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SHORT COMMUNICATION

A RADIOIMMUNOASSAY FOR TESTOSTERONE IN VARIOUS BIOLOGICAL FLUIDS WITHOUT CHROMATOGRAPHY

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This report describes the validation of a testosterone assay suitable for a variety of biological fluids utilising an antiserum raised from an antigen coupled through position 3. The antiserum (E01) was a gift from Dr. S. Tillson and had been raised in the goat against testosterone-3-carboxymethyl oxime-BSA [1].

EXPERIMENTAL

Method

1:2,[^{3}H]-testosterone (ca 1000 cpm) in 20 μl ethanol was added to each sample (100 μl, diluted if necessary with phosphate buffered saline containing 0.1% gelatin (PBS)) and after equilibration extracted with 1 ml hexane-ether 4:1. The aqueous phase was frozen and the organic layer decanted into tubes, evaporated to dryness and the residue dissolved in 200 μl PBS. A 50 μl aliquot was used to determine the recovery and a further aliqout (100 μl) was used for radioimmunoassay.

When further purification was required the hexane-ether extracts were transferred to alumina columns prepared as described by Furuyama, Mayes and Nugent [2]. After the solvent had run into the alumina the columns were washed with 1.6 ml hexane-ether 4:1, followed four times with 1.6 ml of 0.4% ethanol in hexane and the testosterone eluted with 3.2 ml of 1.4% ethanol in hexane. The eluate was evaporated to dryness and dissolved in 200 μl PBS and aliquots taken for recovery and radioimmunoassay as described above.

Standards

A solution containing 1 μg testosterone/ml of ethanol was prepared, 640 μl were removed, evaporated to dryness and the residue dissolved in 100 ml of PBS giving a concentration of 640 pg/100 μl.

Suitable dilutions of this solution were then prepared to give concentrations of 320, 160, 80, 40 and 20 pg/100 μl PBS. These solutions were then aliquoted out in 100 μl quantities, covered with parafilm and stored at 15°C. Tubes were then removed whenever a standard was required.

Radioimmunoassay

A standard curve covering the range 20–640 pg was set up with each assay. Antiserum was then added (100 μl of a 1/6000 dilution) to both the standards and unknowns followed by approximately 10,000 cpm of testosterone in 100 μl PBS. Tubes were included which received 100 μl of PBS in place of the antiserum, as a measure of the non specific binding. After whirlimixing, the tubes were incubated at room temperature for 1 hour or overnight at 4°C. After incubation dextran-coated charcoal suspension (1 ml) was used to separate the free and bound fraction and the bound fraction supernatant counted for 10 minutes or 10,000 counts.

Results were calculated from the standard curve using a log-logit transformation, corrected for recovery and expressed as ng testosterone/ml sample.

RESULTS

Specificity: The antiserum was checked for cross reactivity with 16 steroids likely to be found in plasma. The only compounds that showed a significant cross reaction were 5α-dihydrotestosterone (23.9%), 11β-hydroxy-testosterone (0.4%), oestradiol-17β (0.2%) and androstenedione (0.1%).

Accuracy: The recovery of known amounts (2–6 ng/ml) of testosterone added to plasma was 105.6% ± 9.0 (n = 24) after correction for recovery of ^{3}H-testosterone.

Sensitivity: The sensitivity of the assay, i.e. the precision of the measurement of zero, was 8 pg.

Precision: The between assay variation calculated from the results of a quality control run in each assay gave a value of 7.36 ± 0.85 ng/ml (CV 11.5%) (n = 63).

After the assay had been in routine use the results calculated using the recovery measured for each sample were compared with that calculated using the mean overall recovery for all previous assays. No significant differences were observed (regression analysis $r = 0.984$, $y = 0.996\ x + 0.031$, $n = 150$).

Biological samples. Plasma from a group of 54 men gave a mean value of 5.89 ng testosterone/ml (range 2.51–10.13). The specificity of the assay was checked by comparing the results obtained before and after chromatography of extracts from various biological fluids. No significant differences were observed when the assays were applied to plasma from men, male marmosets, male rats and human follicular fluid. In contrast cord blood and plasma from women when assayed without chromatography seriously overestimated the testosterone level. The results are summarised in Table 1.

In a larger study plasma testosterone levels in male marmosets were found to range from <0.1 to >80 ng/ml. These high values were confirmed by pooling samples containing >25 ng testosterone/ml, the pooled plasma was assayed by immunoassay followed by formation of a t-butyl-dimethylsilyl ether derivative by GLC/MS [3] this being quantitated using a tuned ion technique. The results differed by less than 20%.

DISCUSSION

Previously workers [4] have suggested that antisera to testosterone coupled through the "non functional" positions do not appear to offer any advantage over those coupled through position 3. The results presented above confirm the feasibility of a satisfactory assay system based on an antiserum raised to a T-3-conjugate.

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Table 1. Comparison of testosterone results obtained before and after chromatography of extracts from various biological fluids

Biological material	Testosterone ng/ml Mean ± S.D. before chromatography	Testosterone ng/ml Mean ± S.D. after chromatography	n	Paired t-test t	P
Plasma from men	5.90 ± 1.76	5.98 ± 2.19	19	0.4383	>0.1
Male marmoset plasma	22.01 ± 16.78	22.14 ± 17.18	9	1.6236	>0.1
Male rat plasma	2.78 ± 1.59	2.64 ± 1.97	6	0.7349	>0.1
Human follicular fluid	22.7 ± 11.7	22.7 ± 12.3	6	0.0607	>0.1
Human cord plasma	1.83 ± 0.61	0.23 ± 0.09	7	7.4810	<0.001
Plasma from women	1.08 ± 0.50	0.46 ± 0.14	10	4.6206	<0.01

The technical aspects of the assay have been simplified as far as possible resulting in a robust, reliable assay that has been in use for over one year. Preparing the standards in buffer and storing at −15°C, already aliquoted out, is time-saving and convenient, with no deleterious effect being observed for at least four to six months. The consistency of the recovery makes the use of an internal standard or tracer unnecessary, substantially reducing the amount of counting time required.

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