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Differences in Pituitary and Testicular Function between Diabetic Patients on Insulin and Oral Anti-Diabetic Agents

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Summary. Pituitary responsiveness to thyrotrophin releasing hormone (TRH) and luteinizing hormone releasing hormone (LHRH) was studied in thirty one male diabetics, of whom sixteen were insulin-dependent and fifteen on oral antidiabetic agents. Ten age-matched controls were also studied. TRH and LHRH were simultaneously administered intravenously, each in a small dose of 10 μg followed two hours later by 190 μg and 90 μg respectively. Basal hormone levels were measured in a further group of thirty six patients (twelve on insulin, twelve on oral agents and twelve on dietary restrictions alone).

Higher thyrotrophin (TSH) response was observed following the small dose of TRH in the patients treated with oral agents than in the control subjects. The response of prolactin was lower in patients treated with oral agents compared with those treated with insulin. There was no difference in plasma T_3 and T_4 levels in the patients treated with insulin or oral agents. Significantly higher basal growth hormone (GH) levels were observed in the diabetics. The insulin-dependent group showed a more marked response of GH to TRH/LHRH. No response was observed in the controls. Plasma testosterone levels were significantly lower in the oral agent group (13.8 nmol/l) than in the insulin group (19.4 nmol/l), patients on dietary restrictions (18.4 nmol/l) and the control subjects (19.0 nmol/l). The LH response to the smaller dose of LHRH was impaired in patients on insulin and oral agents. There was a significant difference in FSH response between impotent and sexually normal patients.

Key words: Diabetes, pituitary function, testicular function, testosterone, oral anti-diabetic agents, growth hormone, impotence, gonadotrophins, prolactin, insulin, thyrotrophin.

Disturbance of fertility and sexual function has long been recognised in patients with diabetes mellitus [1, 2]. Impotence in particular is a common complaint which in normal subjects is frequently psychogenic [3, 4] but in diabetes may also be due to autonomic neuropathy [5] or angiopathy [6]. Different workers have sought to implicate endocrine factors in its pathogenesis but without consistency. Normal serum testosterone levels have been frequently reported [5, 7] in impotent diabetic patients. Although Schoffling et al. [8] claimed successful restoration of potency with testosterone therapy, most reports describe it as ineffective [4, 5].

More recently there have been conflicting reports of pituitary gonadal function in diabetic patients. Normal [9] luteinising hormone (LH) and blunted [10] follicular stimulating hormone (FSH) responses to luteinising hormone releasing hormone (LHRH) stimulation have been reported while blunted LH, but normal FSH, responses were demonstrated by Wright et al. [11]. Other aspects of pituitary function have also been studied with the demonstration of raised plasma thyrotrophin (TSH) [12, 13] and growth hormone (GH) levels [14, 15]. In view of the scarcity of dynamic studies of pituitary function in diabetes generally and the conflicting results in the few published reports, it was decided to evaluate pituitary responsiveness to LHRH and to thyrotrophin releasing hormone (TRH) in more detail.

Materials and Methods

1. Patients and Controls

Sixty seven male patients were studied. Twenty eight were insulin-dependent, (Age 50.5 ± 3.5 years), twenty seven on oral anti-diabetic agents (Age 53.6 ± 2.4 years) and twelve on dietary control alone (Age 57.6 ± 3.5 years). The patients were randomly selected from the diabetic clinic of St. Helier Hospital. The main

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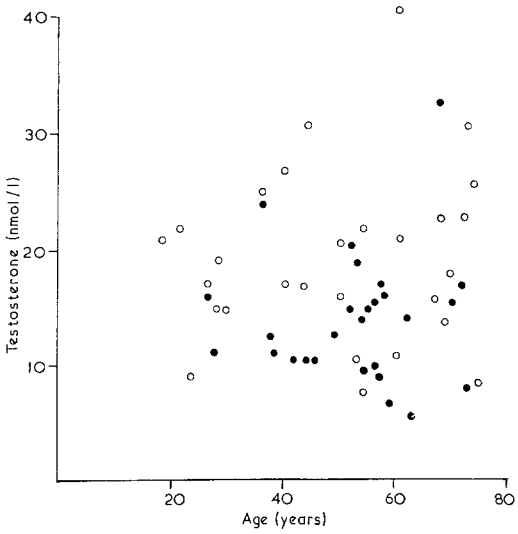

Fig. 1. Scatter diagram showing the relationship between age and plasma testosterone level for the insulin (○) and oral agent (●) treated patients

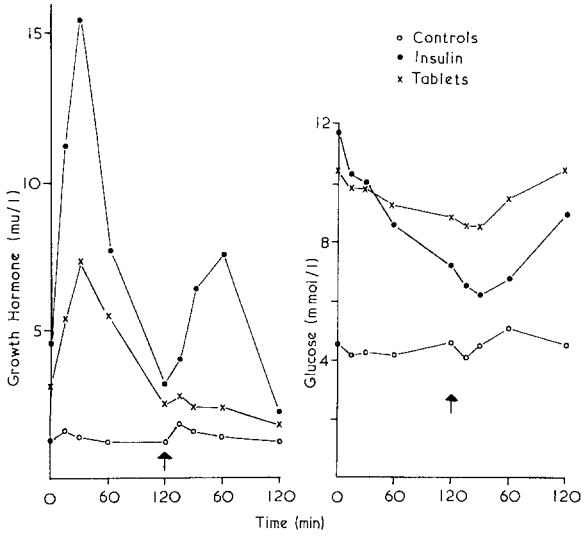

Fig. 2. Growth hormone and glucose response to IV TRH and LHRH in controls and diabetic patients. Zero time: 10 μg TRH and 10 μg LHRH − 120 mins.: 190 μg TRH and 90 μg LHRH

oral anti-diabetic agent used was chlorpropamide. None was on any hormonal therapy other than insulin, nor any other treatment known to affect the blood levels of hormones or the mechanisms regulating their production or release. No patients had any known condition other than diabetes or were receiving other drugs than for the treatment of diabetes. On questioning, six patients on insulin and five on oral agents admitted to impotence, with failure of erection. Informed consent was obtained from all patients after full explanation of the nature and purpose of this study. Ten apparently healthy men served as voluntary controls (Age 41.4 ± 4.3 years). They were not receiving any drugs and were age-matched with the patients. Some were members of the medical and nursing staff and others were patients attending the surgical out-patient clinic for minor surgical problems.

2. Investigations

A combined TRH/LHRH test was carried out on thirty one of the patients (sixteen on insulin (Age 47.0 ± 4.7 years) and fifteen on oral agents (Age 51.7 ± 2.8 years)), and on all ten control subjects. The test was started about 09.30 h. Patients had breakfast and their usual medication before reporting to the investigation unit. On arrival the patient was put to bed and an indwelling plastic cannula inserted into an antecubital vein and kept patent by a slow infusion of 0.154 mol/l saline. Base-line blood samples were collected, 30 minutes apart. Ten μg of TRH and of LHRH were then given from separate syringes through the indwelling cannula, and blood samples collected after 15, 30, 60 and 120 minutes. Further larger doses of TRH (190 μg) and LHRH (90 μg) were given immediately after collection of the 120 minute sample and blood samples again collected over a two hour period. Subjects were allowed to sit up and take mid-morning coffee and lunch. As soon as blood samples were obtained, the plasma was separated and stored at − 20° C. All samples were assayed for prolactin, TSH, GH, LH and FSH. Base-line samples were also assayed for T_4, T_3 and testosterone. Blood glucose was measured in all specimens.

In thirty six other patients (twelve on insulin, twelve on oral agents and twelve on dietary restriction) only basal hormone levels were measured.

3. Assay Methods

Polypeptide and thyroid hormones were measured by radioimmunoassay (RIA). TSH was measured as described by Wood et al. [16], prolactin by the method of Sinha et al. [17] as modified by us [18], GH by the Lepetit Human GH Kit using IRP 66/217 as standard, LH as described by Wilde et al. [19] using LH (MRC 68/40) as standard, FSH using non-LH cross-reacting antiserum and FSH (MRC 69/104) as standard, triiodothyronine (T_3) using an antiserum raised at the University of Surrey and thyroxine (T_4) using the Radiochemical Centre Kit. Testosterone was assayed by a non-chromatographic RIA using an antiserum raised at the University of Surrey and having minimal cross-reactivity with other steroid hormones and metabolites. Glucose was measured using glucose-oxidase. The percentage binding of testosterone in plasma was measured as described by Rosenfield [20].

Statistical Evaluation: All results were reported as the mean ± standard error of the mean. The significance of differences between means was tested using the 't' test. The response of TSH, prolactin, LH and FSH was measured as the sum of the differences from baseline at 15, 30, 60 and 120 minutes for each hormone at both dose levels of TRH/LHRH.

Results

Baseline Plasma Hormone Concentrations (Table 1)

Plasma testosterone was lower in the patients on oral agents compared with both the insulin-treated and diet-controlled patients ($P<0.01$ and <0.05 respectively) who themselves had levels similar to the control group. No correlation was observed between plasma testosterone and age (Fig. 1). Plasma GH was slightly, but significantly raised ($P<0.05$ to <0.01) in the three groups of diabetic patients compared with the control subjects. There was no difference in plasma prolactin, TSH, T_3, LH and FSH levels be-

Table 1. Basal serum hormone levels

		Controls (n = 10)	Patients on insulin (n = 28)	Patients on oral agents (n = 27)	Patients on dietary control (n = 12)
Thyrotrophin[a]	mu/l	1.7 ± 0.6	1.5 ± 0.3	2.4 ± 0.6	–
Prolactin	μg/l	2.3 ± 0.7	3.5 ± 0.4	2.7 ± 0.4	2.7 ± 0.3
Thyroxine	nmol/l	107 ± 7	114 ± 4	110 ± 6	128 ± 4[b]
Triiodothyronine	nmol/l	2.3 ± 0.2	2.1 ± 0.1	1.9 ± 0.1	1.9 ± 0.1
Growth hormone	mu/l	1.3 ± 0.2	4.3 ± 0.9[c]	3.3 ± 0.8[b]	6.0 ± 1.4[c]
Luteinizing hormone	u/l	4.9 ± 0.7	6.4 ± 0.6	6.4 ± 0.5	6.7 ± 0.7
Follicle stimulating hormone	u/l	2.6 ± 0.6	3.1 ± 0.3	3.9 ± 0.5	3.8 ± 1.3
Testosterone	nmol/l	19.0 ± 2.0	19.4 ± 1.4	13.8 ± 1.1[b]	18.4 ± 2.2
Testosterone binding	%		54.9 ± 1.7 (n = 11)	49.9 ± 1.9 (n = 11)	50.3 ± 1.8 (n = 10)
Glucose	mmol/l	4.6 ± 0.4	11.8 ± 1.4	10.5 ± 0.9	

[a] This was measured only in controls and those patients receiving TRH
[b] $P < 0.05$ compared with controls
[c] $P < 0.01$

Table 2. Sum of responses of TSH, Prolactin, LH and FSH to LHRH/TRH in diabetic patients and control subjects

	Control (n = 10)	Insulin (n = 16)	Oral agent (n = 15)	Control (n = 10)	Insulin (n = 16)	Oral agent (n = 15)
TSH mu/l				Prolactin μg/l		
a)	6.1 ± 2.0	4.6 ± 1.0	11.4 ± 2.3[c]	13.2 ± 3.7	8.2 ± 3.0	4.1 ± 1.6[a]
b)	28.0 ± 4.8	24.5 ± 2.6	30.7 ± 4.1	24.0 ± 6.0	20.3 ± 5.8	19.4 ± 2.0
LH u/l				FSH u/l		
a)	45.8 ± 4.8	28.1 ± 2.8[b]	26.4 ± 3.3[b]	8.4 ± 1.8	5.0 ± 1.2	8.4 ± 1.4
b)	50.8 ± 7.9	38.4 ± 4.3	31.8 ± 5.8	12.6 ± 2.8	8.1 ± 2.0	14.9 ± 2.8
	Non-impotent (n = 20)	Impotent (n = 11)		Non-impotent (n = 20)	Impotent (n = 11)	
LH u/l				FSH u/l		
a)	30.7 ± 3.1	22.7 ± 2.3		5.4 ± 1.2	8.4 ± 1.8	
b)	37.5 ± 5.0	32.0 ± 4.8		8.6 ± 1.8	16.6 ± 3.7[d]	

a) Administration of 10 μg LHRH and 10 μg TRH
b) Administration of 90 μg LHRH and 190 μg TRH

[a] $P < 0.05$ compared with control subjects
[b] $P < 0.01$ compared with control subjects
[c] $P < 0.01$ compared with insulin subjects
[d] $P < 0.05$ compared with non-impotent patients

tween the three groups of diabetic patients, nor between them and the control subjects. The T_4 levels in the patients on dietary control was significantly raised when compared with control subjects.

Responses of TRH/LHRH at Low and Standard Dose Levels (Table 2)

The TSH response to the low dose of TRH in the patients on oral agents was greater than that of the patients on insulin ($P<0.01$). In contrast, the prolactin response in the patients on oral agents under these conditions was less than that of the control subjects ($P<0.02$). Both groups of patients had a lower LH response than the control subjects to the low dose of LHRH ($P<0.01$), but the FSH responses were not significantly different.

There was no GH response in control subjects (Fig. 2). The insulin treated patients had a rise at both dose levels. The peak value at the low dose was 15.5 ± 4.0 mu/l and at the standard dose 7.6 ± 1.8 mu/l. Patients on oral agents had a rise only after the low dose with a peak value of 7.4 ± 2.0 mu/l. At no stage were the patients hypoglycaemic nor did the GH responses correlate with changes in blood glucose.

Table 3. Serum testosterone levels in impotent and sexually normal diabetic patients and control subjects

	Testosterone nmol/l	
	Patients on insulin	Patients on oral agents
Sexually normal diabetics (n = 20)	19.6 ± 3.0 (n = 10)	11.6 ± 1.8 (n = 10)
Impotent diabetics (n = 11)	18.0 ± 3.5 (n = 6)	14.5 ± 1.6 (n = 6)
Controls (n = 10)	19.0 ± 2.0	

Impotent Patients (Table 2 and 3)

Basal testosterone, LH and FSH levels in impotent and non-impotent diabetics showed no significant difference from control subjects. The percentage binding of testosterone was the same in all patient groups. The FSH response to LHRH was greater in the impotent than in the non-impotent patients ($P<0.05$) but there was no difference in the LH responses.

Discussion

As far as the authors are aware, assessment of the pituitary responsiveness to TRH/LHRH stimulation in diabetic patients, as part of an overall assessment of pituitary function, has not been previously reported. Because of conflicting results in the literature, we surmised that minor alterations in pituitary function might not be consistently shown by the usual standard tests. For this reason we gave both a small and a standard dose of the releasing hormones to see if minor changes in pituitary function would be more readily demonstrable. The results show significant differences with the lower dose but not the standard dose.

The TSH response to TRH was greater in patients on oral agents than in the insulin group ($P<0.01$) after the low dose of TRH, suggesting a minor difference in responsiveness between the two groups (Table 2). The insulin and the control groups were not different at any point of the test. Raised TSH levels in diabetic patients have been reported by Hunton et al. [12] who attributed this to a goitrogenic effect of the sulphonylurea drugs. On the other hand, Kaufman et al. [13] produced convincing evidence implicating autoimmune factors in patients treated with oral agents. All our patients were clinically euthyroid and had normal levels of T_4 and T_3 (Table 1).

Increased prolactin responses concordant with those of TSH are commonly observed in primary hypothyroid states [21]. Despite the increased TSH response to small doses of TRH in the tablet group, the prolactin response was significantly lower than in the insulin-dependent group ($P<0.02$). This anomalous dissociation between the TSH and prolactin responses has been found in other conditions [22, 23].

Most of the diabetic patients studied showed a definite GH response to TRH/LHRH while none of the control subjects showed any response. It is clear from Fig. 2 that the changes in plasma GH levels are unrelated to changes in blood glucose levels. TRH-mediated GH response has been reported in other pathological conditions, e. g. chronic renal failure [24], acromegaly [25], anorexia nervosa [26], and depression [27] while an LHRH-mediated response has been observed in acromegaly [28]. This abnormal response of GH indicates, we believe, a disorder in hypothalamic pituitary function in diabetics. It is interesting that a greater response was observed in the insulin-dependent patients who have greater impairment of carbohydrate metabolism. The absence of any response in the controls excludes a non-specific effect of stress. Our results are consistent with the findings of others that GH release is facilitated in diabetics. This may explain why GH has been frequently, but inconsistently implicated as a cause of many diabetic complications.

Further evidence of hypothalamic pituitary dysfunction is shown by the reduced LH response in both insulin ($P<0.01$) and oral agents ($P<0.01$) groups following the smaller dose of LHRH.

Wright et al. [11] and Ellenberg [5] have found normal plasma testosterone levels in insulin-treated patients. This has been confirmed by us. However, we observed significantly ($P<0.01$) lower plasma testosterone levels in the group on oral agents. With two exceptions, the patients treated with oral agents had mature-onset diabetes, and it is possible that the observed low testosterone level was a reflection of basic differences between the mature-onset and juvenile-onset diabetes, perhaps due to autoimmune mechanisms. Against this, is the finding of normal testosterone levels in the group of mature-onset diabetics on dietary control alone. It is unlikely that this observation is due to differences in the mean age of the two groups (50.5 ± 3.5 and 53.6 ± 2.4 years for the insulin-treated and oral agent-treated groups respectively) as there is no correlation between age and plasma testosterone levels. Moreover, the insulin-treated impotent and non-impotent diabetics had similar mean plasma testosterone levels (Table 3) despite a big difference in the mean age of the two groups (55.8 ± 5.0 and 41.7 ± 8.0 years respectively). A drug effect on the level of sex hormone binding globulin (SHBG) is excluded by our finding of similar percentage binding of testosterone in the two groups. Any interference with the testosterone

assay method has also been excluded by in-vitro experiments. The possibility remains that the sulphonylureas may have a direct inhibitory action on testosterone production by the testis, through interference with intracellular enzymes. That drugs may act in this way is well documented [29]. On the other hand, the subnormal LH response to LHRH suggests an associated defect at the pituitary level which is even more significant than the figures suggest, since an increased LH response would be expected in the presence of a low testosterone level.

We observed an increased response of FSH in impotent when compared with non-impotent diabetics, in contrast to earlier findings [9, 11]. This would suggest the possibility of some testicular dysfunction.

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