Comparison of Corticotrophin and Corticosteroid Response to Lysine Vasopressin, Insulin, and Pyrogen in Man

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Summary

Plasma corticotrophin (ACTH) and corticosteroid levels in response to lysine vasopressin (LVP), insulin hypoglycaemia, and pyrogen have been compared in seven subjects with normal pituitary adrenal function. Intramuscular vasopressin was a weak stimulus to corticotrophin release, peak values lying within the range 49 to 141 pg/ml. Insulin hypoglycaemia consistently caused a more noticeable increase, with peak levels between 114 and 364 pg/ml, while pyrogen was the most powerful, corticotrophin levels rising to between 209 and 1,725 pg/ml. Peak plasma corticosteroid levels showed less pronounced differences between the three tests, and correlated poorly with peak ACTH levels. Thus, relatively small acute changes in corticotrophin levels produce near-maximal adrenal stimulation. Under these conditions, plasma corticosteroid measurements do not accurately reflect circulating corticotrophin levels. These findings help to explain the physiological basis of several observations on the corticosteroid responses to these clinical test procedures.

Introduction

The corticosteroid responses to lysine vasopressin (LVP), insulin hypoglycaemia, and pyrogen have been used clinically as tests of hypothalamic-pituitary-adrenal function for many years. Several comparative studies of plasma corticosteroid levels during these tests have been reported in normal subjects and in patients with pituitary adrenal dysfunction (Jenkins and Else, 1968; Carroll *et al.*, 1969; Jacobs and Nabarro, 1969; Jenkins *et al.*, 1972). Corticotrophin (ACTH) responses to these stimuli have not, however, been compared partly because it is often assumed that plasma corticosteroid levels reflect circulating ACTH concentrations. During periods of acute pituitary stimulation, and when plasma ACTH levels exceed the adrenal threshold for maximal stimulation, this direct relation may no longer pertain. Accurate assessment of the pituitary response must then depend on direct measurement of plasma ACTH levels.

This paper reports the plasma immunoreactive ACTH and corticosteroid responses to intramuscular LVP, insulin, and pyrogen tests in seven patients with normal pituitary-adrenal function.

Subjects and Methods

Seven patients (two women and five men, aged between 16 and 55 years) volunteered for the tests. They showed no evidence of

endocrine, hepatic, or renal disease, except one patient who had an isolated growth hormone deficiency but normal adrenocortical function. The tests were performed at intervals of two days in the order: LVP; insulin; pyrogen. All were started between 09.00 and 10.00 hours using an indwelling venous cannula inserted into a forearm vein at least 30 min previously. Two basal samples were taken. Synthetic LVP (Sandoz, 10 pressor units) was given by intramuscular injection and samples were taken every 15 min for 90 min (Gwinup, 1965; Brostoff *et al.*, 1968). Soluble insulin (Organon, 0.15 units/kg body weight) was injected intravenously (Greenwood *et al.*, 1966) and samples were taken 30, 45, 60, and 90 min later. Adequate hypoglycaemia was achieved in all subjects, the blood sugar falling to below 40 mg/100 ml, associated with sweating. Bacterial pyrogen (Organon, 0.005 µg/kg body weight) was given intravenously together with aspirin (Jenkins and Else, 1968) and blood was taken 120, 180, 240, and 360 min later. Samples were collected in cooled heparinized plastic syringes, separated immediately, deep frozen in dry ice, and stored at -20° until assay.

Plasma corticosteroids were determined by a modification of the method of Mattingly (1962) (Spencer-Peet *et al.*, 1965). Interassay variation at levels between 10 and 30 µg/100 ml was 0.8 µg (S.D.). Plasma ACTH was measured by radioimmunoassay (Landon and Greenwood, 1968) after extraction with porous glass (Ratcliffe and Edwards, 1971). The antiserum used reacts with amino-acid sequences within the steroidogenic part of the molecule (amino-acids 1-24). All values are expressed relative to a natural human ACTH standard (Lerner *et al.*, 1968). The coefficient of variation was 7% at a level of 1,000 pg/ml (intra-assay) and 19% at a level of 153 pg/ml (interassay). All samples from a single subject were measured in the same assay to minimize methodological variations. The significance of differences between mean levels were analysed by Student's *t* test (two-tailed) and linear regressions were calculated by the method of least squares.

Results

Changes in Plasma ACTH.—A small but significant increment in immunoreactive ACTH over the lowest basal level was found in all except one subject after LVP (table I). The peak responses generally occurred at 30 min and ranged between 49 and 141 pg/ml (mean 95 pg/ml). After insulin, peak ACTH levels ranged from 114 to 364 pg/ml (mean 256 pg/ml) and occurred consistently at 45 min. The smallest peak response occurred in patient 7, in whom the test was terminated prematurely with intravenous glucose. The levels were still raised at 90 minutes in most subjects. The peak ACTH response to pyrogen was greater and more variable than that achieved after insulin (209 to 1,725 pg/ml, mean 643 pg/ml) and occurred either 2 or 3 hr after the stimulus. The plasma ACTH levels had returned to normal by 4 to 6 hr in all except one subject.

Changes in Plasma Corticosteroids.—After LVP, peak corticosteroid levels ranged from 16.9 to 28.7 µg/100 ml (mean 23.5 µg/100 ml) and occurred 30 to 45 min after the stimulus. After insulin, peak corticosteroid levels were achieved usually at 60 min, ranged from 18.9 to 35.3 µg/100 ml (mean 26.7 µg/100 ml), and showed considerable overlap with the maximum levels after LVP. The rise in corticosteroid levels after pyrogen occurred at 2 hr and reached maximum values 2, 3, or 4 hr after the stimulus. Corticosteroid levels exceeded 20 µg/100 ml after

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TABLE I—Plasma ACTH and Fluorogenic Corticosteroid Responses to the Three Tests

Subject No.	Age and Sex		Response to Vasopressin (Time in min)							Response to Insulin (Time in min)						Response to Pyrogen (Time in min)							
			-30	0	15	30	45	60	90	-30	0	30	45	60	90	-30	0	120	150	180	240	360	
1	50 M.	ACTH*	59	101	70	104	—	72	—	57	96	149	—	235	103	48	—	205	393	1,725	—	—	
		C-S†	—	—	—	—	—	—	—	23.3	24.2	19.5	—	35.3	22.3	15.5	—	33.3	40.0	48.3	—	—	
2	37 M.	ACTH	39	47	—	83	57	55	67	74	61	94	272	282	82	159	73	362	—	109	78	56	
		C-S	10.8	7.0	—	25.5	19.1	17.7	18.2	9.4	6.3	8.8	11.9	25.1	22.6	17.6	16.5	24.0	—	29.4	17.5	9.6	
3‡	22 M.	ACTH	33	24	—	55	96	63	51	97	79	171	310§	208	120	40	45	291	—	696	356	281	
		C-S	17.4	12.6	—	22.1	22.1	15.2	10.4	22.4	17.6	18.4	25.2	25.4	25.8	9.5	8.0	26.0	—	33.0	36.3	22.3	
4	48 F.	ACTH	51	48	41	73	80	74	—	44	38	82	364§	184	96	64	62	362	—	—	62	59	
		C-S	13.6	13.6	16.6	26.2	28.7	23.0	—	18.6	14.3	14.3	21.4	24.7	27.3	21.4	22.9	43.6	—	—	38.6	20.0	
5	16 M.	ACTH	61	94	97	141	103	68	—	95	93	60	265	204	130	97	111	635	—	102	92	39	
		C-S	11.7	13.1	13.8	18.6	13.8	11.7	—	15.1	11.4	9.2	16.2	18.9	15.7	18.3	15.4	22.3	—	19.2	16.2	13.8	
6	55 M.	ACTH	41	46	49	46	29	23	—	51	29	34	217§	109	58	49	26	515	—	230	174	47	
		C-S	22.7	15.5	16.9	15.6	10.9	9.5	—	17.5	12.6	14.7	24.7	27.4	26.3	9.4	6.9	25.0	—	29.2	29.3	19.1	
7	21 F.	ACTH	45	69	91	112	81	92	—	90	73	80§	114	79	67	58	28	209	—	195	62	48	
		C-S	18.4	18.7	19.4	20.9	23.0	28.1	—	17.6	17.8	17.8	27.0	25.4	20.5	24.2	23.4	33.7	—	51.4	46.6	20.6	

* ACTH in pg/ml.

† C-S = Fluorogenic corticosteroids in µg/100 ml.

‡ Patient with isolated growth hormone deficiency.

§ Glucose administered intravenously.

6 hr in two subjects. The peak levels ranged between 22.3 and 51.4 µg/100 ml (mean 37.2 µg/100 ml) and showed greater variation than the peak levels after the other two tests (chart). Subject 5, who had the greatest ACTH response to LVP and substantial increases after insulin and pyrogen, showed the lowest corticosteroid response to all three tests and a low normal level after the administration of exogenous ACTH in the form of Synacthen (250 µg intramuscularly).

Peak plasma immunoreactive ACTH (hatched) and corticosteroid (stippled) levels after LVP, insulin hypoglycaemia, and pyrogen tests in seven subjects. Mean values are shown as columns and the standard error of the mean as vertical bars.

The maximum increments in plasma corticosteroids were not significantly different ($P > 0.05$) in the three tests and did not correlate with the maximum increments in plasma ACTH or the logarithm of the plasma ACTH levels.

Discussion

Previous data on the plasma ACTH responses to LVP, insulin hypoglycaemia, and pyrogen are summarized in table II. Several methods of ACTH assay have been used, some being insufficiently sensitive to measure basal levels. The wide scatter in reported values derives partly from the imprecision of many assays near their limits of sensitivity and partly from differences in the performance of the test procedures and variation in individual responses. There are no previous reports comparing ACTH levels achieved by these tests in the same patient.

The present study shows that the ACTH response to intra-

TABLE II—Peak Plasma ACTH Levels after Lysine Vasopressin, Insulin, and Pyrogen

Tests and Authors	Peak Plasma ACTH Level (pg/ml)*	Time of Peak (min)	Method of Assay	No. of Subjects
Lysine Vasopressin:				
Gwinup <i>et al.</i> , 1967	180-450†	10-30	B.A.	7
Strott <i>et al.</i> , 1967	85-110	45-60	R.I.A.	3
Berson and Yalow, 1968	28-130	20-30	R.I.A.	3
Landon and Greenwood, 1968	76-210	—	R.I.A.	3
Takebe <i>et al.</i> , 1968	40-69‡	—	B.A.	5
Binoux <i>et al.</i> , 1971	16-60‡	15-30	B.A.	9
Present study	49-141	30-45	R.I.A.	7
Insulin-Hypoglycaemia:				
Berson and Yalow, 1968	110-360§	30-60	R.I.A.	7
Landon and Greenwood, 1968	210-400	40	R.I.A.	3
Donald, 1971; Donald <i>et al.</i> , 1972	147-431	45	R.I.A.	13
Ichikawa <i>et al.</i> , 1972	362 ± 51.7 (S.E.)	45	R.I.A.	5
Present study	114-364	45	R.I.A.	7
Pyrogen:				
Vance <i>et al.</i> , 1962	U.D.	—	B.A.	1
Takebe <i>et al.</i> , 1966	U.D.	—	B.A.	3
Takebe <i>et al.</i> , 1966¶	110-160	3 hr	B.A.	3
Litta-Modignani and Badoni, 1968	50-250	3 hr	B.A.	5
Matsukura <i>et al.</i> , 1970	100-1,150	2-3 hr	R.I.A.	4
Present study	209-1,725	2-3 hr	R.I.A.	7

* Bioassay values are expressed in gravimetric terms assuming that 1 mg of highly purified natural ACTH has a biological potency of 100 I.U. (Besser *et al.*, 1971). This may be only an approximate estimate of the potency of standard used by some authors.

† Raised basal levels in all subjects.

‡ Undetectable basal levels in most subjects.

§ Only subjects receiving adequate hypoglycaemia included (blood sugar less than 40 mg/100 ml).

¶ Pyrogen administered at 09.00 hours.

|| Pyrogen administered at 23.00 hours.

U.D. = Undetectable. B.A. = Bioassay. R.I.A. = Radioimmunoassay.

muscular LVP is smaller in magnitude and duration than that to insulin or pyrogen. The insulin test produced a more consistent ACTH response than either LVP or pyrogen, although the latter was the most powerful stimulus to ACTH release in six subjects and as potent as insulin in the remaining subject. The ACTH levels after pyrogen were similar to those found during thoracotomy (215-949 pg/ml: mean 478 pg/ml, Ratcliffe and Staub, unpublished observations), cholecystectomy (Newsome and Rose, 1971), or electroconvulsive shock (Berson and Yalow, 1968). Thus, pyrogen provides the most potent test of ACTH secretion used clinically. These results contrast with some of the ACTH levels reported previously during pyrogen stimulation (table II). Such differences may be due to the different ACTH assay procedures and the use of less potent pyrogens—for example, piromen. It is of interest that the metyrapone test produces an ACTH response similar to insulin hypoglycaemia (Donald *et al.*, 1972) but, since it acts through the negative feedback mechanism, it is not an appropriate indicator of stress responsiveness.

The plasma corticosteroid responses were within the range obtained in a large series of normal subjects (Jenkins *et al.*,

1972) and the peak levels achieved in all three tests showed considerable overlap. Thus, the hypothalamic-pituitary-adrenal response to LVP was better judged by the increment in corticosteroids rather than the change in plasma ACTH levels, whereas the ACTH responses to insulin and pyrogen were not accurately reflected by circulating corticosteroid levels. This suggests that a near-maximal adrenal response is achieved by quite small acute increments in plasma ACTH levels which is consistent with the finding (Landon *et al.*, 1967) that a dose of 250 ng α^{1-24} ACTH intravenously produced a similar increment in plasma corticosteroids to that produced by a pharmacological dose (250 μ g). Corticosteroid and ACTH levels may also correlate poorly due to a suppressive effect of rising cortisol levels on further ACTH release through the negative feedback mechanism. This may operate especially on a weak stimulus such as vasopressin, but may also modify the ACTH response to pyrogen if the corticosteroid response is particularly great (as in subject 7).

The present finding of pronounced differences in ACTH release by LVP, insulin, and pyrogen helps explain several puzzling clinical observations. Thus, the vigorous stimulus to ACTH release by pyrogen could account for the failure of small doses of glucocorticoids or morphine to suppress the corticosteroid response to this test (Jenkins, 1968). The positive corticosteroid response to pyrogen noted after pituitary stalk section (van Wyk *et al.*, 1960), might be explained by the release of sufficient corticotrophin releasing factor to bypass the stalk section locally or reach the pituitary via the general circulation. In patients with pituitary tumours, before and after irradiation, the corticosteroid response to LVP is more frequently abnormal than that to insulin, with the pyrogen test showing the highest proportion of normal responses (Jenkins and Else, 1968; Jenkins *et al.*, 1972). A similar pattern is found in patients treated with long-term steroids (Daly *et al.*, 1967; Jasani *et al.*, 1967). Finally, it is known that patients with an impaired corticosteroid response to insulin may have a normal response to surgery or infection (Wynn, 1968). Again the strength of the various stimuli, rather than the pathways through which they operate, may explain these observations.

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Defects and Disabilities of Thalidomide Children

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Summary

The range of defects and disabilities in thalidomide children is very much wider than is generally realized. The defects of 154 children are described and classified. Their disabilities range from incapacitating to negligible.

Introduction

It has become customary to refer to "thalidomide children" as if they formed a homogeneous group with similar defects of more or less comparable severity. This is far from being the

case. The defects may be single or multiple and they may involve almost any system of the body. The perinatal mortality among these children was very high. The disabilities among survivors range from total incapacity to no disability at all.

In an earlier paper (Smithells, 1962) I reported on children with limb and ear defects ascertained on a population basis in Liverpool during the thalidomide epidemic. After publication a few further affected children were born. Further experience also made it possible to exclude with reasonable confidence a few cases in the earlier report which should not have been attributed to thalidomide. The revised original group comprises 29 children, 17 of whom were examined personally. Of these 29 children four were stillborn, eight died in the neonatal period, and one died later in the first year. The combined stillbirth and neonatal mortality was therefore 45%. The causes of death were not known in all cases but included congenital anomalies of the heart, kidneys, and alimentary tract.

Since 1968 I have examined personally a further 159 children at the request of solicitors either to assess the extent of

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