A radioimmunoassay for tauro-β-muricholic acid suitable for use with isolated rat liver cells

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A new radioimmunoassay which can be used to measure the amounts of tauro-β-muricholic acid produced by isolated rat hepatocytes in vitro is described. Cross reactivities of other bile acids known to be present in rat liver with the antiserum used in the assay were not sufficient to interfere with the measurement of tauro-β-muricholic acid. Exogenous taurochenodeoxycholic acid was metabolised by isolated rat hepatocytes concurrently with the appearance of tauro-β-muricholic acid in the cell.

Radioimmunoassay *Tauro-β-muricholic acid* *Taurochenodeoxycholic acid* *Bile acid* *Isolated hepatocyte* *Rat liver*

1. INTRODUCTION

Isolated rat hepatocytes have been shown to synthesise cholic and chenodeoxycholic acids [1–3] and are widely used to study bile acid synthesis. Glycochenodeoxycholic acid, however, is rapidly metabolised by the cells to more polar products [4]. Chenodeoxycholic acid is known to be converted to α- and β-muricholic acid in rat liver [5,6], and it has been recently reported that β-muricholic acid is synthesised in isolated rat hepatocytes [3]. The synthesis of chenodeoxycholic acid in isolated rat liver cells, therefore, cannot be studied unless its conversion to the muricholic acids is taken into account. This paper describes a new radioimmunoassay for tauro-β-muricholic acid that can be used to measure specifically the amounts of this bile acid produced by rat hepatocytes in vitro.

2. MATERIALS AND METHODS

2.1. *Chemicals*

Collagenase type II was obtained from Sigma (London). Bile acids were removed from bovine serum albumin fraction V powder as in [2]. α- and β-muricholic acids were synthesised as in [7] and tauro- and glyco-conjugates of the two bile acids were prepared as in [8]. The unconjugated and conjugated bile acids were purified by repeated thin-layer chromatography in solvent systems consisting of toluene–isopropanol–acetic acid, 30:10:1 and ethyl acetate–methanol–acetic acid–water, 35:12:2:2, respectively [9,10]. The chromatograms were prepared before application of the samples and the bile acids were eluted from the silica gel as recommended in [11].

Conjugated and unconjugated α- and β-muricholic acids were identified by their Rf values relative to a β-muricholic acid standard (Steraloids, Wilton NH) or to tauro- or glycocholic acid [9,10] and by their colour reaction with anisaldehyde [12]. Solutions of both unconjugated and conjugated α- and β-muricholic acids were standardised with 3α-hydroxysteroid dehydrogenase (Sterognost 3α Flu, Nyegaard, Oslo) using taurocholic acid as a standard.

2.2. *Cell preparation*

Female rats of the Wistar strain (150–250 g) were maintained on a pellet diet (Oxoid 86).

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Hepatocytes were prepared [13], and incubated as in [2]. Cell viability was 85% as determined by trypan blue exclusion. Taurochenodeoxycholic acid was added to the incubations in a small volume of the incubation buffer. Duplicate cell samples were taken at the appropriate times and prepared for radioimmunoassay as in [2]. The protein content of the cell samples was determined as in [14].

2.3. *Radioimmunoassay*

The conjugated chenodeoxycholic acid content of the cells was measured by radioimmunoassay as in [15].

Antiserum was raised in rabbits to β-muricholic acid–bovine serum albumin immunogen (10:1 molar ratio) as in [16]. This antiserum was used in conjunction with a β-muricholic acid–histamine ligand prepared by the mixed anhydride method [16] and purified and characterised as in [15]. The β-muricholic acid–histamine ligand was labelled with ^{125}I as in [15]. The radioimmunoassay of tauro-β-muricholic acid was performed as for conjugated cholic acid [15], but using a 2-h incubation, and a sample volume of 10 μl in a final volume of 210 μl.

3. RESULTS AND DISCUSSION

The optimal dilution of the antiserum required to make the detection limit of the assay most sensitive [15] was found to be 1:2000. At this titre the minimum detection limit, defined as the mass of tauro-β-muricholic acid, which gives a response within 2.5 × standard deviation (SD) below the zero standard, was 0.11 ± 0.03 pmol (mean ± SD from 6 determinations).

The intra-assay precision of the assay is shown in the precision profile derived from 7 consecutive assays (fig.1). The coefficient of variation was 10% over 40–700 nmol/l. The inter-assay precision was assessed by repeated analysis of 2 pools of tauro-β-muricholic acid. At 100 nmol/l and 400 nmol/l the inter-assay coefficients of variation were 14% (9 consecutive assays) and 6% (15 consecutive assays), respectively.

The relative percentage cross reactivities of the antiserum with other bile acids compared to tauro-β-muricholic acid are shown in table 1. As in [16],

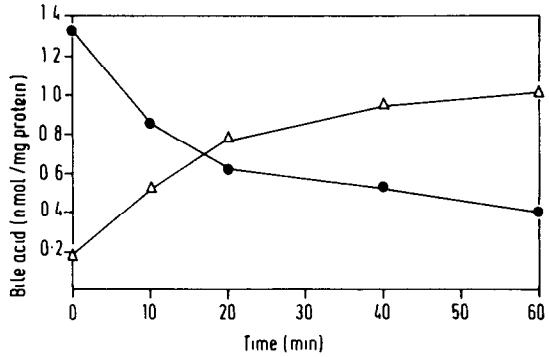

Fig.1. Precision profile for the radioimmunoassay of tauro-β-muricholic acid derived from 7 consecutive assays (mean ± SD).

Table 1

Relative cross reactivities (%) of various bile acids with the antiserum. Tauro-β-muricholic acid being assigned a 100% cross reactivity

Tauro-β-muricholic acid	100	Cholic acid	<0.001
Glyco-β-muricholic acid	20	Taurochenodeoxycholic acid	0.006
β-Muricholic acid	20	Glycochenodeoxycholic acid	0.006
Tauro-α-muricholic acid	2.0	Chenodeoxycholic acid	0.006
Glyco-α-muricholic acid	1.0	Taurodeoxycholic acid	<0.001
α-Muricholic acid	1.0	Glycodeoxycholic acid	<0.001
Ursodeoxycholic acid	0.1	Deoxycholic acid	<0.001
Hyodeoxycholic acid	<0.01	Taurolithocholic acid	0.04
Hyocholic acid	<0.01	Glycolithocholic acid	0.04
Taurocholic acid	<0.001	Lithocholic acid	0.01
Glycocholic acid	<0.001		

injection of an unconjugated bile acid immunogen produced an antiserum specific for the taurine conjugate. Cross reactivities with the majority of bile acids likely to be present in rat liver were insignificant (< 0.04%). Conjugated and unconjugated α-muricholic acids, however, showed cross reactivities of 1–2% and that of ursodeoxycholic acid was 0.1%. β-Muricholic acid has been reported to represent 34.8% of the total bile acids in rat liver, while α-muricholic acid represents 2.6% [17]. The concentration of ursodeoxycholic acid found in rat liver is also very much lower than that of β-muricholic acid [17,18]. These bile acids, therefore, are not present in the liver in sufficient quantities to interfere with the measurement of tauro-β-muricholic acid by this radioimmunoassay.

Fig.2 shows the metabolism of taurochenodeoxycholic acid and the synthesis of tauro-β-muricholic acid as measured by the radioimmunoassay in isolated hepatocytes when 10 μM taurochenodeoxycholic acid was added to the incubations. The disappearance of taurochenodeoxycholic acid coincided with the appearance of tauro-β-muricholic acid in the cells. After 1 h about 80% of the metabolism of taurochenodeoxycholic acid was accounted for by tauro-β-muricholic acid. These results suggest that the assay measures a product formed in isolated hepatocytes from taurochenodeoxycholic acid. The radioimmunoassay is sufficiently specific, precise and sensitive to measure the amount of tauro-β-muricholic acid produced by isolated hepatocytes and should be a valuable tool in the study of the synthesis of bile acids by rat liver cells in vitro.

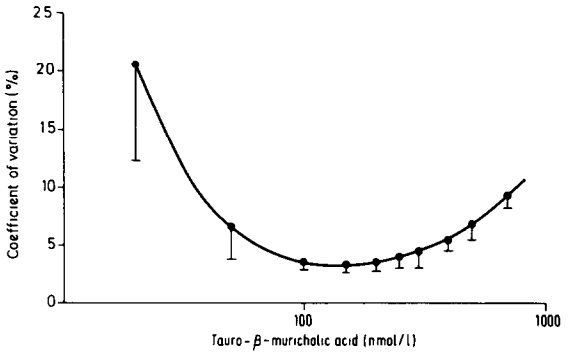

Fig.2. Conversion of taurochenodeoxycholic acid to tauro-β-muricholic acid by isolated hepatocytes. Taurochenodeoxycholic acid (●—●); tauro-β-muricholic acid (△—△).

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