Failure to suppress GH secretion after 2 weeks treatment with atropine or propanthelene in diabetics with proliferative retinopathy

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Abstract. Complete suppression of GH secretion may halt the development of retinal new vessels in patients with diabetic proliferative retinopathy. We have investigated the effectiveness of two cholinergic antagonists, atropine and propanthelene given orally for 2 weeks, in suppressing 24-h GH and IGF-I levels. Seven insulin-dependent diabetics (3 males, 4 females; aged 22–34 years) with active proliferative retinopathy and 6 matched non-diabetic normal subjects were studied in random order with at least 2 weeks between treatments. Suppression of GHRH-induced GH release was demonstrated in both groups of subjects. Twenty-four hour GH secretion was not, however, suppressed in either the patient group (mean area under the GH curve mU·l⁻¹·h⁻¹ ± sd; baseline: 251 ± 108.7; after atropine: 174 ± 106.9; after propanthelene: 180 ± 72.4; P > 0.05) or in the control group (baseline: 103 ± 53.1; after atropine: 73 ± 83.6; after propanthelene: 122 ± 71.6; P > 0.05). GH release at times of hypoglycemia was not suppressed. Mean IGF-I concentration was not significantly reduced. Two subjects (one patient and one control) could not tolerate atropine for more than one week. We conclude that repeated doses of atropine and propanthelene do not achieve complete 24-h GH suppression and are associated with a high incidence of unpleasant adverse reactions.

Several studies have demonstrated that panretinophysectomy or pituitary ablation with ⁹⁰Y can arrest or reverse the progression of proliferative retinopathy and that this beneficial effect persists at 10 year follow-up (Lundbæk et al. 1969; Kohner et al. 1976; Sharp et al. 1987). The degree of GH deficiency achieved correlated with the beneficial effect on retinopathy (Wright et al. 1969; Adams et al. 1974). More recently, elevated circulating and vitreous concentrations of GH dependent insulin-like growth factor I have been found in diabetics with rapidly progressive proliferative retinopathy (Merimee et al. 1983; Grant et al. 1986).

These findings suggest a role for GH directly or via IGF-I in promoting the development of proliferative retinopathy. Selective GH suppression, if equally effective, would be preferable to pituitary ablation in patients with retinal new vessels persisting despite laser treatment.

Cholinergic muscarinic receptor antagonists are potent and selective inhibitors of GH release under a variety of conditions in both normal and diabetic subjects (Delitala et al. 1983; Casanueva et al. 1984; Davis & Davis 1986; Page et al. 1987). The aim of this study was to assess the feasibility of using two such cholinergic antagonists, atropine and propanthelene, to suppress GH in patients with active proliferative retinopathy despite repeated laser therapy.

Patients and Methods

Seven patients with insulin-dependent diabetes and active proliferative retinopathy despite repeated laser therapy were selected for study. Serum creatinine was...
within the normal range although one patient had proteinuria. The clinical details are shown in Table 1. Six non-diabetic normal subjects (4 males, 2 females; aged 24–33 years) were also investigated by the same procedure. The study was approved by the Ethical Committee of the Royal Postgraduate Medical School and Hammersmith Hospital.

Subjects were admitted to the Metabolic Unit for 24-h studies of GH secretion after an overnight fast. An indwelling catheter was inserted into a forearm vein for the purpose of blood sampling. Blood was drawn without disturbing the subjects for measurement of serum GH, IGF-I and plasma glucose of hourly (06.00–24.00) or 2-hourly intervals (24.00–06.00) with at least 21 samples over 24-h. Subjects were encouraged to remain ambulant throughout the day and retired to bed at 22.00 h.

Baseline 24-h sampling was performed on two occasions separated by at least 2 weeks. Patients and control subjects then received, on separate occasions, in randomised order, oral atropine sulphate 1.2 mg to be taken at 4-h intervals starting at 06.00 h, and oral propanthelene bromide 30 mg taken from 06.00 h at 6-h intervals. After 2 weeks of treatment, 24-h GH and IGF-I profiles were repeated. The analysis was performed masked to the subjects' current treatment.

Studies of stimulated GH release were performed at the end of the 24-h baseline studies. Blood samples were drawn at 15-min intervals for 30 min before and 90 min after a bolus of GH-releasing hormone 1–29 NH2 (GHRH) 1 µg/kg iv dissolved in 10 ml of acidified saline and given over 2 min. This test was repeated after pretreatment with atropine 1.2 mg im at −15 min or propanthelene 45 mg po at −30 min.

Assays
Serum was stored at −20°C until assayed. Serum GH was measured with a specific double-antibody radioimmunoassay by an automated system (Kemtek, Burgess Hill, Sussex, UK) using the WHO International Reference Preparation for human growth hormone (66/217, 350 mU = 175 µg) as standard (Burrin et al. 1985). The within and between assay coefficients of variation were 4.7 and 11.4% at a GH concentration of 14 mU/l. The detection limit of the assay was 0.5 mU/l. Samples from a particular subject before and after a test drug were analysed on the same assay.

Serum IGF-I was measured by double-antibody RIA using the monoclonal antiserum 3D 1/2/1 supplied through the National Hormone and Pituitary Program. Before measurement, samples were acid ethanol stripped to separate IGF-I from its binding protein (Daughaday et al. 1980). At an IGF-I concentration of 200 µg/l, the intra- and inter-assay coefficients of variation were 5.3 and 10.2%, respectively; normal range: 100–350 µg/l. HbA1 was measured by agar gel electrophoresis; normal range: 5–8%. Plasma glucose was measured by the glucose oxidase method using the Beckman Glucose Analyser (Beckman Instruments, Palo Alto, CA).

Analysis
The 24-h GH secretion was measured as the area under the GH curve calculated by the trapezoidal rule. Results were analysed non-parametrically; comparisons before and after treatments within the same group were made by the Wilcoxon test for paired data and between groups by the Mann-Whitney test. P < 0.05 was taken as the level of significance. Body mass index was calculated as wt (kg)/height (m)2. Values in the text are expressed as mean ± sd.

Results
Atropine, 1.2 mg by im injection, and propanthelene, 45 mg po, were effective in suppressing the GH response to GHRH in the patient and
control groups (Fig. 1). Table 2 gives the results of the 24-h studies. Baseline values taken on two control 24-h periods did not differ significantly in either control subjects or patients.

All subjects experienced unpleasant dryness of mouth, difficulty of swallowing, blurring of vision and tachycardia. Two subjects (one patient and one control) could not tolerate oral atropine for more than a week; studies were performed at one week on them.

After treatment with oral atropine, the mean area under the GH curve (AUC) ± SD in the control subjects was reduced from 103 ± 53.1 (baseline) to 73 ± 83.57 μU·l⁻¹·h⁻¹, but this was not statistically significant (P > 0.05). Mean AUC after propanthelene treatment was 122 ± 71.6 μU·l⁻¹·h⁻¹, which was not significantly different from baseline values or from results after atropine (P > 0.05). There was no significant difference in numbers of GH peaks during either treatment compared to baseline.

Baseline 24-h GH secretion was greater in the 7 patients compared with the control subjects, although this did not achieve statistical significance (251 ± 108.7 vs 103 ± 53.1 μU·l⁻¹·h⁻¹; P > 0.05).

Mean AUC compared with baseline values was not significantly reduced by either atropine (251 ± 108.7 vs 174 ± 106.9; P > 0.05) or propanthelene (251 ± 108.7 vs 180 ± 72.4; P > 0.05) in the patient group. The number of GH peaks during treatment compared with baseline values was not significantly reduced after atropine (4.14 ± 1.2 vs 2.7 ± 1.1; P > 0.05) or after propanthelene (4.14 ± 1.2 vs 2.86 ± 1.1; P > 0.05).

The pattern of GH secretion is illustrated in Fig. 2 (control subjects) and Fig. 3 (patients). GH
peaks associated with hypoglycemia (plasma glucose < 2.2 mmol/l) were not suppressed by atropine or propanthelene as illustrated by 4 representative patients shown in Fig. 4. There was no difference in frequency of hypoglycemic episodes during baseline measurements or during treatment with either drug.

Mean 24-h serum IGF-I (µg/l) was not significantly reduced after either drug in the normal subjects (baseline: 203 ± 47.9; after atropine: 166 ± 34.3; after propanthelene: 214 ± 57.4; P > 0.05) or in the patient group (baseline: 279 ± 136.4; after atropine: 195 ± 36.6; after propanthelene: 180 ± 47.4; P > 0.05). Changes in serum IGF-I concentration are summarised in Table 3.

**Table 2.**

Area under the 24-h GH curve (AUC) mU·l⁻¹·h⁻¹ before and after atropine and propanthelene.

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<td>Mean ± sd AUC</td>
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<td>Mean ± sd AUC</td>
<td>251 ± 108.7</td>
<td>174 ± 106.9</td>
<td>180 ± 72.38</td>
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* Mean of two control nights.

** Indicates subjecte who only tolerated atropine for 1 week.

**Discussion**

Blunting of the GH response to GHRH in the presence of anticholinergic agents is now well recognised (Casanueva et al. 1986; Pietschmann et al. 1986) and was demonstrated in both groups of subjects. There have been no previous reports of chronic treatment with these drugs on 24-h GH levels.

In a previous study in normal subjects (Taylor et al. 1985), daytime GH secretion was inhibited for 5 h after the acute administration of atropine, 0.6 mg orally, and completely suppressed in two out of four subjects given 1.2 mg by mouth. We gave atropine, 1.2 mg 4-hourly orally, in an effort to abolish GH secretion throughout 24 h. This dose was not effective in totally suppressing 24-h GH levels in normal or diabetic subjects.

With more frequent sampling, particularly at night, we might have been able to demonstrate periods of GH suppression. Nevertheless, the finding of high GH levels in our samples demonstrates that GH secretion was not totally suppressed. This is disappointing, as pituitary ablation was only really effective in the treatment of
24-h GH profiles in 6 normal subjects at baseline (closed circles), after 2 weeks treatment with atropine 1.2 mg x 4 h (triangles) and after 2 weeks treatment with propanthelene 30 mg x 6 h (open circles). Baseline profiles are represented as the mean of 2 control days.

Fig. 2.

24-h GH profiles in 7 diabetic patients at baseline (closed circles), after 2 weeks treatment with atropine 1.2 mg x 4 h (triangles) and after 2 weeks treatment with propanthelene 30 mg x 6 h (open circles).

Fig. 3.
proliferative diabetic retinopathy when GH secretion was totally abolished (Wright et al. 1969; Adams et al. 1974). There was no discernable effect on retinopathy appearance over the study period.

Failure to suppress GH release during hypoglycemia was noted in this study with either treatment and has been previously reported (Blackard & Waddell 1969; Evans et al. 1985). This would make it difficult to maintain total 24-h GH suppression in patients liable to hypoglycemic attacks.

Atropine would not be suitable for long-term use because of severe adverse effects. Propantheline, a synthetic cholinergic antagonist, was found to be better tolerated than atropine. It has been reported acutely to reduce basal GH levels in normal subjects (Davis et al. 1983). More recently, in a study of four insulin-dependent diabetic men, a significant reduction of night-time GH secretion was observed after a single 45 mg bedtime dose (Davis & Davis 1986).

We did not find propanthelene in the maximally recommended daily dose of 120 mg (British National Formulary 1987) to suppress 24-h GH levels effectively in either the control subjects or

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* Mean of two control nights.
in this group of diabetic patients. A lower dose given more frequently might have been more effective.

The acute administration of a similar drug, pirenzepine, abolished sleep-related GH release in five diabetics (Page et al. 1987). In another study, nocturnal GH secretion was inhibited but not totally suppressed, in eight insulin-dependent diabetics with this drug (Martina et al. 1987). The effect of chronic administration of pirenzepine on 24-h GH secretion is not known.

In conclusion, it is unlikely that long-term treatment with atropine or propantheline will influence the course of diabetic proliferative retinopathy. Whether these agents will be more effective in better controlled patients with milder grades of retinopathy is at present, not known.

Acknowledgments

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