EFFECT OF PHENYTOIN THERAPY ON THYROID FUNCTION

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1 Serum total and free fraction of thyroxine and triiodothyronine and urinary losses of unconjugated hormones in normal subjects and in patients treated long-term with therapeutic doses of phenytoin have been measured.

2 Decreases in serum total hormone concentrations with increased free fractions and resultant significant increase in the concentration of free thyroxine but not triiodothyronine were apparent in phenytoin-treated subjects. However, serum free hormone concentrations remained within the euthyroid range.

3 These changes in serum free hormone concentration were reflected by an increased urinary loss of unconjugated thyroxine, but normal excretion of unconjugated triiodothyronine.

4 Phenytoin in therapeutic doses displaces thyroxine and to a lesser extent, triiodothyronine from binding proteins in serum and thus increases peripheral clearance of thyroid hormones.

Introduction

Reduction in serum protein-bound iodine (PBI) levels in phenytoin-treated individuals was first reported by Oppenheimer, Fisher, Nelson & Jailer, (1961). This phenomenon was apparent even in hypothyroid patients maintained on a constant exogenous dose of thyroid hormone thus indicating an extrathyroidal drug effect. Subsequent *in vitro* studies showed that phenytoin displaces thyroxine from thyroxine-binding globulin (Wolff, Standaert & Rall, 1961) and increases the dialysable fraction of thyroxine (Oppenheimer & Tavernetti 1962). More recent *in vivo* studies suggested that while total serum thyroxine is lowered, its non-protein bound (free) fraction remains unaltered during phenytoin therapy with resultant reduction in the physiologically important absolute free (.1. non protein-bound) hormone concentration (Chin & Schussler, 1968; Larsen, Atkinson, Wellman & Goldsmith, 1970). These findings are somewhat at variance with the further findings of Larsen *et al.*, (1970) that the rate of thyroxine degradation is unchanged and urine and faecal losses of hormone actually increase during phenytoin administration. Furthermore, patients receiving long-term phenytoin therapy remain clinically euthyroid.

The advent of reliable radioimmunoassay techniques for measurement of serum triiodothyronine together with the recent availability of methods for assay of unconjugated thyroid

hormones in urine (Chan & Landon, 1972; Burke, Shakespeare & Fraser, 1972; Black, Griffiths, Finucane & Hoffenberg, 1975) prompted this study on the effects of long term phenytoin therapy on serum total and non protein-bound thyroxine and triiodothyronine. It was hoped that useful additional data might be obtained from estimation of urine losses of unconjugated thyroid hormones in normal and phenytoin-treated subjects.

Methods

Subjects

The following groups were studied:

(a) Twenty healthy laboratory workers and students (thirteen male; seven female) aged from 20-35 years (mean 27 years). These subjects had not taken any drug preparation for several weeks prior to the study.

(b) Ten patients (eight female; two male) with presumed idiopathic grand-mal epilepsy, and no intercurrent illness, who were receiving as their only treatment, phenytoin (200-400 mg/day) for at least 6 weeks prior to investigation. Ages ranged from 23-54 years (mean 37 years).

Procedure

Single 24 h urine collections, commencing at 08.00 h were carried out. Voided urine was

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Figure 1 Serum total, percentage free fraction and absolute serum free hormone concentrations of thyroxine and triiodothyronine in (A) normal and (B) phenytoin-treated subjects. Mean values $\pm$ 1 s.d. are shown. The significance of between-group differences assessed by an unpaired Student's *t*-test is indicated at the foot of each column.

immediately placed in opaque plastic receptacles containing 10 ml of 0.01 M methylthiouracil in 0.1 M sodium hydroxide to ensure stability of thyroid hormones and to minimise spontaneous deiodination. Aliquots of urine were stored at -20°C , as soon as collection was complete. A mid-morning specimen of blood was obtained on the day urine collection was carried out. Serum was stored at -20°C . Specimens of urine and serum were thawed immediately prior to use.

Assay methods

Serum total and urine unconjugated thyroxine and triiodothyronine were respectively measured by competitive protein-binding and radioimmunoassay techniques of high specificity and sensitivity (Black *et al.*, 1975).

Serum free fractions of thyroxine and triiodothyronine were determined by a modification of

Figure 2 Twenty-four hour urinary losses of thyroxine and triiodothyronine in (A) normal and (B) phenytoin-treated subjects. Mean values $\pm$ s.d. are shown. Groups were compared by means of an unpaired Student's *t*-test and the significance of differences is indicated at the foot of each column.

the Sephadex gel filtration technique described by Lee, Henry & Golub, (1964).

Serum and urine creatinine concentrations were measured by an Autoanalyser method.

Urine was screened for the presence of protein by dropwise addition of 25% sulfosalicylic acid (0.5 ml) to urine (5 ml) following prior acidification with dilute hydrochloric acid. Absence of turbidity indicated a protein content of less than 10 mg/100 ml.

Results

All subjects appeared euthyroid on clinical examination.

Serum total, percentage free fraction and absolute free concentrations of thyroxine and triiodothyronine in normal and phenytoin-treated subjects are shown in Figure 1. Mean normal values (range in brackets) were respectively 94 ng/ml (73-120 ng/ml); 0.052% (0.044-0.062%) and 49 pg/ml (40-67 pg/ml) for thyroxine, and 1.48 ng/ml (1.08-2.17 ng/ml); 0.40% (0.29-0.50%) and 5.9 pg/ml (3.5-7.9 pg/ml) for triiodothyronine. A highly significant decrease in serum total thyroxine with a comparable increase in free thyroxine fraction is apparent in phenytoin-treated subjects. This resulted in an appreciable

increase over normal in the concentration of serum free thyroxine. A similar but less striking pattern is seen in the case of triiodothyronine except that the slight increase in serum free triiodothyronine does not achieve statistical significance. Mean values for serum total thyroxine were two thirds of normal and six individual values were in the hypothyroid range for this laboratory. In the case of triiodothyronine mean values were 15% below normal and three values were in the hypothyroid range. It is noteworthy that all individual values for serum free thyroxine and triiodothyronine concentrations in phenytoin-treated subjects were within our normal range and clearly indicated the euthyroid status of these patients.

Daily urine losses of unconjugated thyroid hormones are presented in Figure 2. Mean normal values (range in brackets) were 1.95 μg (0.85-3.08 μg) for thyroxine and 1.07 μg (0.66-1.73 μg) for triiodothyronine. The increased thyroxine losses in phenytoin-treated patients were highly significant ($P < 0.001$) but triiodothyronine losses did not differ significantly from normal. Accuracy of urine collection was monitored by simultaneously determining creatinine clearance. In every case, this exceeded 80 ml/minute.

Discussion

Results in the present study are at variance with previous studies by Chin & Schussler (1968) and Larsen *et al.*, (1970) which suggested that no significant competitive displacement of thyroid hormones from serum binding proteins occurs *in vivo* following the administration of phenytoin in therapeutic doses. Because free hormone fraction was apparently unchanged from normal, serum free hormone concentration was reportedly reduced in proportion to the depression in serum total hormone levels. However, the ultrafiltration method used by both groups of workers to measure free thyroxine fraction is methodologically suspect (Spaulding & Gregerman, 1972). Furthermore, no adequate explanation was offered for the other findings of Larsen *et al.*, (1970) that absolute thyroxine degradation rate, generally assumed to be a function of free hormone concentration, was unchanged, and that urine and faecal losses of radioactive hormone were increased in phenytoin-treated subjects. In a recent study, Hansen, Skovsted, Lavridsen, Kirkegaard & Siersbaek-Nielsen (1974) found significantly

reduced serum total concentrations both of thyroxine and triiodothyronine in patients treated longterm with phenytoin. Free thyroxine index was reduced and basal serum levels of thyroid stimulating hormone were increased. This was interpreted as indicating that serum free thyroxine concentration is lowered in patients treated long-term with phenytoin. However, it is doubtful that the triiodothyronine resin test used by these workers to calculate a free thyroxine index is sufficiently sensitive to detect a phenytoin-induced decrease in thyroxine binding from a normal of 99.95% to 99.9% as found in the present study. Furthermore, the authors were unable to explain the lack of an exaggerated thyroid-stimulating hormone response to administered thyrotropin releasing-hormone in their phenytoin-treated patients.

Urinary levels of unconjugated thyroid hormones, in the absence of proteinuria, should reflect serum concentrations of unbound (i.e. filterable) thyroxine and triiodothyronine. Chan, Besser & Landon (1972) recorded a marked increase in urinary thyroxine in one individual following the institution of phenytoin therapy. A subsequent study (Chan, 1974) showed slight but insignificant increases in thyroxine excretion in a group of phenytoin-treated subjects. Chan's (1974) assay method for urine unconjugated thyroxine involved acidification and alcohol extraction of urine—steps which lead to artefactually high estimations (Black, Griffiths, Hoffenberg & Leatherdale, 1973).

The present study suggests that therapeutic levels of phenytoin significantly displace thyroid hormones from binding proteins resulting in reduced serum total hormone, increased free fraction and resultant increased free hormone concentration. These effects are more marked with regard to thyroxine than to triiodothyronine and are reflected in the urinary losses of unconjugated hormones which result from glomerular filtration of serum free hormones. Despite these phenomena, serum free hormone concentrations which probably determine the thyroid status of the individual, remain in the euthyroid range, unlike serum total hormone concentrations which may overlap with values in the hypothyroid range.

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