Anticonvulsant therapy and cortisol elimination

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1 The effect of anticonvulsant therapy on early morning concentration of cortisol in saliva and plasma was assessed in a group of epileptic patients receiving regular phenytoin medication and the results compared with those obtained from a group of normal subjects not receiving drug therapy.

2 Values of cortisol in matched samples of plasma ($331 \pm 23 \text{ nmol l}^{-1}$, mean $\pm$ s.e. mean, $n = 6$) and saliva ($11.4 \pm 0.9 \text{ nmol l}^{-1}$, mean $\pm$ s.e. mean, $n = 9$) provided by epileptics did not differ significantly from those in the plasma ($334 \pm 41 \text{ nmol l}^{-1}$, mean $\pm$ s.e. mean) and saliva ($12.0 \pm 2.0 \text{ nmol l}^{-1}$, mean $\pm$ s.e. mean) of healthy volunteers ($n = 12$).

3 Six anticonvulsant-treated epileptics, together with six age and sex matched normal volunteers, each received intravenous dexamethasone (1 mg h^{-1}) to determine the half-life of cortisol in plasma and saliva. In the anticonvulsant-treated group, the half-life of cortisol in plasma ($73 \pm 5 \text{ min}$, mean $\pm$ s.e. mean) and saliva ($83 \pm 5 \text{ min}$, mean $\pm$ s.e. mean) was reduced significantly ($P < 0.01$ plasma, $P < 0.05$ saliva) from that observed in healthy volunteers. In patients, the half life of cortisol and antipyrine showed a significant correlation ($r^2 = 0.75$, $P < 0.05$ plasma, $r^2 = 0.71$, $P < 0.05$ saliva).

4 The antipyrine half-life in saliva was reduced significantly ($P < 0.02$) and the antipyrine clearance rate, increased significantly ($P < 0.005$) in the treated epileptic group, reflecting drug-induced microsomal enzyme production.

5 The half-life of cortisol in plasma and saliva in subjects receiving anticonvulsant therapy probably depends on the degree of microsomal enzyme induction occurring in response to anticonvulsant therapy.

Keywords anticonvulsants cortisol enzyme induction

Introduction

A number of endocrine and metabolic effects have been associated with anticonvulsant therapy, in particular phenytoin (Gharib & Munoz, 1974). In 1958 Kivalo *et al.* demonstrated changes in hypothalamo-hypophyseal function in mice after phenytoin administration and since then it has been shown that the formation and transport of a number of substances in the hypothalamus is affected (Bonnycastle, 1956; Fichman,

1967). Chronic administration of phenytoin also leads to increased hepatic microsomal enzyme induction and this results in an increased rate of cortisol clearance (Choi *et al.*, 1971). This is compensated for by an increased cortisol secretion rate (Werk *et al.*, 1971) which, in animals, produces hyperplasia of the adrenal glands. In the presence of an increased rate of cortisol clearance and the maintenance of normal plasma

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cortisol levels it has to be assumed that ACTH secretion from the anterior pituitary must be increased in a compensatory way. Four such cases where ACTH was increased were described by Berson & Yalow (1968). In contrast Krieger (1962) has reported that phenytoin therapy prevented the normal urinary steroid response to oral metyrapone and it was suggested there was some interference with ACTH release at a hypothalamic or pituitary level. This subnormal increase in urinary 17-hydroxycorticosteroids during the metyrapone test can now be explained as being due to a decreased concentration of unconjugated metyrapone in the plasma as a result of an increased rate of clearance, since increasing the oral dose of metyrapone resulted in a normal plasma ACTH and urinary steroid response (Meikle, 1969). This observation along with a normal responsiveness to vasopressin and pyrogen suggests that phenytoin does not interfere with hypothalamic-pituitary-adrenal control.

The purpose of this study was to assess the effect of anticonvulsant therapy on salivary cortisol and thus free physiologically active cortisol. Saliva cortisol concentrations reflect the free physiologically active fraction of cortisol in the plasma (Peters *et al.*, 1982) and there is excellent agreement between plasma free and saliva cortisol levels over a wide range of concentrations (Umeda *et al.*, 1981).

To measure the half-life of plasma and salivary cortisol an intravenous infusion of dexamethasone was employed to block ACTH release by negative feed-back inhibition. This in turn will prevent cortisol secretion by the adrenal glands. Dexamethasone does not interfere with the radioimmunoassay of cortisol but may possibly interfere with the normal distribution and metabolism of steroids and have an effect on the metabolic clearance rate. By collecting serial samples of plasma and saliva during the dexamethasone infusion it is possible to determine the clearance and half-life of endogenous cortisol from the circulation and saliva.

Methods

Plasma and salivary cortisol at 09.00 h

Matched samples of plasma and saliva were collected at 09.00 h from 12 normal, rested volunteers (five males, seven females; mean age 29; range 22–57 years). All nine epileptic patients (five males, four females; mean age 38; range 20–68 years) provided a sample of saliva but only six provided matched samples of saliva and

plasma. To aid collection saliva flow was stimulated by chewing on Teflon tape. Each epileptic subject had been receiving phenytoin for at least 1 year at a minimum daily dose of 200 mg (range 200–400 mg daily). Each epileptic subject studied showed no clinical or radiological evidence of hypothalamic-pituitary disease. Saliva was collected by direct salivation into disposable glass tubes and at the same time blood was obtained by venepuncture of an antecubital vein and collected into lithium heparin tubes. Plasma and saliva were stored at -20°C until assayed. The assays for plasma total (Riad-Fahmy *et al.*, 1979) and salivary (Walker *et al.*, 1978) cortisol have been described elsewhere.

Dexamethasone suppression test

Six epileptic patients, together with six age and sex-matched normal volunteers (three females, three males; mean age 31; age range 22–58 years) each gave informed consent for an intravenous dexamethasone suppression test. Dexamethasone sodium phosphate was infused intravenously at the rate of 1 mg h^{-1} via a Vicker's infusion pump. Matched samples of plasma and saliva were collected at 08.30 h and 09.00 h before the infusion was started and thereafter every hour for 5 h. The results obtained were plotted semilogarithmically and the half-life of cortisol in plasma and saliva calculated.

Antipyrine test

The same six epileptic patients, together with the six age and sex matched controls, underwent an antipyrine test. Each subject fasted overnight and had abstained from ethanol for 24 h. Antipyrine (phenazone BP, 600 mg) was given orally in aqueous solution and saliva samples collected at time 0, +1, +2, +3, +5, +8, +13, +24 and +32 h. Teflon tape was chewed to stimulate saliva secretion and approximately 5 ml of saliva obtained for each collection. Antipyrine in saliva was assayed by gas liquid chromatography (Fraser *et al.*, 1976). The results obtained were used to calculate the antipyrine half-life and antipyrine clearance. The half-life of antipyrine was obtained from a semilogarithmic plot of the concentration-time curve. Antipyrine clearance was calculated from the following formula:

$$\text{clearance} = \frac{\text{Dose antipyrine}}{\text{AUC}_{0-\infty}}$$

where $\text{AUC}_{0-\infty}$ is the area under the concentration-time curve between time zero and infinity. The $\text{AUC}_{0-\infty}$ is calculated by adding the

$AUC_{0-32\text{ h}}$ measured by the trapezoidal method to the AUC_{∞} which is calculated as C at 32 h divided by the elimination rate constant.

Statistical methods

Statistical significance was determined using Students *t*-test for unpaired data.

Results

Plasma and salivary cortisol levels at 09.00 h

Plasma and salivary cortisol levels at 09.00 h in the group of epileptics ($331 \pm 23\text{ nmol l}^{-1}$ plasma, $n = 6$; $11.4 \pm 0.9\text{ nmol l}^{-1}$ saliva, $n = 9$, mean $\pm$ s.e. mean) did not differ significantly from those in the group of normal subjects ($334 \pm 41\text{ nmol l}^{-1}$ plasma; $12.0 \pm 2.0\text{ nmol l}^{-1}$ saliva; $n = 12$, mean $\pm$ s.e. mean).

Dexamethasone suppression test

A significantly shorter half-life was demonstrated for cortisol in both plasma ($P < 0.01$) and saliva ($P < 0.05$) in the phenytoin-treated group. The half-life of plasma cortisol was $73 \pm 5\text{ min}$ (mean $\pm$ s.e. mean) in the phenytoin-treated group, compared with $116 \pm 7\text{ min}$ (mean $\pm$ s.e. mean) in the normal group. The saliva cortisol half-life was $83 \pm 5\text{ min}$ (mean $\pm$ s.e. mean) in the phenytoin-treated group compared with $106 \pm 9\text{ min}$ (mean $\pm$ s.e. mean) in the normal group.

Antipyrine test (Table 1)

The antipyrine half-life and clearance rate were calculated for each of the six phenytoin-treated epileptic subjects and the results compared with those obtained from the six normal age and sex-matched volunteers. The half-life of antipyrine was significantly reduced ($P < 0.02$) in the phenytoin-treated group ($5 \pm 1\text{ h}$, mean $\pm$ s.e.

mean) compared with the normal group ($11 \pm 2\text{ h}$, mean $\pm$ s.e. mean). A finding in keeping with the reduced half-life of plasma and saliva cortisol was the significant increase ($P < 0.005$) in the antipyrine clearance rate in the phenytoin-treated group ($130 \pm 24\text{ ml min}^{-1}$, mean $\pm$ s.e. mean) when compared with that in healthy volunteers ($36 \pm 4\text{ ml min}^{-1}$, mean $\pm$ s.e. mean). The half-life for antipyrine correlated significantly with the half-life of plasma ($r^2 = 0.75$, $P = 0.05$) and saliva ($r^2 = 0.71$, $P = 0.05$) cortisol in the phenytoin-treated group, but not in the normal group. In contrast, the half-life of plasma and saliva cortisol showed no correlation with antipyrine clearance in either group.

Discussion

The determination of the half-life of antipyrine and antipyrine clearance provides a sensitive index of hepatic microsomal induction (Stevenson *et al.*, 1972). Patients receiving anticonvulsant drugs have increased microsomal enzyme activity and therefore increased ability to metabolise drugs and endogenous substances including cortisol. Cortisol metabolism has been evaluated in patients receiving phenytoin by measuring the ratio in urine of 6-hydroxycortisol (6-OHF) to 17-hydrocorticosteroid (17 OHCS) (Werk *et al.*, 1971). During phenytoin administration there was a positive correlation of cortisol secretion rate with the increase in ratio of 6-OHF/17 OHCS. The increase in ratio is probably related to enhancement of hepatic microsomal enzyme activity by the phenytoin. The group of epileptics receiving anticonvulsant therapy demonstrated a significantly reduced half-life of cortisol in plasma and saliva which correlated with a reduced half-life of antipyrine. The significantly reduced half-life of plasma cortisol in the epileptic group is in agreement with previous studies in rodents by Choi *et al.* (1971). Since anticonvulsant therapy causes considerable but unpredictable variation in the degree of

Table 1 The half-life values for cortisol in plasma ($t_{1/2}\text{ P}$) and saliva ($t_{1/2}\text{ S}$), the half-life of antipyrine and antipyrine clearance in the two groups studied. The results are expressed as mean $\pm$ s.e. mean. Statistical significance was obtained using Student's *t*-test for unpaired data.

	Normals	Epileptics	
Cortisol $t_{1/2}\text{ P}$ (min)	116 ± 7	73 ± 5	($P < 0.01$)
Cortisol $t_{1/2}\text{ S}$ (min)	106 ± 9	83 ± 5	($P < 0.05$)
Antipyrine $t_{1/2}$ (h)	11 ± 2	5 ± 1	($P < 0.02$)
Antipyrine clearance (ml min^{-1})	36 ± 4	130 ± 24	($P < 0.005$)

stimulation of hepatic microsomal enzymes (Choi *et al.*, 1971; Brooks *et al.*, 1972; Hague *et al.*, 1972) care has to be taken with patients receiving steroid replacement therapy.

In subjects with a normally functioning hypothalamic-pituitary-adrenal axis changes in cortisol clearance would be followed by compensatory changes in cortisol secretion rate to maintain normal plasma cortisol concentrations, as occurs in hyperthyroidism (Gordon & Southren, 1977). However there is evidence that phenytoin may suppress the release of TSH directly at pituitary level as chronic treatment

with phenytoin leads to a decrease in the mean serum free T_4 concentration. In addition phenytoin inhibits TRH induced TSH release in hypothyroid subjects (Surks *et al.*, 1983). Since salivary cortisol concentrations reflect free and thus physiologically active cortisol it is an important observation that similar suppression of ACTH release does not occur and salivary cortisol levels are not different to those of subjects not on treatment. However dynamic testing of the hypothalamo-pituitary-adrenal axis may be required to demonstrate an effect of phenytoin on ACTH secretion.

References

- Berson, S. A. & Yalow, R. S. (1968). Radioimmunoassay of ACTH in plasma. *J. clin. Invest.*, **47**, 2725–2751.
- Bonnycastle, D. D., Paasonen, M. K. & Giarman, N. J. (1956). Diphenylhydantoin and brain levels of 5-hydroxytryptamine. *Nature (Lond.)*, **178**, 990.
- Brooks, S. M., Werk, E. E. Jr., Ackerman, S. J., Sullivan, I. & Thrasher, K. (1972). Adverse effects of phenobarbitone on corticosteroid metabolism in patients with bronchial asthma. *New Engl. J. Med.*, **286**, 1125–1128.
- Choi, Y., Thrasher, K., Werk, E. E. Jr., Sholiton, L. J. & Olinger, C. (1971). Effect of diphenylhydantoin on cortisol kinetics in humans. *J. Pharmac. exp. Ther.*, **176**, 27–34.
- Fichman, M. P., Bethune, J. E. & Kleeman, C. R. (1967). Inhibition of antidiuretic hormone by diphenylhydantoin (abstract). *Clin. Res.*, **15**, 141.
- Fraser, H. S., Mucklow, J. C., Murray, S. & Davies, D. S. (1976). Assessment of antipyrine kinetics by measurement in saliva. *Br. J. clin. Pharmacol.*, **3**, 321–325.
- Gharib, H. & Munoz, J. M. (1974). Endocrine manifestations of diphenylhydantoin therapy. *Metabolism*, **23**, 515–524.
- Gordon, G. G. & Southren, A. L. (1977). Thyroid hormone effects on steroid hormone metabolism. *Bull. N. Y. Academic Medicine*, **53**, 241.
- Hague, N., Thrasher, K., Werk, E. E. Jr., Knowles, H. C. & Sholiton, L. J. (1972). Studies of dexamethasone metabolism in man; Effect of diphenylhydantoin. *J. clin. Endocrinol. Metab.*, **34**, 44–50.
- Kivalo, E., Tyrkko, J. & Marjanen, P. (1958). The response to diphenylhydantoin of the neurosecretory substance in the mouse. *Ann. Med. Internae, Fenn.*, **47**, 169.
- Krieger, D. T. (1962). Effect of diphenylhydantoin on pituitary-adrenal inter-relations. *J. clin. Endocrinol. Metab.*, **22**, 490.
- Meikle, A. W., Jubiz, W., Matsukura, S., West, C. D. & Tyler, F. H. (1969). Effect of diphenylhydantoin on the metabolism of metyrapone and release of ACTH in man. *J. clin. Endocrinol.*, **29**, 1553.
- Peters, J. R., Walker, R. F., Riad-Fahmy, D. & Hall, R. (1982). Salivary cortisol assays for assessing pituitary-adrenal reserve. *Clin. Endocrinol.*, **17**, 583–592.
- Riad-Fahmy, D., Read, G. F., Gaskell, S. J., Dyas, J. & Hindawi, R. (1979). A simple, direct radioimmunoassay for plasma cortisol featuring a ^{125}I radioligand and a solid phase separation technique. *Clin. Chem.*, **25**, 665–668.
- Stevenson, I. H., Browning, M., Crooks, J. & O'Malley, K. (1972). Changes in human drug metabolism following long term exposure to hypnotics. *Br. med. J.*, **4**, 322–324.
- Surks, M. I., Ordene, K. W., Mann, D. N. & Kumara-Siri, M. H. (1983). Diphenylhydantoin inhibits the thyrotropin response to thyrotropin-releasing hormone in man and rat. *J. clin. Endocrinol. Metab.*, **56**, 940–945.
- Umeda, T., Hirumatsu, A., Iwaoka, T., Shimada, T., Muira, F. & Sato, T. (1981). Use of saliva for monitoring unbound free cortisol levels in serum. *Clin. Chim. Acta*, **10**, 245–253.
- Walker, R. F., Riad-Fahmy, D. & Read, G. F. (1978). Adrenal status assessed by direct radioimmunoassay of cortisol in whole saliva or parotid fluid. *Clin. Chem.*, **24**, 1460–1463.
- Werk, E. E. Jr., Thrasher, K., Sholiton, L. J., Olinger, C. & Choi, Y. (1971). Cortisol production in epileptic patients treated with diphenylhydantoin. *Clin. Pharmac. Ther.*, **12**, 698–703.

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