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ABSTRACTS VOLUME

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T035

MEASUREMENT OF DEHYDROEPIANDROSTERONE SULPHATE (DHEAS): A COMPARISON OF ISOTOPE-DILUTION LIQUID CHROMATOGRAPHY TANDEM MASS SPECTROMETRY (ID-LC-MS/MS) AND SEVEN CURRENTLY AVAILABLE COMMERCIAL IMMUNOASSAYS

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Background: Dehydroepiandrosterone sulphate (DHEAS) is an important marker of the adrenal gland. Its assessment is required in several adrenal diseases, such as adrenal tumours, adrenal insufficiency and congenital adrenal hyperplasia. Most clinical laboratories assess DHEAS using commercially available immunoassays. The aim of the present study was to develop an ID-LC-MS/MS method for the assessment of DHEAS in serum and to use this method to investigate the accuracy of currently available commercial DHEAS assays.
Methods: Our ID-LC-MS/MS method consisted of a sample preparation with addition of deuterated internal standard ([2H6]DHEAS) to the serum samples and a protein precipitation using acetonitrile, where after the samples were analyzed using a symbiosis online SPE system (Spark Holland, Emmen, the Netherlands) coupled to a Quattro Premier XE tandem mass spectrometer (Waters Corp., Milford, MA). Intra-assay CV was 4.1%. Seven currently available DHEAS assays were compared to our ID-LC-MS/MS method by assessing 75 serum samples (concentration range 0.06- 20.6 µmol/L measured by ID-LC-MS/MS) by each method as well as performing recovery experiments and dilution series. Data obtained in the present study were also compared to data of three EQAS's.
Results: Three methods agreed well with ID-LC-MS/MS (R between 0.93 and 0.99 and slopes ranging from 0.92 to 1.07) and showed good recoveries (range 76-115%). Four methods showed standardization problems (slopes were 0.84, 1.14, 1.20 and 1.28) and recoveries in these methods were 61-85%, 103-126%, 115-136% and 126-142%, respectively. Intra-assay coefficient of variation were <5.5% in six methods; one assay had an unacceptably high intra-assay coefficient of variation of 18%. Linearity was good in all methods. Our data are in agreement with data obtained in three EQAS's.
Conclusion: Some of the currently available DHEAS methods show standardization problems and/or a high variation. These problems have potentially adverse clinical consequences. We advise the manufacturers to improve their assays and laboratorians to scrutinize the DHEAS method they employ.

T036

PITUITARY SOMATOTROPES IN OVARIECTOMIZED RATS AFTER TREATMENT WITH COMBINED LHRH AND ESTRADIOL

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Background: Estradiol is important hormone which controls the secretory activity of hormone producing cells in the rat female pituitaries. Luteinizing hormone-releasing hormone (LHRH), found in hypothalamus, primarily controls the release of gonadotropic hormones from the anterior pituitary gland, but also affects some other pituitary cells. In the present study, the influence of estradiol dipropionate (EDP), combined with LHRH, on the morphology and secretory activity of pituitary somatotropes, in ovarictomized (ovx) Wistar rat females, was studied.
Methods: Female Wistar rats were ovx at 12 weeks of age. Animals were divided into two groups, each comprising seven females. The first group of ovx females received EDP (i.p. 250 µg) daily for 4 weeks, combined with LHRH (i.p. 25 µg), during the last three days of the fourth week after the surgery. The second group were ovx controls, who i.p. received the adeqate volume of sterile olive oil and saline during the same period. All females were sacrificed 24 h after the last injection. Pituitary somatotropes were studied using the peroxidase-antiperoxidase (PAP) immunohistochemical procedure. Serum concentrations of growth hormone in control and EDP+LHRH treated female rats were measured by the hGH-Delfia kit.
Results: The absolute and relative pituitary weights in EDP+LHRH treated females were significantly increased ($P < 0.05$) by 229.7 and 273.7% respectively, in comparison with the controls. Immunopositive pituitary somatotropes in the control rats were round to pyramidal in shape, usually located in close proximity to the blood vessels. In the EDP+LHRH treted ovx females somatotropes were elongated, irregularly shaped, with more intensely stained cytoplasm. Concentration of growth hormone in the serum of EDP+LHRH treated ovx females was significantly increased ($P < 0.05$) by 60.0%, compared to the Ovx controls.
Conclusions: Our findings show that EDP+LHRH application has caused the change in the morphology and stimulated hormone secretion of pituitary somatotropes, in ovx females.