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CONCENTRATION OF ESTRADIOL, TESTOSTERONE AND PROGESTERONE IN CEREBROSPINAL FLUID COMPARED TO PLASMA UNBOUND AND TOTAL CONCENTRATIONS

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SUMMARY

Estradiol, progesterone and testosterone are measured in plasma and CSF in 17 women and 11 men. The results show a transfer from plasma to CSF of about 4% for estradiol, 10% for progesterone and 2.5% for testosterone. There was found to be a clear correlation between the plasma and CSF levels of these steroids. A comparison is also made between the calculated levels of unbound estradiol and testosterone in plasma and the levels in the CSF. The results show approximately the same concentrations of these steroids in the CSF as the calculated levels of unbound steroids in the plasma.

INTRODUCTION

When working with psychological or neurological abnormalities in which the hormone balance can be of importance it is of interest to determine the extent of hormone penetration of the blood-brain barrier, and whether changes in the blood hormone levels are reflected in the brain hormone levels. An indication of this can be obtained by simultaneous measurement of hormone levels in plasma and cerebrospinal fluid (CSF). There is a possibility that variations in the total hormone levels are accompanied by corresponding changes in the plasma binding capacity and will not, therefore, be reflected in the target organs, for which the free hormone fraction is believed to be the physiologically active agent. The hormone levels in the CSF may be taken as levels of free hormone since the blood-CSF barrier is not normally penetrated by proteins and functions as a natural filter.

It is also of interest to compare the levels of free hormone in plasma with the levels in the CSF to see whether they agree. This can be achieved using the method developed by us for calculation of free estradiol and testosterone in plasma. The aim of this work is therefore two-fold:

(a) To analyse to what extent variations in plasma hormone levels are reflected in the CSF, and (b) to compare the calculated levels of free hormone in plasma with the levels in the CSF.

METHOD

Blood and CSF were drawn from 17 women and 11 men between the ages of 19–65 years, within 0–65 min, between the time of drawing blood and CSF. Mainly patients from the neurological and psychiatric clinics with no underlying disease which could influence the results were used, e.g. headache investigations with normal clinical findings or patients with neuroses and no somatic illness. Estradiol and testosterone were measured in duplicate samples using radioimmunoassay and competitive protein binding, respectively, after paper chromatography [1]. Progesterone was measured in duplicate samples using radioimmunoassay after direct extraction [2]. In the case of CSF 4 ml was extracted for estradiol and testosterone determinations and 1.5–4 ml for progesterone. The method for calculated unbound hormone will be described elsewhere (Södergård *et al.*, to be published). In principle, the method is based on measurement of binding capacity of testosterone-estradiol binding globulin (TeBG), albumin concentrations and total concentrations of testosterone and estradiol. In addition, published maximal and minimal concentrations of dihydrotestosterone [3, 4], 3β, 17β-dihydroxy-androst-5-en [5], 3α,17β-dihydroxy-5α-androstan [6] and 3β,17β-dihydroxy-5α-androstan [7] were taken into account, and calculations of unbound hormone concentrations of estradiol and testosterone were made with either max. or min. plasma values of these androgen metabolites. Knowing the association constants of the steroids to TeBG and albumin at 37°C one can calculate the concentrations of differently bound fractions and unbound hormone. Two values of unbound estradiol and testosterone were calculated for each individual sample, depending on the min. or max. plasma level of the androgen metabolites. Regression lines were calculated for the total hormone levels in plasma, compared to those in CSF. For the calculated, unbound plasma hormone levels, the concentrations calculated taking into account the max. or min. androgen metabolite levels, were compared to hormone levels determined in CSF.

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The correlations were calculated using product-moment correlation. Differences between women and men were calculated according to Wilcoxon's ranking method, and between calculated unbound levels in plasma and CSF levels using the paired t-test.

RESULTS

The concentrations of estradiol, testosterone and progesterone in the CSF and in plasma are seen in Fig. 1. The ratio between the concentrations in CSF and the total concentrations in plasma showed no differences between women and men, except for progesterone where the relative concentration in CSF was significantly higher in men (Table 1). The regression line for the relation between total estradiol levels in plasma (x) and the concentrations in CSF (y) was $y = 0.245 + 0.034\,x$ with residual standard error (SE) of 0.88 pg/ml. The correlation coefficient between the total levels in plasma and CSF levels was 0.731 ($P < 0.0005$, Fig. 1). In the case of testosterone the corresponding line was $y = 1.259 + 0.017\,x$ with SE of 3.0 ng/100 ml and correlation coefficient of 0.831 ($P < 0.0005$, Fig. 1). For progesterone the regression line was $y = 0.018 + 0.049\,x$, SE 0.01 ng/ml and the correlation coefficient 0.913 ($P < 0.0005$, Fig. 1).

The mean percentage of calculated unbound estradiol out of total plasma concentrations in female and male plasma showed small variations between max. or min. metabolite levels; in women 3.4–3.6% and in men 3.3%. The mean percentage of calculated unbound testosterone in female plasma out of total concentration varied between 2.1–2.5% and between 2.1–2.3% in men depending on the metabolite concentrations. Regression lines for min. and max. metabolite concentrations respectively are shown in Fig. 2. There was no significant difference either for estradiol or testosterone between the levels in the CSF and the two calculated unbound levels in plasma, using min. or max. levels of androgen metabolites in plasma in the calculations of unbound. When accounting for minimal levels of androgen metabolites in plasma, the regression line for the relation between calculated free estradiol in plasma (x) and estradiol in CSF (y) was $y = 0.11 + 1.05\,x$, SE 0.89 pg/ml. The correlation coefficient was 0.73 ($P < 0.0005$, Fig. 2). For maximal levels of androgen metabolites the regression line was $y = 0.20 + 0.98\,x$, SE 0.90 pg/ml and correlation coefficient 0.72 ($P < 0.0005$, Fig. 2). Corresponding regression lines for testosterone were $y = 0.49 + 0.96\,x$, SE 1.75 ng/100 ml, $r = 0.92$ ($P < 0.0005$, Fig. 2) for min. metabolite concentrations and $y = 0.40 + 0.86\,x$, SE 1.80 ng/100 ml and $r = 0.92$ ($P < 0.0005$, Fig. 2) for max. metabolite values. The slopes of the regression lines of both estradiol and testosterone were close to one in all

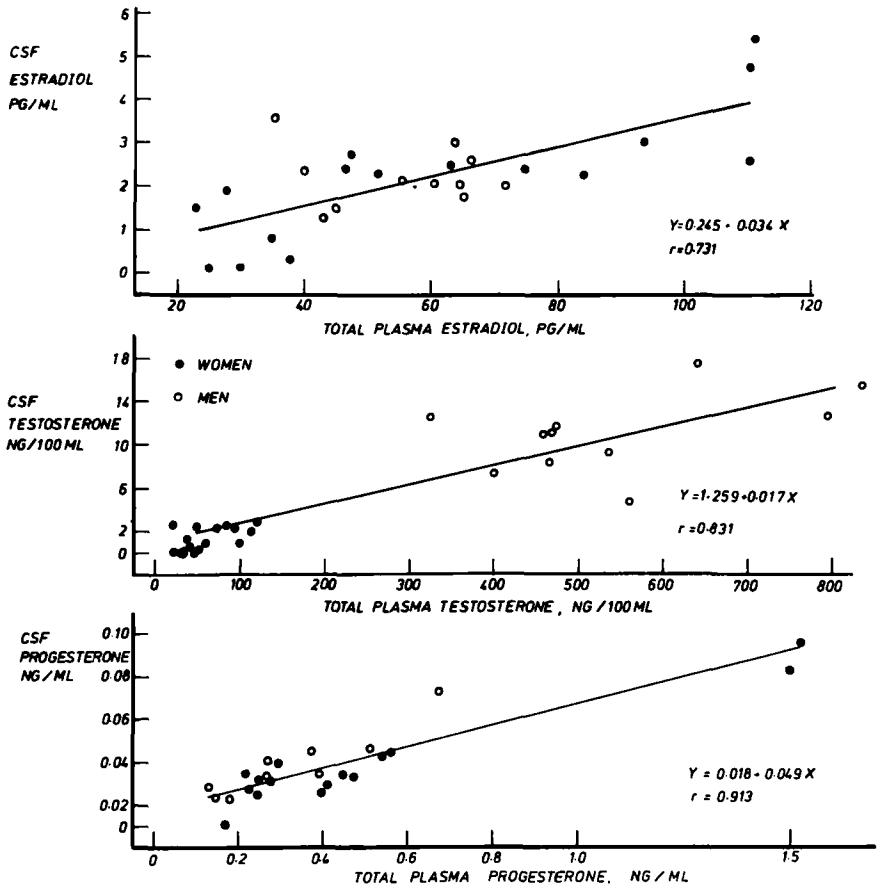

Fig. 1. Relation and regression lines between total plasma hormone levels and concentrations in CSF.

Table 1. Hormone concentrations in CSF and their percentage of total plasma concentrations

	Women		Men		Women and men together		Significance between women and men	
	Conc. ± SE	% ± SE	Conc. ± SE	% ± SE	Conc. ± SE	% ± SE	Conc.	%
Estradiol (pg/ml)	2.4 ± 0.4	3.8 ± 0.5	2.2 ± 0.2	4.3 ± 0.7	2.3 ± 0.2	4.0 ± 0.4	NS	NS
Testosterone (ng/100 ml)	1.4 ± 0.3	2.9 ± 0.9	11.1 ± 1.1	2.2 ± 0.2	5.1 ± 1.0	2.5 ± 0.5	$P < 0.005$	NS
Progesterone (ng/100 ml)	4.5 ± 0.8[1]	9.3 ± 0.8	3.9 ± 0.5[2]	13.2 ± 1.3	4.3 ± 0.5	10.8 ± 0.8	NS	$P < 0.025$

[1] $n = 16$. [2] $n = 9$.

cases suggesting a 1:1 ratio between free hormone concentrations in plasma and concentrations in the CSF.

DISCUSSION

There would appear to be a clear relationship between the total levels in plasma and the levels in the CSF in the case of all three hormones which we have measured. The correlation coefficients were highly significant. This means that variations measured in the total levels will be reflected in the CSF's hormone content. The fraction of the plasma level of estradiol measured in the CSF is approximately the same in men and women and is about 4%. The corresponding testosterone fraction is somewhat lower, about 2.5%. Our ratio between testosterone levels in CSF and total levels in plasma agree relatively well with the ratio obtained by David *et al.*[8] between the activity of ^{3}H-testosterone in the CSF and in plasma after injection of ^{3}H-testosterone i.v. to intact male apes, however, not to the ratios obtained by them in orchidectomized apes [8]. However, the results are not altogether comparable since David *et al.*[8] had a completely different approach to their study. Their results were obtained by injecting a bolus of radioactive steroids i.v. upon which they measured the distribution of radioactivity into different compartments. The fraction of progesterone found by us in the CSF is lower than that found by David *et al.*[8], but corresponds relatively well with that found by Curie and Weiss[9] in pregnant women. There may be differences between species which can explain the difference between our results and those of David *et al.*[8], or possibly the difference in the methods used may be of relevance. It would be interesting to know if the concentrations of hormones in CSF are the same or if they change proportionally with hormone concentrations in the brain water. Penetration of ions, e.g. radioactive Na^+ and Cl^- to CSF is slow compared to the penetration to the brain water [10]. Steroids may however be assumed to pass the blood–brain barrier in the same way as other nonionic organic substances, e.g. thiourea which penetrates both CSF and brain water at a similar rate. It has also been shown that labelled corticosterone injected into the lateral ventricle of rats can be found in similar concentrations in cerebral cortex as in whole blood [11].

The calculated level of free estradiol in plasma seems to be the same as that in the CSF. The slopes of the two regression lines between calculated unbound and CSF levels were close to one (0.98–1.05). The regression lines had small parallel shifts upwards (0.11–0.20 pg/ml), however, which must be considered to lie within the margin of error. In the case of testosterone the correspondence was good between the calculated unbound levels and those in the CSF. The slopes of the regression lines were 0.86–0.96 and even here there was a slight parallel shift upwards (0.40–0.49 ng/100 ml) of the regression lines. There was no significant difference between the CSF levels and the unbound hormone levels when either max. or min. androgen metabolite levels were used in the calculation. For estradiol the regression lines between the CSF concentrations and the unbound concentrations calculated using min. or max. androgen metabolite values were very close to each other (Fig. 2). The levels of androgen metabolites were thus of minor importance for unbound estradiol. In the case of un-

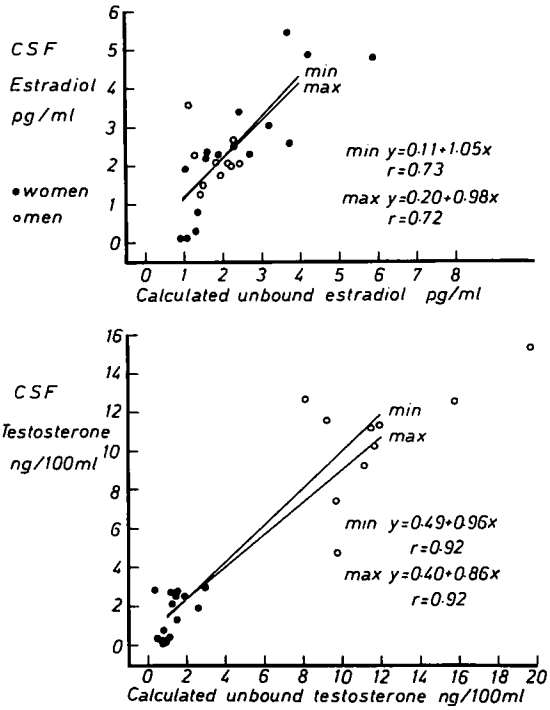

Fig. 2. Relation between unbound hormone levels calculated using mean published levels of androgen metabolites and concentrations in CSF. Regression lines shown are between CSF concentrations and the levels of plasma unbound hormones when using both max. and min. androgen metabolite levels in plasma in the calculations.

bound testosterone the corresponding regression lines deviated to a moderate extent. Hence androgen metabolites binding to TeBG may be of relevance for the calculation of unbound testosterone levels. However, the true levels of unbound testosterone must lie in between the levels obtained when taking into account the range of androgen metabolites as was done in the above results. There was no significant difference between the levels in CSF and the unbound levels in plasma calculated with either max. or min. plasma metabolite levels.. Therefore the true levels of unbound testosterone in plasma will not differ from the levels in CSF. Cortisol may be assumed to have a similar ratio between CSF and plasma unbound concentrations as testosterone and estradiol. Measurements of ultrafiltered plasma cortisol, which is proposed to be the unbound concentration, resulted in 1.5 times [12] higher concentration in the plasma fraction. This deviation from our results may be due to differences in methodology [13]. A method to calculate unbound progesterone in plasma has not been successfully worked out so far.

In the light of the above results we conclude that the variation in the total plasma testosterone, estradiol and progesterone levels are reflected in the CSF and that the calculated free levels of estradiol and testosterone seem to agree with those found in the CSF.

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