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SHORT COMMUNICATION

RADIOIMMUNOASSAY OF 17-HYDROXY-PROGESTERONE: PHYSIOLOGICAL VARIATIONS AND PATHOLOGICAL VALUES IN MAN

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SUMMARY

Highly specific antisera, directed against 17 hydroxyprogesterone (17-OH P), were produced in rabbits. They were tested for their cross-reactivity with 29 steroids and the development of the specificity of four antisera for progesterone, 11 deoxycortisol, cortisol and 17-OH pregnenolone was followed throughout the year of immunization. Plasma 17-OH P was then determined and the values obtained from healthy adults, children and patients suffering from hirsutism and 21-hydroxylase deficiency are given.

INTRODUCTION

17-Hydroxy-progesterone (17-OH P), as a precursor of cortisol and testosterone and a metabolite of progesterone, is involved in any variations of these steroids that may be observed and its measurement could give useful information about the adrenal and gonadal production of these hormones. We have obtained highly specific antisera directed against 17-OH P, from rabbits. The present communication reports a method for measuring this steroid and its variations in man, under physiological and pathological conditions. In routine clinical assays, the omission of the preliminary chromatographic step could be considered.

MATERIALS AND METHOD

Eighteen mature white New Zealand rabbits were immunized against 17-hydroxy-progesterone 3-(o-carboxymethyl-oxime, bovine serum albumin according to Vaitukaitis' technique [1] with 0.5 mg of immunogen in 1 ml complete Freund's adjuvent. In addition, once a month, an identical booster dose was administered in the same way. After each booster injection, the animal was bled from the marginal vein of the ear, after five days in order to check the antibody titer and after 10 days to collect blood when the titer had reached 1/10,000. After several months of immunization, the rabbits were sacrificed and bled completely. Antisera were taken up in volume of 0.1 ml, lyophilized and stored at 4°C.

Half to one milliliter of plasma was extracted with 10 ml diethyl-ether. The organic phase was evaporated to dryness and passed through a mini-celite column with ethylene glycol as stationary phase, according to Abraham's technique [2] (celite, from Johns Manville Company, has been washed by benzene and dried at 500°C before use to eliminate the "blank values").

The 17-OH P fraction [isooctane 85-Ethylacetate 15 (V/V)] was evaporated in siliconized tubes (Siliclad, from Clay Adams) and the dry residue was redissolved in 2 ml gelatin phosphate buffer (phosphate buffer: 0.4 M, pH 7.4, gelatin 1% and sodium azide 0.2%). Two different aliquot volumes, in duplicate, were incubated with the diluted antiserum at the final volume of 0.7 ml.

RESULTS AND COMMENTS

Characterization of the antisera

The titers of anti 17-OH P antisera increased progressively after each monthly booster injection in six of the 18 rabbits. After 12 months of immunization, titers as high as 1/45,000 and 1/90,000 were obtained.

Five antisera were tested for their cross reactivity with 29 steroids. With respect to cortisol, corticosterone, mineralocorticoids, androgens, estrogens and contraceptive drugs (Dydrogesterone, Quingestanol, Norethisterone, Lynestrenol, Norgestrel), the specificity was good (% of cross-reaction <0.01). The most positively cross-reacting steroid appeared to be 17α-hydroxy-pregnenolone and thereafter, in decreasing order, 5α-dihydro-progesterone, progesterone, 11-deoxycortisol and pregnenolone (1.80, 0.5, 0.4, 0.15 and 0.05% respectively, with the best antiserum obtained). The specificity was established after the first immunization and did not vary through the remainder of the study. This last point was already observed in the search for aldosterone antibody [3] and it is in agreement with Wicking's findings [4]. However, according to Forest *et al.*, the specificity could be improved by repeated injections [5].

The affinity constant, calculated from a Scatchard plot, ranged from 0.5 to 4×10^{10} l/mol. It did not change significantly during the year of immunization and there was no difference between the five antisera tested.

Reliability of the method

After extraction and chromatography, the mean recovery of [^{3}H]-17 OH P was $82.6 \pm 4.8\%$ ($n = 150$). The intra-assay variation was 3% for a mean level of 0.86 ng/ml ($n = 15$) and the inter-assay variation was 8% for a mean level of 0.88 ng/ml ($n = 22$). Known amounts of 17-OH P (0.2, 0.5 and 1 ng/ml) were added to a pool of plasma. The values found experimentally and expressed in ng/ml ± S.D., were: 0.51 ± 0.03 for the plasma pool and 0.71 ± 0.06, 1.03 ± 0.06 and 1.47 ± 0.09 for the three added plasmas respectively. Compared with predicted values, the regression equation obtained was: $y = 0.964\,x + 0.523$ ($R = 0.998$).

17-OH P in normal steroidogenesis

Blood samples were collected between 8 a.m. and 10 a.m. in heparinized syringes and the plasma separated by centrifugation at 4°C.

Table 1. Determination of 17-OH P plasma concentrations with (1) and without (2) determination step

(1)	(2)
200	211
400	417
440	434
185	180
240	239
72	50
88	84
88	90
170	153

Eight healthy young women were studied throughout their menstrual cycle. Although some women showed a 17-OH P peak which coincided with the LH peak, this was not a general phenomenon and, on the whole, the 17-OH P pattern through the cycle involved two distinguishable levels. Starting from 0.43 ± 0.22 (S.D.) ng/ml during the follicular phase, 17-OH P increased sharply to 0.91 ± 0.38 (S.D.) ng/ml at mid cycle and reached the level of 1.52 ± 0.57 (S.D.) ng/ml at the luteal phase. These values are in agreement with those reported by other authors [6, 7, 8].

This pattern disappeared in four women using oral contraceptives (ethinylestradiol 50 μg plus quingestanol acetate 500 μg) and in 15 menopausal women: their 17-OH P mean levels were: 0.32 ± 0.15 (S.D.) ng/ml and 0.32 ± 0.19 (S.D.) ng/ml respectively. This is consistent with a suppression of ovarian function. 17-OH P can thus be used as a valid index of corpus luteum function like progesterone.

Six children aged from 1 to 11 years exhibited 17-OH P values from 0.10 to 0.71 ng/ml with a mean level of 0.38 ± 0.10 (S.D.) ng/ml.

Daily variations of 17-OH P were studied in 7 healthy men, sampled every four hours over a period of 24 hours. The highest values occurred either at 8 a.m. or at 12 a.m. (1.65 ± 0.35 (S.D.) and 1.47 ± 0.49 (S.D.) ng/ml respectively) and the lowest was found at 12 p.m. (0.62 ± 0.22 (S.D.) ng/ml). In addition, the plasma 17-OH P of 22 other young men determined at 8 a.m. was 1.54 ± 0.46 (S.D.) ng/ml.

17-OH P in abnormal steroidogenesis

In 21 women suffering from ovarian or idiopathic hirsutism plasma 17-OH P measured on the 6th, 7th and 8th days after the temperature rise, ranged from 1.08 to 3.30 (S.D.) ng/ml with a mean of 1.94 ± 0.65 (S.D.) ng/ml. This value was not significantly different from that observed in normal women. However, values above 1 ng/ml during the follicular phase or above 3 ng/ml during the luteal phase are an indication of a possible adrenal origin of the hirsutism. In such cases, subsequent ACTH stimulation should provoke a marked increase in 17-OH P, reaching up to tenfold the basal level [9]. In untreated patients suffering from congenital 21-hydroxylase deficiency, there was a striking increase in 17-OH P and the values fluctuated widely from 6 ng/ml to 1260 ng/ml. Practically, there is no need to carry out the chromatographic step if a rapid screening for diagnosis purposes is required (Table 1). During the treatment of the disease 17-OH P remains a biological parameter which should be checked at regular intervals as is the case for testosterone and plasma renin activity [10].

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