Cortisol in the cerebrospinal fluid of patients suffering from affective disorders

ALEC COPPEN, B. W. L. BROOKSBANK, R. NOGUERA, AND D. A. WILSON

Summary Cortisol concentration was estimated by a competitive protein-binding method in the lumbar cerebrospinal fluid (CSF) of patients suffering from depression or from mania, and compared with the CSF cortisol concentration of neurological control patients not suffering from an affective disorder. There were no significant differences between the groups.

There have been numerous investigations into adrenocortical function in affective disorders using either 17-hydroxycorticosteroid excretion rate, measurement of plasma cortisol, or estimates of the secretion rate of cortisol by isotope dilution techniques (see reviews by Sachar, 1967; Coppen, 1970). Reports have been conflicting, but on the whole the evidence has suggested that there is a moderate increase in adrenocortical activity in a proportion of patients admitted to hospital suffering from depression, although this decreases as the patient adapts to his new environment. The increased adrenocortical activity decreases in most, though not in all cases, with clinical recovery. Most investigations of adrenocortical activity have been concerned with peripheral measures as opposed to its estimation in the central nervous system where it is possible that it could influence the formation of 5-hydroxytryptamine (Curzon, 1969; Lapin and Oxenkrug, 1969), which is known to be abnormal in the affective disorders (Coppen, 1968).

One approach to the study of cortisol in the central nervous system is to measure its concentration in cerebrospinal fluid (CSF). CSF contains unbound cortisol which is probably in equilibrium with the unbound cortisol in blood (Murphy, Cosgrove, McIquham, and Pattee, 1967). McClure (1969) in a small series of five depressed patients found them to have considerably lower levels than those found in five control subjects.

During an investigation into indoleamine metabolism in the affective disorders, samples of lumbar CSF were obtained from patients suffering from depression, or mania, and from neurological patients who were not affectively disturbed who acted as a control group. The opportunity was taken to examine many of these samples of CSF for their concentration of cortisol.

Methods

Patients Samples of CSF were obtained from 23 patients suffering from depression, 16 manic patients, and 18 control patients with a variety of neurological conditions but who did not have any psychiatric illness. Severity of depression was measured by the Hamilton Rating Scale (Hamilton, 1967) and the self-rating inventory devised by Beck, Ward, Mendelson, Mock, and Erbaugh (1961). Severity of mania was assessed on a five-point (0-4) global rating scale. CSF was obtained from a lumbar tap a few days after admission (mean 2.2 days after admission), and care was taken to collect the first 11 ml. of fluid. The CSF was then stored at -15°C until assay.

All patients suffering from affective disorders were taken off their drugs one to two days before the test and were sedated at night with chloral hydrate. Nine patients suffering from depression had not received any drugs for their psychiatric condition but their mean cortisol level did not differ from the rest of the patients who had been treated with a variety of antidepressant drugs before admission.

Estimation of Cortisol in CSF Cortisol concentration in CSF specimens was estimated by competitive protein-binding radioassay (Murphy, 1967) using modifications of the methods described by Murphy et al. (1967) for CSF and by Beardwell, Burke, and Cope (1968) for urine. The principal modifications were the addition of an internal standard and the use of Fuller's earth in place of Florisil for adsorption of unbound cortisol. The procedure was as follows:

To 1 ml. samples of CSF, duplicated if possible, was added an accurately measured quantity of 1,2-3H cortisol (Radiochemical Centre, Amersham, U.K., 2 c/mM) (0.1 ml. ethanol containing about 10 μCi) and 7.5 ml 432
Cortisol in the cerebrospinal fluid of patients suffering from affective disorders

433

dichloromethane (Merck, ‗pro analysis‘). The mixture was rotated on a Matburn mixer for 20 min. The aqueous phase was removed and 5.0 ml of the dichloromethane extract taken to dryness in an assay tube under a nitrogen stream at 35°C. The residue was taken up in 1.0 ml of 2% ethanol in 0.9% saline, and 0.2 ml was removed for determination of recovery of 3H. The percent recovery ranged from 66 to 100.

Corticosteroid-binding globulin (CBG) isotope solution was prepared as described by Beardwell et al. (1968) but utilizing human late pregnancy plasma. One millilitre of CBG isotope solution was added to the remaining 0.8 ml of the extract and to 0.8 ml 2% (v/v) ethanol in saline containing 0, 5, 10, and 15 μg cortisol; the standards were each run in duplicate. The assay tubes were held at 45°C for 5 min, then at 5°C for 10 min. Fuller’s earth (B.D.H. ‗for adsorption‘, 30 mg) was added to each tube, which was shaken for 2 min, centrifuged for 2 min at 5°C, and then returned to the 5°C water-bath for a further 10 min. A sample (0.5 ml) of the supernatant was taken for scintillation counting. The scintillator mixture consisted of 13 ml toluene-ethoxy-ethanol (7:6) containing 0.4% 2,5-diphenyloxazole (PPO) and 0.01% 1,4-bis-(4-methyl-5-phenyloxazolyl)-benzene (Dimethyl-POPOP). Calculations were based on a minimum of 20,000 counts in a 3H channel, using a Packard Tricarb Model 3375 liquid scintillation spectrometer.

The standard curve, set up with each batch of samples, was linear over the range 0 to 15 μg cortisol and the coefficient of variation of the standard counts per min was ±1.6%. The mean percent bound 3H-cortisol was 68.9 ± 3.4% (SD), with a range of 63.0-73.1.

The concentration of cortisol in CSF was calculated from the standard curve for the day, after correcting for cortisol added as internal standard before extraction, the percentage of this bound to protein and the losses on extraction. In the range 0 to 6 μg/ml CSF, the SD of the difference between duplicates was ±1.26 μg/ml. As the greater part of each CSF sample was required for other assays, the number of samples that could be assayed for cortisol in duplicate was only 13. It was, therefore, not possible to determine whether the accuracy of the method differed significantly over the range of observed values.

RESULTS

Table 1 shows the data obtained for the concentration of cortisol in CSF for each group, subdivided according to sex, together with the ages of each group and the correlations between CSF cortisol and age. It is apparent that there were no significant differences either between controls, depressives, and manics or between sexes. Correlations with age were significant only for the male controls (negative) and male manics (positive). In view of the relatively high methodological variance of the figures for CSF cortisol concentration, no attempt at interpretation of these unexpected correlations with age seems warranted: in the male manic group the significance vanishes if one aberrant value (9.9 μg/ml) is eliminated.

The Figure depicts the concentration of CSF cortisol plotted against time of day of lumbar puncture in the subjects for whom this time was known. It is clear that there is no diurnal trend discernible in the whole population of figures, and that there is insufficient information to tell whether diurnal swings might differ between the clinical groups. No patient was tested more than once on the same day.

There was also no significant correlation between CSF cortisol concentration and severity of depression as measured by the Hamilton Scale (n = 13, rs = ±0.152) or by the Beck Scale (n = 20, rs = +0.333). In measuring the severity of mania in the

<p>| TABLE 1 |
| CSF CORTISOL CONCENTRATION IN CONTROL PATIENTS AND IN PATIENTS SUFFERING FROM DEPRESSION OR MANIA, AND SPEARMAN RANK CORRELATIONS BETWEEN CSF CORTISOL CONCENTRATION AND AGE |
|</p>
<table>
<thead>
<tr>
<th>Group</th>
<th>Number</th>
<th>Lumbar CSF cortisol (μg/ml)</th>
<th>Age (yr.)</th>
<th>Correlation CSF cortisol</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>mean  SD</td>
<td>mean SD</td>
<td>rs vs. age, P</td>
</tr>
<tr>
<td>Controls</td>
<td>13</td>
<td>2.93 ± 2.09</td>
<td>46.5 ± 11.6</td>
<td>-0.687 &lt;0.01</td>
</tr>
<tr>
<td>Males</td>
<td>5</td>
<td>3.12 ± 2.62</td>
<td>51.6 ± 13.0</td>
<td>0.500 NS</td>
</tr>
<tr>
<td>Females</td>
<td>18</td>
<td>2.98 ± 2.17</td>
<td>47.9 ± 12.1</td>
<td>-0.442 NS</td>
</tr>
<tr>
<td>All subjects</td>
<td>18</td>
<td>2.98 ± 2.17</td>
<td>47.9 ± 12.1</td>
<td>-0.442 NS</td>
</tr>
<tr>
<td>Depressives</td>
<td>11</td>
<td>2.89 ± 1.39</td>
<td>56.4 ± 6.1</td>
<td>0.300 NS</td>
</tr>
<tr>
<td>Males</td>
<td>12</td>
<td>3.68 ± 2.35</td>
<td>52.3 ± 10.3</td>
<td>0.523 NS</td>
</tr>
<tr>
<td>Females</td>
<td>23</td>
<td>3.30 ± 1.95</td>
<td>54.3 ± 8.6</td>
<td>0.407 NS</td>
</tr>
<tr>
<td>All patients</td>
<td>23</td>
<td>3.30 ± 1.95</td>
<td>54.3 ± 8.6</td>
<td>0.407 NS</td>
</tr>
<tr>
<td>Manics</td>
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<td>3.38 ± 2.69</td>
<td>45.7 ± 10.4</td>
<td>0.659 &lt;0.05</td>
</tr>
<tr>
<td>Males</td>
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<td>1.96 ± 1.72</td>
<td>42.4 ± 15.5</td>
<td>-0.075 NS</td>
</tr>
<tr>
<td>Females</td>
<td>16</td>
<td>2.94 ± 2.47</td>
<td>44.7 ± 11.8</td>
<td>0.248 NS</td>
</tr>
</tbody>
</table>

1Controls vs. depressives, NS,
Controls vs. manics, NS.
Alec Coppen, B. W. L. Brooksbank, R. Noguera, and D. A. Wilson

manic patients there were only three scale points to compare, and so a chi-square test was carried out on a null hypothesis: this gave a non-significant value of $\chi^2 = 1.5$ (n = 16).

Finally, in the group of patients suffering from depression the CSF cortisol concentration was found not to be related to the length of the interval between admission to hospital and the lumbar tap (Table 2).

**TABLE 2**

<table>
<thead>
<tr>
<th>Days</th>
<th>Number of patients</th>
<th>CSF Cortisol Concentration (mg/ml.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>5</td>
<td>3.34</td>
</tr>
<tr>
<td>1</td>
<td>6</td>
<td>3.42</td>
</tr>
<tr>
<td>2</td>
<td>3</td>
<td>3.13</td>
</tr>
<tr>
<td>3+4</td>
<td>6</td>
<td>2.97</td>
</tr>
<tr>
<td>5+</td>
<td></td>
<td>3.40</td>
</tr>
</tbody>
</table>

**DISCUSSION**

The control figures we have obtained (2.98 ± 2.17 (SD) mg/ml.) for cortisol concentration in lumbar CSF are in broad agreement with those reported by Murphy et al. (1967) for a similar variety of patients suffering from various non-inflammatory neurological disorders, though Uete, Nishimura, Ohya, Shimomura, and Tatebayashi (1970), who used a fluorimetric method, have reported somewhat lower control figures including those from healthy subjects.

The failure to find any significant shift in the mean CSF cortisol concentration, either upwards or downwards, from the control mean, in depressed or manic patients is of considerable interest. Patients with depression are indeed suffering, and some disorder of the brain-pituitary-adrenal cortex control system would be expected, if only as a secondary phenomenon related to stress. Our data do not support the implications of McClure's observations that the brain in depression is lacking the level of cortisol necessary for its proper functioning (McClure and Cleghorn, 1969), nor do they reflect the increase in total corticosteroids in the plasma found in some depressives by many investigators. Moreover, we found no correlation between severity of depression and CSF cortisol concentration. As previous studies (Brooksbank and Coppen, 1967; Sachar, 1967) have shown that plasma and urinary corticosteroids in depressives tend to fall during the first few days after admission to hospital before any marked clinical improvement has occurred, the relationship between CSF cortisol level and the interval between admission and lumbar puncture was examined in the present series: again no definite trend was discernible.

It is probable that the concentration of cortisol in CSF is directly related to that of the non protein-bound fraction in plasma (Murphy et al., 1967; Uete et al., 1970). Unbound cortisol is likely to be the form that is physiologically active in the brain (Yates and Urquhart, 1962; Kawai and Yates, 1966): the impermeability of the blood-brain barrier to protein would seem to preclude any cerebral effect.
of protein-bound cortisol (Keller, Richardson, and Yates, 1969). Our results therefore indicate that the physiologically active level of cortisol in the brain as a whole is unlikely to be grossly abnormal in affective illness.

Uete and his collaborators (1970) did observe a diurnal variation in the level of cortisol in the CSF of five human subjects in whom continual sampling of CSF was possible, but the fluctuations were small compared with those found simultaneously in the blood plasma. It is therefore not surprising that we failed to find a definite relationship between time of day and CSF cortisol concentration in our samples taken from different individuals. The fact that the CSF cortisol concentration in our control males, who after all were not healthy, was apparently correlated negatively with age should be noted with caution. Total plasma cortisol and cortisol secretion rate are not lower in the elderly when allowance is made for changes in body mass (Bliss, Sandberg, Nelson, and Eik-Nes, 1953; Romanoff, Morris, Welch, Rodriguez, and Pincus, 1961).

We wish to thank our colleagues at the Atkinson-Morley and Graylingwell Hospitals for their collaboration in the collection of samples of CSF, and Mr. J. Bailey for his assistance with the statistical computations.

REFERENCES


Cortisol in the cerebrospinal fluid of patients suffering from affective disorders 435
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Alec Coppen, B. W. L. Brooksbank, R. Noguera and D. A. Wilson

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