THE EFFECT OF INTRAVENOUS HEPARIN INFUSIONS ON THE THYROID STIMULATING HORMONE RESPONSE TO THYROTROPHIN RELEASING HORMONE

J.E. THOMSON*, S.G. BAIRD, G.H. BEASTALL,

W.A. RATCLIFFE & J.A. THOMSON

University Department of Medicine and Radioimmunoassay Unit,
Royal Infirmary, Glasgow

- 1 The TSH response to TRH is suppressed 24 h after the commencement of i.v. heparin infusion in euthyroid subjects, as compared to repeat testing, both after 7 days of continuous i.v. heparin and after 7 days without heparin but while on warfarin.
- 2 This suggests that the rise in serum free thyroid hormones, which heparin produces, has a 'metabolic effect'. Possible clinical effects of this action must be considered.
- 3 Heparin has no effect on the radio-immunoassay of TSH.

Introduction

Intravenously administered heparin produces a marked rise in serum free thyroxine (FT_4) levels and a definite but less marked rise in serum free triiodothyronine (FT_3) levels. (Hollander, Scott, Burgess, Rabinowitz, Merimee & Oppenheimer, 1967; Schatz, Sheppard, Steiner, Chandarlapaty & de Veber, 1969; Hershman, Jones & Bailey, 1972; Thomson, Baird & Thomson, 1977). With bolus doses of the drug, this effect is relatively transient but intravenous heparin infusions result in elevated FT_4 levels for up to 12 h (Saeed-Uz-Zafar, Miller, Branaman & Mansour, 1971). Hershman *et al.* (1972) have reported that this rise in free thyroid hormone levels results in a 'metabolic effect' in the form of suppression of the serum thyroid stimulating hormone (TSH). Having been unable to confirm the latter finding (Thomson *et al.*, 1977), the present study was undertaken in an effort to demonstrate such a 'metabolic effect,' using the TSH response to thyrotrophin releasing hormone (TRH), the most sensitive available indicator of thyroid status.

Methods

Sixteen subjects were studied, informed consent having been obtained from each. These were clinically euthyroid patients, admitted to hospital for therapeutic anti-coagulation because of deep venous thrombosis. Anti-coagulant therapy consisted of continuous

intravenous heparin infusions, in a dose of 10,000 IU 6 hourly for either 48 h or 7 days. This was followed by anticoagulation with oral warfarin. TRH tests were performed 24 h after starting the heparin infusion (Test 1) in fifteen subjects, after 7 days of continuous intravenous heparin (Test 2) in the nine subjects in whom heparin was continued for this period, and 7 days after stopping heparin (Test 3) but when on oral warfarin in fourteen subjects. No other drugs known to affect thyroid function tests were administered during the period of the study.

TRH tests were performed after the method of Ormston, Garry, Cryer, Besser & Hall (1971) with serum TSH being measured at 0, 20 and 60 min after the administration of the TRH.

Thyroid stimulating hormone levels were determined by a double antibody radioimmunoassay method based on that of Hall, Amos & Ormston (1971) using MRC 68/38 standard TSH, Hartree DE-32-3 TSH for iodination with ^{125}I by a modification of the method of Hunter & Greenwood (1962) and a rabbit antiserum kindly donated by Professor W.R. Butt (Birmingham and Midland Hospital for Women, Birmingham B11 4HL). The interassay and intra-assay coefficients of variation for the TSH method are 13.6% and 6.3% respectively and the upper limit of normality is 8.0 mU/l. A normal TSH response to TRH is a 20 min TSH increment of between 5 and 20 mU/l.

Calculation of results was achieved by the automated technique of Challand, Spencer & Ratcliffe (1976) which uses a linear interpolation method. All

* Present address: Stobhill Hospital, Glasgow G21 3UW.

samples for individual subjects were measured in the same assays having been stored at -4°C .

Results

The results are recorded in Tables 1, 2 and 3. Paired data, to which Student's *t*-test has been applied, are compared throughout, each subject acting as his own control.

Eight subjects had both tests 1 and 2 performed. The mean values for the 20 min and 60 min TSH levels and 20 min TSH increment were significantly lower for test 1 than for test 2. In addition although the mean TSH increment was within the normal range at both tests, three of the eight subjects had an absent or subnormal TSH response at test 1 and a normal response at test 2. There was non-significant difference between the mean values for basal TSH.

Table 1 Comparison of TSH response (mean $\pm$ s.d.) to TRH after 24 h and 7 days of heparin therapy

Test	Therapy	Number of subjects	TSH (mu/l) Time (min)			TSH Δ 20
			0	20	60	
1	24 h on heparin	8	3.9 ± 2.0	10.3 ± 6.7	8.8 ± 5.1	6.8 ± 5.7
2	7 days on heparin	8	3.4 ± 2.8	18.8 ± 13.2	14.1 ± 8.6	15.4 ± 11.5
Significance		<i>P</i>	NS	<0.05	<0.05	<0.01

Table 2 Comparison of TSH response (mean $\pm$ s.d.) to TRH after 24 h of heparin therapy and while on oral warfarin.

Test	Therapy	Number of subjects	TSH (mu/l) Time (min)			TSH Δ 20
			0	20	60	
1	24 h on heparin	13	3.2 ± 2.5	10.6 ± 6.7	7.8 ± 5.1	7.7 ± 6.1
3	on warfarin only	13	4.1 ± 2.4	15.2 ± 6.1	9.6 ± 4.2	11.0 ± 7.7
Significance		<i>P</i>	NS	<0.001	NS	<0.01

Table 3 Comparison of TSH response (mean $\pm$ s.d.) to TRH after 7 days of heparin therapy and while on oral warfarin

Test	Therapy	Number of subjects	TSH (mu/l) Time (min)			TSH Δ 20
			0	20	60	
2	7 days on heparin	7	3.0 ± 3.2	21.9 ± 12.9	15.8 ± 8.4	18.9 ± 10.9
3	on warfarin only	7	4.0 ± 2.5	18.3 ± 8.9	12.0 ± 7.6	14.2 ± 8.5
Significance		<i>P</i>	NS	NS	NS	NS

Thirteen subjects had both test 1 and 3 performed. Again the mean values for the 20 min TSH and the 20 min TSH increment were significantly lower for test 1 than for test 3. Three of these subjects had a subnormal response at test 1 and a normal response later at test 3, although again the mean TSH increment was within the normal range at both tests. There was no significant difference between the mean values for basal or 60 min TSH.

Seven subjects had both tests 2 and 3 performed. No significant differences were observed between these two tests and all TSH responses were within the normal range.

In order to assess that heparin has no direct effect of the assay of TSH, serum was assayed both in the absence and presence of known amounts of heparin. No change in antibody binding was observed in the presence of heparin levels equivalent to 400 u/ml and fifteen random serum samples assayed for TSH in the presence (y) and absence (x) of 100 u/ml heparin produced a correlation coefficient of 0.994 and a linear equation $y = 0.94x + 0.2$.

Discussion

This investigation has shown a significant impairment of TSH response to TRH 24 h after starting i.v. heparin therapy, when compared to repeat testing after 7 days of continuous i.v. heparin or after 7 days without heparin but while on oral warfarin. In contrast to the report of Hershman *et al.* (1972) but in agreement with our previous findings (Thomson *et al.*, 1977), suppression of basal TSH has not been demonstrated.

The absence of any detectable suppression of response after seven days of continuous heparin therapy suggests that a direct action of heparin is not involved. It seems likely that the known relatively transient rise in free thyroid hormones which occurs after heparinisation may be responsible, by suppressing the hypothalamic-pituitary axis.

Other possible explanations for these findings must, however, be considered. A mild antithyroid action by warfarin would have produced an exaggeration of the response at test 3 and thus apparent suppression at test 1. However, this possibility is excluded by the fact that the response 24 h after starting heparin is suppressed, in comparison to test 2 when warfarin was not involved; in addition all responses on warfarin were normal whereas a number of absent or subnormal responses were found at test 1; furthermore, no difference is detectable between tests 2 and 3, and any effect of warfarin would be expected

to apply equally to a comparison of test 3 with both test 1 or test 2. Finally, Williams & Doniach (1961) demonstrated that warfarin had no effect on thyroid function.

The results could be explained if heparin interfered with the TSH assay but again such an effect would apply equally to tests performed after 24 h and 7 days of heparin therapy and no difference between tests 2 and 3 was found. Moreover a formal investigation of the effect of heparin on the TSH assay failed to demonstrate any significant effect.

These results therefore, suggest that the rise in free thyroid hormones produced by heparin has a detectable 'metabolic effect' in the form of suppression of pituitary release of TSH. It seems reasonable to ask whether this phenomenon affects other tissues. Thyroid hormones specifically stimulate one of the adenyl cyclase systems in myocardium (Levey, 1971; Epstein, Skelton, Levey & Eutman, 1970); and work in dogs with acute myocardial infarction, has shown that heparin induced elevations of free fatty acids are associated with the development of ventricular arrhythmias (Kurien Yates & Oliver, 1969; Kurien & Oliver, 1970). It is possible that elevated levels of free thyroid hormones might contribute to this effect of heparin on myocardium. An increased incidence of arrhythmias in patients with acute myocardial infarction given heparin, has not, however, been demonstrated (Nelson, 1970; Russo, Friesinger, Margolia & Ross, 1970).

The mechanism by which heparin produces its effect on free thyroid hormones is by no means certain. It has been suggested that changes in hormone binding may be responsible (Hollander *et al.*, 1967) but this has not been confirmed by other reports (Schatz *et al.*, 1969) and indeed, Tabachnick, Haeo Yu-Lee & Karcek (1973) have shown that heparin is a relatively ineffective inhibitor of thyroxine (T_4) binding to thyroxine binding globulin (TBG). The thyroid is not involved since the same phenomenon occurs in athyreotic individuals (Saeed-Uz-Zafar *et al.*, 1971). Heparin does however, reduce cellular binding of T_4 (Schwartz, Schadow, Faierman, Surks & Oppenheimer, 1973; Tabachnick *et al.*, 1973) and it may be that the elevation of free thyroid hormones is due to the release of these hormones from the tissues. The liver contains large stores of T_4 (Oppenheimer, 1972) and ether and halothane anaesthesia have been shown to produce a rise in serum FT_4 by causing release of T_4 from liver (Oyama, Shibata, Matsubi & Kudo, 1969). It seems reasonable to postulate that heparin has a similar action.

References

- CHALLAND, G.S., SPENCER, C.A. & RATCLIFFE, J.G. (1976). Observations on the automated calculation of radioimmunoassay results. *Ann. clin. Biochem.*, **13**, 354–360.
- EPSTEIN, S.E., SKELTON C.L., LEVEY, G.S. & EUTMAN M., (1970). Adenyl cyclase and myocardial contractility. *Ann. int. Med.*, **72**, 561–780.
- HALL, R., AMOS, J. & ORMSTON, B. (1971). Radioimmunoassay of human serum thyrotrophin. *Br. med. J.*, **1**, 582–585.
- HERSHMAN, J.M., JONES C.M., & BAILEY A.L. (1972). Reciprocal changes in serum thyrotrophin and free thyroxine produced by heparin. *J. clin. Endocrinol. Metab.*, **34**, 574–579.
- HOLLANDER, C.S., SCOTT, R.L., BURGESS, J.A., RABINOWITZ, T.J. MERIMEE, T.J. & OPPENHEIMER, J.H. (1967). Free fatty acids: a possible regulator of free thyroid hormone levels in man. *J. clin. Endocrinol. Metab.*, **27**, 1219–1223.
- HUNTER, W.M. & GREENWOOD, F.C. (1962). Preparation of iodine-131 labelled human growth hormone of high specific activity. *Nature, (Lond.)* **194**, 495–496.
- KURIEN, V.A. & OLIVER, M.F., (1970). A metabolic cause for arrhythmias during acute myocardial hypoxia. *Lancet*, **i**, 813–815.
- KURIEN, V.A., YATES, P.A., & OLIVER, M.F. (1969). Free fatty acids, heparin, and arrhythmias during experimental myocardial infarction. *Lancet*, **ii**, 185–187.
- LEVEY, G.S. (1971). Catecholamine sensitivity, thyroid hormones and the heart. A revaluation. *Am. J. Med.*, **50**, 413–420.
- NELSON, P.G. (1970). Effect of heparin on serum free fatty acids, plasma catecholamines and the incidence of arrhythmia following acute myocardial infarction. *Br. med. J.*, **2**, 735–737.
- OPPENHEIMER, J.H. (1972). Thyroid hormones in liver. *Mayo Clin. Proc.*, **47**, 854–863.
- ORMSTON, B.J., GARRY, R., CRYER, R.J., BESSER, G.M. & HALL, R. (1971). Thyrotrophin in releasing hormone in a thyroid function test. *Lancet*, **ii**, 10–14.
- OYAMA, T.S., SHIBATA, S., MATSUBI, A. & KUDO, T. (1969). Thyroxine distribution during halothane anaesthesia in man. *Anaesth. Analg., (Cleveland)*, **48**, 715–719.
- RUSSO, J.V., FRIESINGER, G.D., MARGOLIA, S. & ROSS, R.S. (1970). Heparin and ventricular arrhythmias after myocardial infarction. *Lancet*, **ii**, 1271–1275.
- SAEED-UZ-ZAFAR, M., MILLER, J.M., BRANAMAN, G.M. & MANSOUR, J. (1971). Observations on the effect of heparin on free and total thyroxine. *J. clin. Endocrinol. Metab.*, **32**, 633–640.
- SCHATZ, D.L., SHEPPARD, R.H., STEINER, G., CHANDARLAPATY, C.S. & DE VEBER, G.A. (1969). Influence of heparin on serum free thyroxine. *J. clin. Endocrinol. Metab.*, **29**, 1015–1022.
- SCHWARTZ, H.L., SCHADLOW, A.R., FAIERMAN, D., SURKS, M.I. & OPPENHEIMER, J.H. (1973). Heparin administration appears to decrease cellular binding of thyroxine. *J. clin. Endocrinol. Metab.*, **36**, 398–600.
- TABACHNICK, M., HAO YU-LEE, W. & KARCEK, L. (1973). Effect of oleate diphenylhydantoin and heparin on the binding of 125-thyroxine to purified thyroxine binding globulin. *J. clin. Endocrinol. Metab.* **36**, 392–394.
- THOMSON, J.E., BAIRD, S.G. & THOMSON, J.A. (1977). Effect of i.v. heparin on serum free tri-iodothyronine levels. *Br. J. clin. Pharmacol.*, **4**, 701–702.
- WILLIAMS, E.D. & DONIACH, I. (1961). The anti-thyroid activity of the anticoagulant phenylindanedione. *J. Endocrinol.*, **21**, 421–423.

(Received August 25, 1977)