PAPERS AND ORIGINALS

Comparison of Effects of Long-term Corticotrophin and Corticosteroid Treatment on Responses of Plasma Growth Hormone, ACTH, and Corticosteroid to Hypoglycaemia

J. R. DALY, M. R. FLETCHER, D. GLASS, D. J. CHAMBERS, LUCILLE BITENSKY, J. CHAYEN

British Medical Journal, 1974, 2, 521-524

Summary

The development of the highly sensitive cytochemical bioassay for ACTH has permitted the measurement of plasma ACTH levels during the insulin hypoglycaemia test (I.H.T.) in patients treated with corticosteroids and corticotrophin. The ACTH, corticosteroid, and growth hormone (GH) responses in the I.H.T. were measured in three groups of 12 rheumatoid arthritis patients. One group was receiving long-term corticotrophin treatment, the second was undergoing long-term corticosteroid treatment, and the third had never received systemic hormone therapy. The increments in plasma ACTH, corticosteroids, and GH were diminished in the corticosteroid-treated group, as were increments in plasma GH and ACTH in the corticotrophin-treated group; but in this group the corticosteroid increment was normal. Examination of the area under the curve of the ACTH response showed that the total amount of ACTH secreted was normal though the rate of secretion was reduced. In the corticosteroid-treated group both rate and total secretion were diminished.

Introduction

A major problem of prolonged corticosteroid therapy is the resulting suppression of hypothalamic-pituitary-adrenal (H.P.A.) function. Consequently, some clinicians prefer corticotrophin treatment where possible because it seems to produce less inhibition of the H.P.A. axis than corticosteroids themselves. The evidence favouring corticotrophin therapy is that the plasma corticosteroid response to insulin-induced hypoglycaemia is virtually normal in most patients treated with corticotrophin (Bacon *et al.*, 1968; Carter and James, 1970) whereas this response is often considerably impaired in those treated with corticosteroids (Daly *et al.*, 1967; Livanou *et al.*, 1967).

Though now generally accepted this fact is very surprising. Both forms of treatment result in high circulating levels of corticosteroid, either exogenous or endogenous, and would therefore be expected to have an equally suppressive effect on H.P.A. function. Experiments carried out in an attempt to discover why they do not in fact do so have so far failed to resolve the problem (Carter and James, 1971).

One suggestion has been that corticotrophin treatment might suppress pituitary function but that it also might cause adrenal hyperplasia and hypersensitivity. Thus the ACTH response to hypoglycaemia would be reduced, but it would be compensated for by the enhanced adrenal sensitivity, and so a normal, or nearly normal, rise in plasma cortisol might be achieved. Indirect support for this hypothesis was provided by the observation that another aspect of the pituitary response to hypoglycaemia—namely, growth hormone (GH) secretion—seemed to be impaired by corticotrophin treatment (Daly and Glass, 1971).

A major obstacle to the direct testing of the hypothesis was the difficulty of determining plasma ACTH, particularly when it is present in very low concentrations. A sensitive and precise technique for its assay has recently become available, however. This is the cytochemical bioassay which is some hundred times more sensitive than radioimmunoassay, probably more specific, and in our hands, more precise (Daly *et al.*, 1974 b). We therefore determined plasma ACTH during the insulin hypoglycaemia test (I.H.T.) in corticotrophin- and steroid-treated patients, and we measured also the plasma corticosteroid and GH responses. In this way we hoped to make a contribution towards understanding the discrepancy between the effects on the H.P.A. axis produced by corticotrophin and corticosteroid treatment.

Department of Chemical Pathology, Charing Cross Hospital Medical School, London W6 8RF
J. R. DALY, M.R.C.PATH., Senior Lecturer

Kennedy Institute of Rheumatology, London W6 7DW
M. R. FLETCHER, M.R.C.P., Maynard-Jenour Research Fellow, Division of Clinical Research
D. GLASS, M.R.C.P., Senior Registrar, Division of Clinical Research
D. J. CHAMBERS, B.SC., Research Assistant, Division of Cellular Biology
LUCILLE BITENSKY, D.SC., Head of Laboratory of Medical Histochemistry, Division of Cellular Biology
J. CHAYEN, D.SC., Head of Division of Cellular Biology

Patients and Methods

Three groups of 12 patients with rheumatoid arthritis were studied. One group had received long-term corticotrophin treatment, the second long-term corticosteroids, and the third, or control, group had never received systemic hormonal treatment. The groups were matched, as far as possible, for age, sex, and duration and severity of disease (table I). The I.H.T. was performed at 09·00 hours after an overnight fast, no steroids or corticotrophin having been given for the previous 48 hours, a time shown to be adequate for the clearance of the effects of corticotrophin gel (fig. 1). The standard procedure was adopted for the I.H.T. (Landon *et al.*, 1963), blood being sampled via an indwelling needle at 0, 15, 30, 45, 60, and 90 minutes after the administration of 0·15 U/kg body weight of soluble insulin intravenously. The samples at 0, 30, 60, and 90 minutes were analysed for glucose, corticosteroid, ACTH, and GH levels and the samples at 15 and 45 minutes for glucose only. The response to hypoglycaemia was assessed by considering not only the maximum increment —that is, baseline-peak difference—in the plasma level of each hormone but also the quantity of ACTH and corticosteroid secreted by measuring the area under the curve of the response/time graph (Greenwood *et al.*, 1966). The area was measured with a planimeter and the results were expressed in arbitrary units. A correction was made for the basal hormone levels by subtracting an area derived from the half time of the hormone in plasma (fig. 2). The rate of ACTH release was estimated from the maximum slope of the graph of the ACTH response and that of corticosteroids by the maximum slope of their response graph.

TABLE I—*Data on Three Groups of Patients with Rheumatoid Arthritis. Ranges are given in Parentheses*

Rheumatoid Arthritis Patients	No. and Sex		Mean Age (Years)	Mean Duration of Disease (Years)	Mean Duration of Treatment (Years)	Daily Dose at Time of Testing (IU Corticotrophin Gel; mg Prednisolone)
	M.	F.				
Controls	2	10	48·5 (25-64)	10·8 (3·5-19)		
Corticotrophin treated	2	10	51·2 (26-63)	12·4 (3-21)	10·9 (3-18)	15·4 (6-36)
Steroid treated	3	9	46·6 (28-61)	8·5 (2·5-17)	5·1 (1·5-11)	7·3 (5-9·5)

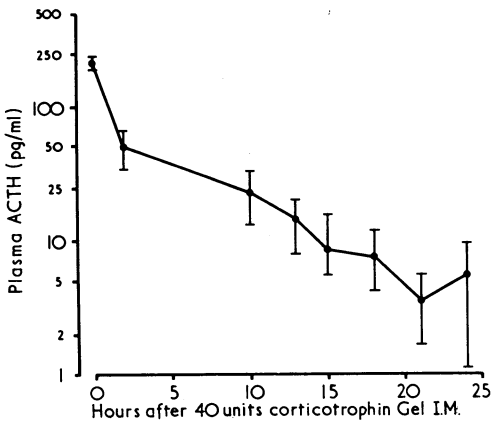

FIG. 1—Rate of decline of plasma ACTH after 40 IU of corticotrophin gel.

Glucose was estimated on an AutoAnalyzer (Technicon) using glucose oxidase, corticosteroids were estimated by fluorimetry (Spencer-Peet *et al.*, 1965), ACTH was determined by cytochemical bioassay (Daly *et al.*, 1974 b), and GH by radioimmunoassay using a double antibody technique. The standard of ACTH was the third international, and that of GH was M.R.C. "A."

Comparison of the results between groups were carried out by Student's *t* test.

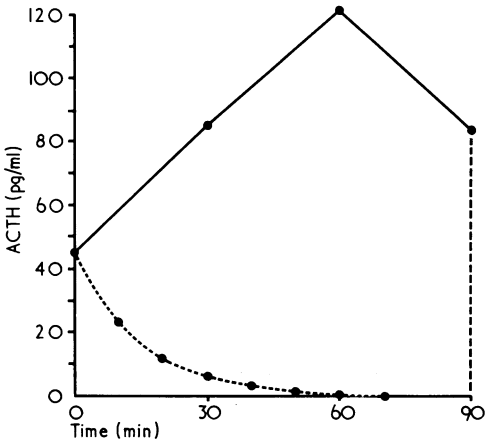

FIG. 2—Method of measuring area under ACTH response/time graph. Dotted line on left demarcates area subtracted from total area to correct for ACTH secreted before insulin injection.

Results

The rate of disappearance of ACTH after intramuscular administration of corticotrophin gel (Acthar Gel, Armour) is shown in fig. 1. Other results are shown in figs. 3 and 4 and summarized in table II.

Discussion

Our results showed once more the marked difference between the plasma corticosteroid response to hypoglycaemia in corticotrophin-treated and corticosteroid-treated patients, the former group being indistinguishable from the controls. (In an earlier study of corticotrophin-treated patients a barely significant depression of the corticosteroid response was found (Daly and Glass, 1971).)

The result of the I.H.T. is usually assessed from the increment in the plasma levels of GH or corticosteroids. Applying this criterion to the plasma ACTH, it is seen that the response of this hormone is markedly impaired in corticotrophin-treated patients (see fig. 3). This impairment is not so marked as in those receiving corticosteroids, but is nevertheless significant. This paradox of a diminished rise in plasma ACTH associated with a normal rise in plasma corticosteroids seems at first to be explicable only if the sensitivity of the adrenal cortex is in fact greatly enhanced, as was previously suggested (Daly and Glass, 1971).

The relation between the levels of ACTH and corticosteroids in plasma, however, is extremely complex (Daly *et al.*, 1974 a). Furthermore, the production of corticosteroids by the adrenal cortex must be a function both of the plasma concentration of ACTH and of the duration of its secretion. Consequently we needed to examine our data more rigourously, taking into account both these factors. It has been suggested previously that an assessment of the total secretion of hormone can be made by measuring the area under the curve of the plasma level/time graph (Greenwood *et al.*, 1966). The complexity of this manoeuvre, however, led to its being abandoned as a routine. When our data were examined in this way

TABLE II—*Results of Insulin Hypoglycaemia Test in Three Groups of Rheumatoid Arthritis Patients. Results expressed as Mean ± S.D. Tests of Significance are versus Control Group*

	Lowest Glucose Level* (mg/100 ml)	Increment in Serum GH (ng/ml)	Increment in Plasma ACTH (pg/ml)	Increment in Plasma Corticosteroids (μg/100 ml)	Area Under ACTH Rise during 90 min. (Arbitrary units)	Maximum Rate of ACTH Secretion (Arbitrary Units)	Maximum Rate of Corticosteroid Secretion (Arbitrary Units)
Control Patients	21 ± 10	52 ± 29	129 ± 63	16 ± 8	88 ± 34	4·3 ± 2·8	3·5 ± 1·8
Corticotrophin-treated patients ..	20 ± 12 (N.S.)	14 ± 13 (P <0·001)	70 ± 22 (P <0·01)	13 ± 4 (N.S.)	68 ± 24 (N.S.)	1·5 ± 0·6 (P <0·005)	2·7 ± 1·0 (N.S.)
Steroid-treated patients	26 ± 14 (N.S.)	4 ± 6 (P<0.001)	37 ± 36 (P <0·001)	4 ± 3 (P <0·001)	34 ± 18 (P <0·001)	0·9 ± 0·9 (P <0·001)	0·9 ± 0·7 (P <0·001)

*There was no difference between groups in timing of lowest plasma glucose level.

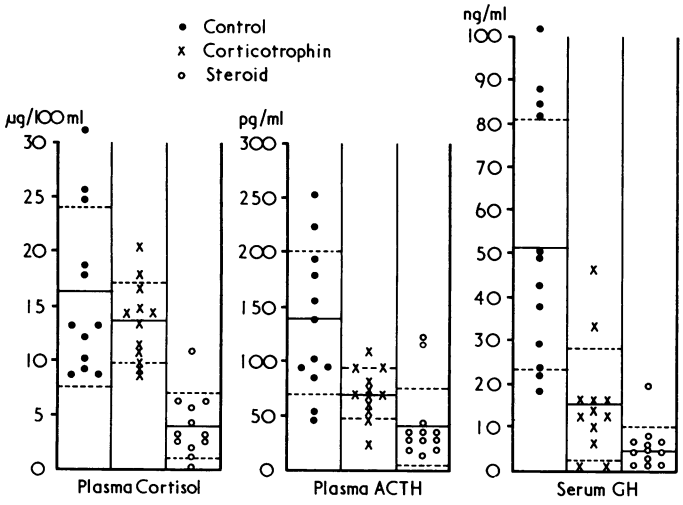

FIG. 3—Maximum increments of plasma corticosteroid, ACTH, and growth hormone (GH) during insulin hypoglycaemia test in control, corticotrophin-treated, and corticosteroid-treated groups.

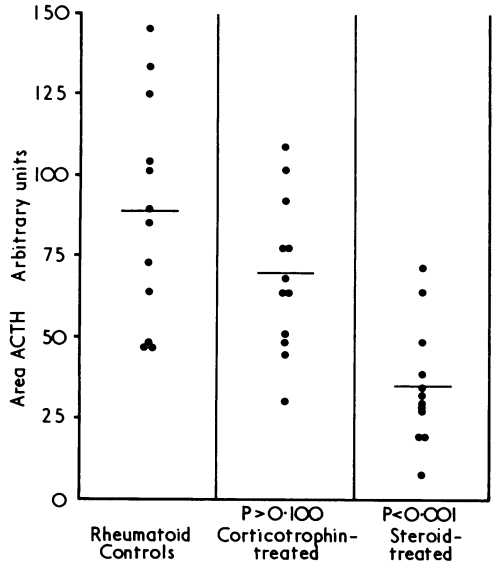

FIG. 4—Area under ACTH response/time graph during insulin hypoglycamia test in control, corticotrophin-treated, and corticosteroid-treated patients.

it was found that despite the smaller increment in plasma ACTH in the corticotrophin-treated group the total quantity secreted during the I.H.T. seemed to be about the same as in the controls (see fig. 4). Thus the stimulus to the adrenal cortex is equivalent in the corticotrophin-treated and the control groups, and this could account completely for the similar corticosteroid production. It seems that corticotrophin treatment reduces the rate of secretion of ACTH but not the total amount secreted, while steroids reduce both. What remains uncertain is whether these two effects differ in kind, or whether the diminished rate of secretion seen in the corticotrophin-treated patients merely represents a less severe form of the suppression seen in the corticosteroid-treated group. If they differ only in degree the difference is possibly purely a reflection of the dosage and frequency of administration of the drug.

It is difficult to equate corticotrophin and prednisolone doses precisely though this has been attempted (Carter and James, 1971). On clinical grounds, however, we believe our doses of corticotrophin and of corticosteroids were comparable. It has been shown that cortisol will inhibit ACTH secretion (Daly *et al.*, 1974 a), hence endogenous corticosteroid, and not merely steroid administered exogenously, should cause pituitary inhibition. Corticotrophin is usually given only once a day during the evening while corticosteroids are often given both morning and evening, as was the case in our steroid-treated group. This may be a crucial factor for there is already evidence (Myles *et al.*, 1971), which work now in progress seems to confirm, that if the corticosteroid is given in the same total dose but only once daily, preferably in the morning, it causes no more H.P.A. suppression than corticotrophin.

The I.H.T. is the most useful of the several available tests of H.P.A. function (Jacobs and Nabarro, 1969) because it seems to be an adequate estimate of a patient's ability to respond to stresses such as surgery (Jasani *et al.*, 1967) and, if the result is normal, indicates that steroid cover for such stresses should not be necessary (Plumpton *et al.*, 1969). The test may be made more discriminating by studying the area under the curve and not merely the baseline-peak difference. Our experience indicates, however, that to obtain the maximum benefit from this method the test should continue for 120 minutes and not end at 90 minutes. This is particularly important in GH studies for the time course of that hormone's response to hypoglycaemia is too slow to allow its total secretion to be assessed in 90 minutes. For this reason we were unable to study it further here.

We thank Dr. J. T. Scott for permission to investigate patients in his care. J.C. was in receipt of a project grant from the Medical Research Council, and the Arthritis and Rheumatism Council for Research gave general support. M.R.F. is a Maynard-Jenour Research Fellow.

References

Bacon, P. A., Daly, J. R., Myles, A. B., and Savage, O. (1968). *Annals of the Rheumatic Diseases*, 27, 7.
Carter, M. E., and James, V. H. T. (1970). *Annals of the Rheumatic Diseases*, 29, 73.
Carter, M. E., and James, V. H. T. (1971). *Annals of the Rheumatic Diseases*, 30, 91.
Daly, J. R., and Glass, D. (1971). *Lancet*, 1, 476.
Daly, J. R., Fleisher, M. R., Chambers, D. J., Bitensky, L., and Chayen, J. (1974 a) *Clinical Endocrinology*. In press.

Daly, J. R., Loveridge, N., Bitensky, L., and Chayen, J. (1974 b). *Clinical Endocrinology*. In press.
Daly, J. R., Myles, A. B., Bacon, P. A., Beardwell, C. G., and Savage, O. (1967). *Annals of the Rheumatic Diseases*, **26**, 18.
Greenwood, F. C., Landon, J., and Stamp, T. C. B. (1966). *Journal of Clinical Investigation*, **27**, 183.
Jacobs, H. S., and Nabarro, J. D. N. (1969). *Quarterly Journal of Medicine*, **38**, 475.
Jasani, M. K., *et al.* (1967). *Quarterly Journal of Medicine*, **36**, 267.
Landon, J., Wynn, V., and James, V. H. T. (1963). *Journal of Endocrinology*, **27**, 183.
Livanou, T., Ferriman, D., and James, V. H.T. (1967). *Lancet*, **2**, 856.
Myles, A. B., Bacon, P. A., and Daly, J. R., (1971). *Annals of the Rheumatic Diseases*, **30**, 149.
Plumpton, F. S., Besser, G. M., and Cole, P. V. (1969). *Anaesthesia*, **24**, 3.
Spencer-Peet, J., Daly, J. R., and Smith, V. (1965). *Journal of Endocrinology*, **31**, 235.

Alpha-Fetoprotein Levels in Amniotic Fluids from Spontaneous Abortions

MARY J. SELLER, M. R. CREASY, EVA D. ALBERMAN

British Medical Journal, 1974, 2, 524-525

Summary

Alpha-fetoprotein (A.F.P.) levels in the amniotic fluid were determined in 54 cases of spontaneous abortion in which the amniotic sac remained intact. These levels were correlated with the morphological and cytogenetic status of the fetus. Of the 29 fetuses with no apparent abnormality 22 had A.F.P. levels below 50 μg/ml, while 10 of the 11 fetuses with severe neural tube defects had raised levels (50-305 μg/ml). Seventeen fetuses had chromosome anomalies of various types. Three out of four which were 45, X had considerably raised A.F.P. levels (78-210 μg/ml) but fetuses with other chromosome constitutions and no neural tube defects had levels no higher than 32 μg/ml.

Introduction

The observation by Brock and Sutcliffe (1972) that there is a connexion between a raised alpha-fetoprotein (A.F.P.) level in the amniotic fluid and severe neural tube defects in the fetus evoked great interest. A number of clinical investigations were instigated, and now amniotic fluid A.F.P. estimations are proving invaluable in the early prenatal diagnosis of fetuses with severe neural tube defects (Seller *et al.*, 1974).

A.F.P. first appears in the amniotic fluid around six weeks of gestation and the levels rise to attain a maximum value between 13 and 15 weeks of gestation (Gitlin and Boesman, 1966). While it is well documented that in the case of fetuses with neural tube defects the A.F.P. level is considerably raised above normal from the 14th week of gestation, because amniocentesis is possible from this time, our knowledge of the situation in the first trimester is very limited (Allan *et al.*, 1973).

This led us to examine the A.F.P. content of the amniotic fluids derived from spontaneous abortions where the aborted material consisted of a fetus inside an intact chorion and amniotic sac. In addition to obtaining data on the levels of A.F.P. in fetuses with neural tube defects we were also interested to discover whether there is any alteration in the amniotic fluid A.F.P. level when there is a chromosome aberration in the fetus. Chromosome anomalies are common in fetuses from spontaneous abortions. More than one estimate has put the overall incidence as high as 40-50% (Boué and Boué, 1973; Alberman and Creasy, 1974).

Paediatric Research Unit, Guy's Hospital Medical School, London SE1 9RT
MARY J. SELLER, B.SC., PH.D., Lecturer
M. R. CREASY, M.SC., Research Assistant
EVA D. ALBERMAN, M.D., D.P.H., Senior Lecturer

Our findings show that amniotic fluid A.F.P. levels are raised in fetuses with neural tube defects which have been spontaneously aborted from the seventh week of gestation onwards and that they are also raised in fetuses with a 45, X chromosome constitution, but the levels in fetuses with other chromosome anomalies are normal.

Methods

The products of spontaneous abortions were collected daily from several hospitals in and around London as part of a survey currently being carried out in the paediatric research unit.

Amniotic fluid was aspirated into a hypodermic syringe from specimens which consisted of an intact chorion and amniotic sac. The fluid was subsequently discarded if it was blood stained or if the sac when opened did not contain a morphologically recognizable fetus.

The crown-rump length of the fetus was measured, and the fetus was examined under a binocular microscope for any gross morphological abnormality. The age of the fetus was calculated from the date of the first day of the last menstrual period of the mother. A small portion of gonad or extraembryonic membrane was set up in tissue culture, and after about three-weeks growth mitotic chromosome preparations were made by conventional methods. Apart from the usual orcein stain, trypsin banding and fluorescent techniques were also used. The chromosomes were analysed separately by two people.

Cellular debris was removed from the amniotic fluid by centrifugation at 2,000 r.p.m. for five minutes. The supernatant was stored in the deep freeze for several days to several weeks before A.F.P. estimation. The A.F.P. was assayed by one-dimensional antigen/antibody-crossed electrophoresis as previously described (Seller *et al.*, 1973). Quantitative estimations were made by reference to a standard provided by Dr. M. Adinolfi. Morphologic and cytogenetic details of the fetuses were withheld from the person performing the A.F.P. assay until after the levels had been determined.

Results

A total of 54 amniotic fluids were assayed for A.F.P. derived from sacs spontaneously aborted from the seventh to the 27th week of gestation. The associated fetuses were almost always macerated and very small in size for gestational age. Despite this, tissue culture was successfully achieved and chromosome analysis was possible in 48 cases (89%). The A.F.P. levels from specimens in which no chromosome results were obtained have been included in the results.

The A.F.P. levels obtained from the amniotic fluids of fetuses with no morphological or chromosomal abnormality and from those with severe neural tube defects are shown in the chart. Out of 29 ostensibly normal fetuses 22 had A.F.P. levels below 50 μg/ml (range <1-49 μg/ml), and the remaining seven fell between 50 and 565 μg/ml. By contrast all eight fetuses with