l)	79	77	45–129	77	78	31–126
Urine:						
Cortisol (nmol/mmol creatinine)	14·7	11·8	4·2–60	20·1	13·1	3·3–76

*Sum of yearly mean B-Hg (nmol/l).
†TSH by IRMA (see methods).

HORMONE AND ANTIBODY ASSAYS

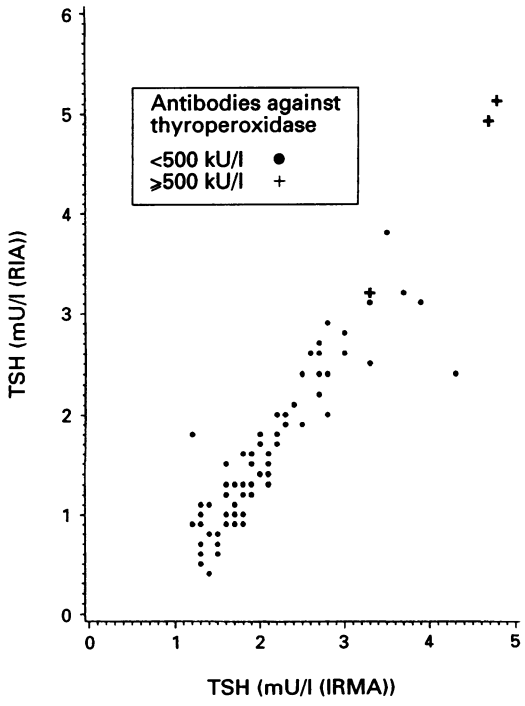
Venous blood samples for the hormone assays were obtained in 10-ml Vacutainer gel-barrier tubes (SST A 3200, Becton-Dickinson), with the subject in a sitting position, on the same occasion as the blood sample for Hg estimation. Aliquots of serum were separated within an hour and transferrred into glass tubes kept at –25°C.

All serum specimens were analysed for TSH by radioimmunoassay (RIA) and by immunoradiometric assay (IRMA), and for free T4, T3, and antibodies against thyroperoxidase as detailed later. Measurement of TSH by RIA was by NHS-TSH double antibody RIA, Diagnostic Products Corp, Los Angeles, CA, USA (detection limit 0·6 mU/l); TSH by IRMA was performed with RIA-gnost hTSH, Behringwerke (analytical detection limit 0·03 mU/l). For TSH, values given refer to the IRMA results unless otherwise stated. The assays of free thyroid hormones were performed with ligand-analogue methods, with due consideration to artefacts such as antithyronine antibodies and thyroxine-binding albumin.[16] Free T4 and free T3 were determined by Amerlex-M methods (Amersham International plc, Amersham, Bucks, UK). Antibodies against thyroperoxidase (anti "microsomal" antibodies) were determined with a ^{125}I-Protein A-binding method (PROMAK, Henning-Berlin). Values are given in arbitrary units, and a decision limit of 500 kU/l has been recommended by the manufacturer (analytical detection limit 50 kU/l).

Serum prolactin was determined with a polyethylene glycol assisted double antibody RIA (Diagnostic Products Corp). Serum testosterone was determined by a non-extraction radioimmunoassay with an antiserum against a testosterone-19-carboxymethyl adduct to bovine serum albumin (RSL 1251 testosterone, ICN Biochemicals Inc, 3·4% cross reactivity with 5α-dihydrotestosterone). Serum free testosterone was assayed by a non-extraction ligand-analogue technique (Coat-A-Count Free Testosterone, Diagnostic Products Corp). As stated by the manufacturer, the central 0·90 fractile reference limits for men vary with age—for example, for the age group 30–39 years they are 62–135 pmol/l; for 60–69 years, 38–90 pmol/l. Urinary cortisol was determined with a non-extraction radioimmunoassay (Farmos Diagnostica).

STATISTICAL ANALYSES

The results for the exposed and reference groups were compared by Wilcoxon's signed rank test for paired observations. Non-paired group differences (smokers *v* non-smokers) were analysed by Wilcoxon's rank sum test. For correlations between single variables, Spearman's rank correlation coefficient (r_s) was used. Associations between more than two variables were analysed by the multiple linear regression technique. Statistically significant refers to $p < 0·05$ in two tailed tests.



Relation between results of thyrotrophin (TSH) concentrations analysed by radioimmunoassay (RIA) and immunoradiometric assay (IRMA).

Results

The Hg concentrations in whole blood (B-Hg), plasma (P-Hg), and urine (U-Hg) were higher in the exposed workers, as would be expected (table 1). The Hg concentrations in these three media were also highly intercorrelated in both groups. The serum concentrations of thyroid hormones, TSH, testosterone, prolactin, and the urinary cortisol excretion were not significantly different between the groups (table 1). Three subjects (two exposed and one referent) had increased concentrations of antithyroperoxidase antibodies (840, 2800, and 3900 kU/l), together with TSH concentrations in the upper part of the distributions for both assays, indicating autoimmune thyroid disease, previously not clinically diagnosed (figure). In the further analysis (table 2), these three subjects were excluded. Two other subjects (one exposed and one referent) had detectable antibody concentrations (>50

Table 2 Median hormone concentrations in Hg exposed workers and referents, after exclusion of three subjects with biochemical evidence of autoimmune thyreoditis

Variables	Referents (n = 40)	Exposed (n = 39)	High B-Hg (n = 13)	High U-Hg (n = 11)	High CEI (n = 15)
Serum:					
Free T4 (pmol/l)	17·3	18·2	18·5*	17·8	17·8
Free T3 (pmol/l)	6·1	6·2	6·2	6·0	5·7
Free T4/Free T3	2·8	3·0	3·3†	3·3‡	3·0
TSH (mU/l)	1·4	1·3	1·1	1·1	1·2
Prolactin (mU/l)	224	204	193	193	178
Testosterone (nmol/l)	17·0	16·0	18·0	18·0	19·0
Free testosterone (pmol/l)	77	78	68	68	80
Urine:					
Cortisol (nmol/mmol creatinine)	11·9	13·1	12·3	18·3	13·1

* p = 0·02; † p = 0·06; ‡ p = 0·04 compared with their respective age matched referents.
Results for subgroups of exposed subjects having the highest Hg concentration in blood (B-Hg > 50 nmol/l), or urine (U-Hg > 20 nmol/mmol creatinine or 35 μg/g creatinine), or the highest cumulative exposure index (CEI = sum of yearly mean B-Hg > 800 nmol/l) are indicated separately.

kU/l), albeit below the decision limit of 500 kU/l. Their TSH concentrations did not differ from the remaining subjects, and these subjects were not excluded.

As shown in table 2, free T4 concentrations were slightly higher in the Hg exposed group, and the difference was statistically significant for the subgroup with B-Hg >50 nmol/l. Free T3 concentrations, however, did not differ. Consequently, the ratio free T4/free T3 was slightly higher in the exposed group. The difference was statistically significant for the subgroup with current U-Hg >20 nmol/mmol creatinine (35 μg/g), and nearly so for the subgroup with B-Hg >50 nmol/l.

Serum prolactin concentrations were lower in smokers (statistically significant in the reference group; p = 0·04), as were TSH concentrations (p = 0·02 in the combined group of exposed subjects and referents). Free testosterone was, however, higher in smokers (p = 0·01 in the combined group).

In the reference group, there was a clear correlation between free T4 and free T3 (r_s = 0·53, p = 0·0005), and free T3 was negatively correlated with age (r_s = –0·40, p = 0·01). Total testosterone and free testosterone were also correlated, (r_s = 0·56, p = 0·0002). If the groups of exposed subjects and referents were combined, the associations mentioned were similar. Furthermore, total testosterone was positively correlated with age (r_s0·34, p = 0·002), and free T4 was negatively associated with age (p = 0·03) when smoking was allowed for.

In the exposed group, there was a trend towards an inverse correlation between free T3 on one hand, and U-Hg and the cumulative exposure index on the other (table 3). When age and smoking were taken into account in a multiple regression model, the regression coefficient for the cumulative exposure index was found to be statistically significant (p = 0·04), which was not the case for U-Hg. Free T4 was not correlated to any exposure variable. The ratio free T4/free T3 was correlated to P-Hg (table 3), but the association was not significant when age and smoking were allowed for. The negative correlation between TSH and cumulative exposure (table 3) was no longer statistically significant when age and smoking were taken into account. There was a statistically significant positive correlation between total testosterone and the cumulative exposure index, and the association remained when age and smoking were taken into account. There were, however, no associations between exposure and free testosterone.

Table 3 Spearman rank correlations between exposure variables and hormone concentrations in Hg exposed chloralkali workers (n = 39) for those hormones in which associations with exposure could be discerned (p < 0·10)

Hormones	B-Hg	P-Hg	U-Hg	CEI
Serum free T3	—	—	−0·28 (p = 0·08)	−0·28* (p = 0·09)
Free T4/free T3	—	0·33 (p = 0·04)	—	—
Serum TSH	—	—	—	−0·36 (p = 0·03)
Serum testosterone	—	—	—	0·49* (p = 0·002)

*Associations that were also statistically significant (p < 0·05) in a multiple linear regression analysis (see text), taking age and smoking into account. CEI = cumulative exposure index.

Discussion

Contamination and poor analytical performance are important potential problems in trace metal studies.[17] The mercury concentrations in the reference group of the present study are consistent with those found in comparable Swedish populations,[18 19] and do not indicate external contamination. The precision was acceptable and the accuracy was checked in two different ways. The cumulative exposure index was, however, dependent on only a few B-Hg measurements per year. The hormone assays were performed in a laboratory with extensive experience from clinical and epidemiological studies involving the compounds analysed in the present study, and the values found were in good agreement with results from these other studies.

Another problem concerns within and between subject variation from other sources than the possible influence of occupational exposure. The day to day variation in B-Hg and U-Hg concentrations on consecutive days is substantial, even at stable exposure conditions.[20] The hormone concentrations in healthy subjects are also subject to within subject variation—for example, a decrease in testosterone concentration occurs by early afternoon. Blood sampling was restricted to a limited part of the day to decrease diurnal variations. Furthermore, special care was taken to investigate the exposed subjects and referents at the same time of day, and to analyse the samples in random order with all samples in one assay. Interindividual variation due to smoking habits and age were allowed for in the data analyses. These sources of variation decrease the power of detecting possible differences between exposed workers and referents, and tend to blur possible dose-response relations. A non-differential misclassification of exposure and effects, however, could not explain differences between groups or correlations between exposure and effects.[21]

Taken together, the group differences in basal hormones were small and the

correlations with exposure indices were weak, bearing in mind that multiple comparisons and correlations were tested. Starting with the pituitary, a reasonable a priori hypothesis should be that accumulation of Hg results in an impaired pituitary function. In animal studies, Hg accumulated in several types of secretory cells (somatotrophs, thyrotrophs, and corticotrophs).[22 23] The slightly lower concentrations of TSH and prolactin in exposed workers (table 2), and the negative correlation between TSH and the cumulative exposure index are therefore noteworthy. Decreased TSH concentrations may, on the one hand, be a result of decreased hypothalamic stimulation or decreased pituitary capacity, or, on the other, of increased feedback inhibition by thyroid hormones, T4 in particular. As discussed later, there was some evidence in favour of the second possibility (increased free T4 concentrations in the presence of an intact negative feedback system). In two recent studies, pituitary function in Hg exposed subjects was found to be normal, compared with non-exposed referents, when tested by stimulation with thyrotrophin releasing hormone (TRH)[6] or TRH and gonadotrophin releasing hormone (GnRH).[5] The number of subjects in these studies was small and most of them were dentists with lower Hg exposure than chloralkali workers. The positive correlation between basal prolactin and U-Hg[5] could not be reproduced in the present study, and may well have been a random finding. In summary, there is no evidence that pituitary function would be affected by Hg exposure of the magnitude reported in the present study. Growth hormone (GH) may be the most vulnerable pituitary hormone,[24] but methods for assessing GH secretion (measurement of urinary GH, serum insulin like growth factor 1, and its main binding protein, BP3) were not available at the time of the present study.

In the exposed workers, free T3 was negatively correlated to the cumulative Hg dose index, whereas free T4 and the ratio free T4/free T3 were slightly higher compared with the referents. This could possibly be explained by an inhibitory effect of Hg on 5'-deiodinases. All T4 is synthesised in the thyroid, but T4 requires deiodination to produce the active hormone T3. There are several types of deiodinases, but more than 80% of plasma T3 is produced in the liver, kidneys, and muscle by the type I enzyme. In animals injected or fed with high doses of inorganic Hg, the uptake of labelled iodine by the thyroid and the concentrations of circulating T4 and T3 were decreased,[9 24] T3 being more affected than T4. Furthermore, inorganic Hg inhibits thyroid peroxidase, responsible for the synthesis of T4 and iodothyronine deiodinase.[8 24 25] Our subjects had a much lower Hg exposure than was the case in the animal studies, but Hg is a potent inhibitor of several enzyme systems, in particular those rich in sulphhydryl groups. In this context, it is interesting to note that the type I iodothyronine deiodinase is a selenoenzyme, and that selenium deficiency in animals partly inhibits the deiodination of T4 to T3, resulting in increased T4 and decreased T3 concentrations in plasma.[26] Furthermore, there is a metabolic interaction between selenium and Hg in animals.[27] A close correlation between Se and Hg concentrations has been found in necropsy samples from subjects exposed to Hg vapour,[1 2] indicating the formation of intracellular mercury-selenium-protein-complexes. Thus one could speculate that Hg exposure in our subjects could interact with the synthesis and/or activity of the selenoenzyme deiodinase type I.

Total testosterone was positively correlated with the cumulative Hg dose index, whereas free testosterone was not. This could possibly reflect increased amounts of sex hormone binding globulin, the main carrier protein in plasma for androgens, which was not measured in the present study. In animals, a peripheral toxic effect of Hg on the Leydig cells of the testes, with decreased testosterone concentrations, has been reported.[10 11] Neither this study nor two previous studies on testicular function,[5 7] provide any evidence for an adverse effect on the testes at these occupational exposure levels of Hg vapour.

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