Determination of the urinary cortisol production rate with [1,2,3,4-13C]cortisol

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CHAPTER 8

Summary

The aim of the studies described in this thesis was to develop and evaluate a stable isotope dilution method using [1,2,3,4-^{13}C] cortisol and gas chromatography/mass spectrometry to measure the urinary cortisol production rate in children and babies. A stable non-radioactive labelled tracer was used as it is not considered medically ethical to administer radioactive labelled ones to children for research purposes. The urinary cortisol production rate method consists of intravenously administering a tracer dose of [1,2,3,4-^{13}C] cortisol to the subject under study and collecting all the urine excreted for the following two to three days, when the excretion of tracer is complete. Subsequently the isotope dilution is measured in the cortisol metabolites. The cortisol production rate can then be calculated knowing the amount of tracer administered the time of urine collection and isotope dilution in the cortisol metabolites.

Chapter 1 included a description of cortisol synthesis, metabolism, production and its biological effects. Different techniques used to measure the cortisol production rate and details of isotope dilution gas chromatography/mass spectrometry methodology were also given. From the different methods used to estimate the cortisol production rate, the urinary CPR method was chosen as method of choice to measure the cortisol production rate with [1,2,3,4-^{13}C] cortisol.

In chapter 2 the extraction and high performance liquid chromatography isolation technique used to obtain urinary cortisol metabolites tetrahydrocortisone and tetrahydrocortisol is described. The method was first validated with piglets as their weight was comparable to that of newborn infants. The liquid chromatographic technique was needed to isolate the urinary steroids of interest from other compounds present in urine and thus reduce the run time on the mass spectrometer.

Chapter 3 describes the complete procedure used to measure the urinary cortisol production rate in patients administered [1,2,3,4-^{13}C] cortisol. The cortisol production rate could be determined from measuring the isotope dilution in the principal urinary cortisol metabolites namely, tetrahydrocortisone, tetrahydrocortisol, 5α-tetrahydrocortisol, α-cortolone, and β-cortolone in a cumulative three day urine collection, when excretion of the tracer is complete. All metabolites could be oxidized to the common product 11-oxo-aetiocholanolone (or 11-oxo-androsterone in the case of 5α-tetrahydrocortisol).

The oxidation products were then analyzed by selective ion monitoring as the highly stable methoxime tertiary butyldimethylsilyl derivative. The quantitation of the tracer enrichment was carried out at m/z 344 $[M-103]^+$ ion for unlabelled — and m/z 348 for labelled — 11-oxo- aetiocholanolone, or m/z 390 $[M-57]^+$ and m/z 394 for unlabelled and labelled 11-oxo-aetiocholanolone or 11-oxo-androsterone, respectively (in the case of oxidized 5α-tetrahydrocortisol). As the isotope dilution in all the urinary cortisol metabolites could be measured in the same product, 11-oxo-aetiocholanolone or 11-oxo-androsterone, only one calibration graph for the mass spectrometer needs to be constructed. The compounds measured were usually free of any interfering co-eluting peaks due to the liquid chromatographic isolation and specific oxidation. Preparation of the calibration graph was a problem as labelled 11-oxo-aetiocholanolone mixtures needed to be prepared from labelled cortisol, and was successfully solved by chemical and biological synthesis. The GC/MS calibration graph mixtures were prepared and resulted in a highly reproducible long term instrumental precision of the gas chromatographic/mass spectrometric analysis. Samples could be accurately and precisely measured, even down to isotope enrichments of 0.1%. The accuracy of the isotope dilution analyses was found to be greater than 5%. Low levels of tracer enrichment needed to be measured as administration of too high a dose to the patient would result in a reduction of the endogenous cortisol secretion by negative feedback. Normally the mass of [1,2,3,4-^{13}C] cortisol administered to the patient was estimated to be 0.3 to 1.0% of the daily CPR. In chapter 3 the cortisol production rate was measured in a child with reduced pituitary function, and the CPR was found to be 4.06 $\pm$ 0.29 mg/day (from three metabolites).

In chapter 4 the occurrence of isotope effects with [1,2,3,4-^{13}C] cortisol was investigated. This was carried out by administering known mixtures of [1,2,3,4-^{13}C] cortisol and unlabelled cortisol to adrenalectomized piglets (no endogenous cortisol secretion by the adrenal). Small *in vivo* isotope effects were observed with tetrahydrocortisone (3%), tetrahydrocortisol (3%), and α- and β-cortolone (12%).

A similar study was also carried out with commercially available [9,12,12-^{2}H] cortisol and presented in chapter 5 and here even larger *in vivo* isotope effects occurred with the metabolism to tetrahydrocortisone and tetrahydrocortisol (10%), the two principal urinary cortisol metabolites. During metabolism of [9,12,12-^{2}H] cortisol one deuterium atom is also lost, thus the isotope enrichment must be measured at an m + 2 instead of m + 4 which can not be so accurate and precise as that measured with [1,2,3,4-^{13}C] labelled cortisol metabolites. Thus it was concluded that [1,2,3,4-^{13}C] cortisol was the best tracer for cortisol production rate measurements.

In chapter 6 the cortisol production rate was measured in two children aged 3 and 11 years suffering from 17α-hydroxylase deficiency. Although very small amounts of cortisol metabolites and larger than usual amounts of other steroid metabolites are excreted in urine of these patients it was possible to measure the very low CPR's of 62 and 600 nmoles cortisol/day secreted. The results

obtained were 2 to 10 times lower than those previously reported in other children with 17α-hydroxylase deficiency where radioactively labelled cortisol was used. This application illustrated that the presence of 1.8 μg of THE in the urine sample (111ml) was sufficient for measurement of the isotope dilution by GC/MS.

These studies have shown that an accurate, sensitive, specific, precise and reproducible method for measurement of the urinary cortisol production rate using [1,2,3,4-^{13}C] cortisol, has been developed.