Thyroid hormone deiodination in the domestic cat

D J Foster¹, K L Thoday¹ and G J Beckett²

¹The University of Edinburgh, Department of Veterinary Clinical Studies, The Hospital for Small Animals, Easter Bush, Roslin, Midlothian EH25 9RG, UK

²The University of Edinburgh, Department of Clinical Biochemistry, Royal Infirmary of Edinburgh, Lauriston Place, Edinburgh EH3 9YW, UK

(Requests for offprints should be addressed to G J Beckett)

ABSTRACT

We have investigated thyroid hormone deiodination in the liver, kidney and thyroid of the domestic cat. Affinity labelling with ¹²⁵I-bromoacetyl reverse T₃ (¹²⁵I-BrAc-rT₃) demonstrated that liver and kidney, but not the thyroid, express type I iodothyronine deiodinase (IDI), results that were confirmed by measuring the activity of the IDI using ¹²⁵I-rT₃ and T₄ as substrate. Feline hepatic and renal IDI metabolised rT₃ at approximately 0.2% of the rate of rat hepatic IDI under identical assay conditions.

The *K_m* of the feline enzyme was at least 500-fold greater than that of rat IDI. However, feline and rat hepatic IDI metabolised T₄ at a similar rate and had similar *K_m* values (1.35 μM and 2.25 μM, respectively). This study demonstrates that cats and rats express IDI in the liver and kidney in similar concentrations; however, the feline enzyme appears unable to utilise rT₃ as a substrate under physiological conditions.

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INTRODUCTION

The thyroid gland provides the sole source of circulating thyroxine (T₄), which is regarded as a prohormone requiring 5'-deiodination to produce the metabolically active hormone, 3,5,3' triiodothyronine (T₃). Conversion of T₄ to T₃ by 5'-mono-deiodination in a number of tissues, including the liver and kidney, is an important source of plasma T₃ (Silva & Larsen 1986, Kohrle 1994, St Germain 1994). There are clear tissue differences in the source of T₃ that is bound to nuclear T₃ receptors: in brain, approximately 80% of T₃ residing on the nuclear receptors appears to arise from deiodination of T₄ within the tissue whereas, in contrast, in the kidney, approximately 90% of nuclear T₃ arises from plasma T₃ (Silva & Larsen 1986). Thyroxine may also undergo 5-deiodination in many tissues to yield the metabolically inactive molecule, 3,3',5' triiodothyronine (reverse T₃; rT₃). Deiodination of thyroid hormones thus plays a crucial role in the regulation of the T₃ supply to both tissue and blood (Kohrle 1994, St Germain 1994).

Deiodination of thyroxine and other iodothyronines is catalysed by the family of iodothyronine deiodinases (ID), and current evidence suggests that these enzymes provide an autoregulatory role

in many tissues to maintain intracellular T₃ in response to altered thyroidal secretion of thyroid hormones (St Germain & Croteau 1989, Kohrle 1994). In addition, the deiodinases appear to be of critical importance during development, ensuring there is a regulated and co-ordinated exposure of specific tissues to thyroid hormones (Mitchell *et al.* 1998, Richard *et al.* 1998). Three selenoenzymes are involved in the deiodination of thyroid hormones, namely iodothyronine deiodinase types I, II and III (IDI, IDII, IDIII). IDI is the major isoenzyme found in liver and kidney and this enzyme appears to provide the major proportion of T₃ found in plasma. IDI is also expressed in high concentrations in the thyroid of some species, including humans, dogs and rodents, but many animal species, including goat, cattle and sheep, fail to express the enzyme, or at least express it at very low levels within the gland (Beckett *et al.* 1993). IDI can catalyse both 5'- and 5-mono-deiodination to produce T₃ and rT₃, respectively, from T₄ (St Germain & Croteau 1989, Beckett & Arthur 1994). In rats and humans, IDI appears to show a substrate preference for rT₃, although it can readily act on T₄ (Visser *et al.* 1988).

IDII is not expressed by liver or kidney, but the enzyme appears to provide an important intracellular source of T₃ in many other tissues,

including the central nervous system, pituitary, thyroid and brown adipose tissue of some species including the rat (St Germain & Croteau 1989, Leonard & Kohrle 1996). IDII catalyses only 5'-deiodination and has T_4 as its preferred substrate. IDIII performs only 5-deiodination and acts on T_3 in preference to T_4 (Leonard & Kohrle 1996).

A number of methods have been used to assay IDI expression and activity. Expression of the enzyme is usually assessed with an affinity labelling technique using ^{125}I -bromoacetyl derivatives of rT_3 , T_3 or T_4 (Kohrle *et al.* 1990, Schoenmakers *et al.* 1992). Activity is measured by assessing the ability of IDI and IDII to metabolise T_4 or rT_3 in the presence of an active thiol agent such as dithiothreitol (DTT) (Leonard & Rosenberg 1978, Visser & Overmeeren-Kaptein 1981, Moreno *et al.* 1994). IDI and IDII show very different sensitivities to inhibition by the addition of propylthiouracil (PTU) and gold-thioglucose (GTG). IDI activity with rT_3 or T_4 as substrate is readily inhibited by both these agents (Santini *et al.* 1992, Beckett & Arthur 1994), whereas IDII is relatively resistant to inhibition by PTU or GTG (St Germain 1994). These inhibitors are thus often used to indicate which isoenzyme is expressed by tissues.

Previous studies have suggested that IDI has similar properties in most species investigated. Apart from canine IDI, the main differences reported have been confined to small variations in molecular mass (Schoenmakers *et al.* 1992), differences in turnover number of approximately 10-fold between species (Santini *et al.* 1992, Schoenmakers *et al.* 1992) and differences in sensitivity to inhibition by GTG (Santini *et al.* 1992). However, there have been reported clear species differences concerning the degree of expression of IDI by the thyroid (Beckett *et al.* 1993, Beech *et al.* 1993).

Feline hyperthyroidism, resulting almost exclusively from hyperfunctional adenomas, is the most common endocrine disease in the cat (Thoday *et al.* 1984). Little is known regarding the pathogenesis of the disease and, at a fundamental level, very little is known regarding thyroid hormone metabolism, and in particular, deiodination, in this animal species. The present study was undertaken to identify the characteristics of thyroid hormone deiodination in the thyroid, liver and kidney of the domestic cat.

MATERIALS AND METHODS

Animal tissues

Tissues (liver, kidney and thyroid) were obtained from five cats immediately after euthanasia by a lethal dose of intravenous barbiturate, placed in

liquid nitrogen and stored at -80°C until assays were carried out. All cats were of mixed breeding and between the ages of 2 and 16 years. They were subject to euthanasia because of diseases that were not affecting the organs studied (two cats had upper respiratory tract disease; three had gastrointestinal disease). Rat tissues were obtained and stored in a similar manner.

Preparation of tissue homogenates, cytosols and microsomal fractions

Portions of tissues were homogenised in 100 mM KH_2PO_4 /1 mM EDTA buffer pH 7.4 (IDI assay buffer), at 20% w/v, and centrifuged at 1500 *g* for 10 min at 5°C . The supernatant was removed and used immediately for the assay of IDI activity. A microsomal fraction of tissue was also prepared by collecting a portion of the post-1500 *g* supernatant and centrifuging it at 12 000 *g* for 20 min, the resulting supernatant then being centrifuged further at 105 000 *g* for 60 min at 5°C . The microsomal pellet was resuspended and washed once in IDI assay buffer and recentrifuged at 105 000 *g*. The washed microsomal pellet was resuspended in IDI assay buffer to a protein concentration of 1 mg/ml.

Protein determinations

Protein concentrations were determined by the method described by Bradford (1976) using bovine serum albumin (BSA) as standard.

Assay of deiodinase activity using ^{125}I - rT_3 as substrate

The assay procedure was based on that of Sawada *et al.* (1986). Before use, ^{125}I - rT_3 (NEN biochemicals, Hounslow, Middx, UK; 9250 KBq/1.25 ml, 27.8–46.3 MBq/ μg) was purified as follows. Free iodine-125 was removed by passing the tracer down a 2 ml Sephadex G25 column in IDI assay buffer. The column was washed with 20 ml IDI assay buffer to remove contaminating iodine-125 and the ^{125}I - rT_3 was eluted with a 1% (w/v) solution of BSA prepared in IDI assay buffer.

Tissue homogenates (200 μl ; $n=5$, post-1500 *g* centrifugation) in duplicate were incubated with 100 μl purified ^{125}I - rT_3 at a final activity of 0.5 $\mu\text{Ci/ml}$ in the presence of 20 mM DTT and a final rT_3 concentration of 6 nM. The final volume of the incubation mix was 900 μl . For kinetic experiments, rT_3 was added in concentrations spanning the range 5 nM to 500 μM . Samples were incubated at 37°C for the times indicated in the

results section, and the reaction was stopped by the addition of 0.4 ml human sera, followed by 0.4 ml 40% trichloroacetic acid. After centrifugation at 1500 *g* for 10 min, 0.8 ml supernatant were removed and counted together with tubes containing the remaining supernatant and pellet. The percentage of iodine-125 released was calculated and used to determine the mass of rT_3 that had been metabolised.

Effects of PTU and GTG on deiodinase activity

The inhibition of IDI in activity assays was investigated by the addition of 1 mM PTU or 12 nM GTG (final concentration) as described previously (Berry *et al.* 1991, Santini *et al.* 1992).

Assay of IDI activity in tissue homogenates using T_4 as substrate

This was performed as described previously (Beckett *et al.* 1987). A solution of T_4 (56 μ M) was prepared in 0.25 M sucrose/0.05 M Tris/1 mM EDTA buffer pH 7.4 containing 3% BSA to ensure that the T_4 remained in solution. Tissue homogenates (2 ml) prepared as described above were then preincubated for 10 min at 37 °C and T_4 (final concentration 5.1 μ M; 200 μ l) and DTT (20 mM final concentration) were added to start the reaction. At designated time points, 200 μ l samples were removed in duplicate into 400 μ l ethanol and centrifuged at 1500 *g* for 5 min. The concentration of T_3 was then determined by immunoassay using a Vitros Eci immunoassay analyser (Ortho Clinical Diagnostics, Rochester, NY, USA).

Affinity labelling of IDI in microsomal liver fraction

^{125} I-*N*-bromoacetyl- rT_3 (^{125} I-BrAc- rT_3) was prepared as described previously (Schoenmakers *et al.* 1992). Two hundred microlitres ^{125} I- rT_3 were evaporated to dryness under a stream of dry nitrogen and 20 μ l 1.5 mg/ml bromoacetic acid *N*-hydroxysuccinamide ester in dimethylformamide were added to the dried tracer. To this, 5 μ l 10% (v/v) triethylamine in dimethylformamide was subsequently added and the mixture left at room temperature for 50 min. The iodinated affinity label was then purified by passing it through an LH20 Sephadex column (2 ml) equilibrated with 0.1 M HCl. The label was eluted with 95% ethanol and stored at -20 °C until used (within 7 days).

Microsomal fractions isolated from tissues as described above were diluted to a final concen-

tration of 1 mg/ml protein in 50 mM Tris HCl, 3 mM EDTA, 3 mM DTT. Microsomes (50 μ l; 50 μ g protein) were then added to 0.6 μ Ci ^{125} I-BrAc- rT_3 affinity label, which had been evaporated to dryness under dry nitrogen. The reaction was allowed to proceed for 15 min.

For experiments designed to study the effects of PTU and GTG on IDI affinity labelling, either 1 mM PTU or 40 nM GTG (Santini *et al.* 1992) were added to each test before addition of the affinity label. The effects of rT_3 and PTU on affinity labelling were also studied with the addition of 6 nM rT_3 together with 1 mM PTU. Samples were then diluted 2:1 with 'boiling mix' consisting of SDS (35 mM), glycerol (1.4 mM), 2-mercaptoethanol (0.3 mM) and bromophenol blue (15 mM), and then heated for 10 min at 90 °C.

After affinity labelling, samples were subjected to SDS polyacrylamide gel electrophoresis using a 12% (w/v) gel, and the ^{125} I-labelled proteins visualised using autoradiography.

Determination of deiodination kinetics of rT_3 and T_4 for IDI

K_m and V_{max} were determined by construction of Lineweaver-Burke plots. Final concentrations of rT_3 and T_4 ranged from 100 nM to 50 μ M and 5 nM to 5.1 μ M respectively.

Serum rT_3 concentrations

A commercial rT_3 RIA kit (Biostat Diagnostics, Stockport, Cheshire, UK) was used to measure the rT_3 concentrations in serum from 10 euthyroid cats and 10 euthyroid rats. The Mann-Whitney *U* test was used to test for significant difference between the rT_3 concentrations in cats and rats.

RESULTS

IDI activity in tissue homogenates and microsomal fractions using rT_3 and T_4 as substrates

^{125} I- rT_3 as substrate

When ^{125} I- rT_3 (6 nM) was used as substrate, feline liver and kidney homogenates showed approximately 0.2% of the deiodinase activity found in rat liver (Table 1). Because of the low deiodinase activity found in feline liver and kidney, it was necessary to use an incubation period of between 4 and 24 h whereas, using rat liver, an incubation period of only 5 min with a 1:10 dilution of homogenate was required. In each case,

TABLE 1. IDI activity in tissue homogenates (*n*=5) with rT₃ (6 nM) and T₄ (5·1 μM) as substrates

	Deiodinase activity	
	rT ₃ (fmol/mg per min)	T ₄ (nmol/mg per min)
Rat liver	23800 ± 990	3·71 ± 0·49
Rat liver plus PTU (1 mM)	ND	0·382 ± 0·003
Rat liver plus GTG (12 nM)	ND	0·02 ± 0·03
Cat liver	42·33 ± 7·16	3·61 ± 0·06
Cat liver plus PTU (1 mM)	0·07 ± 0·06	0·002 ± 0·01
Cat liver plus GTG (12 nM)	0·05 ± 0·04	ND
Cat kidney	68·79 ± 6·26	—
Cat thyroid (2 euthyroid, 3 thyrotoxic)	ND	—

ND, not detectable.

deiodination was found to proceed at a linear rate. No deiodinase activity could be found in thyroid homogenates from two euthyroid and three thyrotoxic cats, even when the incubation periods were as long as 48 h (Table 1). PTU (1 mM) and GTG (12 nM) completely inhibited the deiodinase activity in rat liver homogenates and caused a reduction in activity to approximately 0·2% of the basal activity in cat liver (Table 1).

T₄ as substrate

When T₄ (5·1 μM) was used as substrate, the deiodinase activity in rat and feline liver was not significantly different (Table 1). PTU and GTG markedly inhibited deiodination in cat and rat liver, such that less than 10% of the initial activity remained.

Kinetic studies

The kinetic data for rat and feline liver homogenates for rT₃ and T₄ are shown in Table 2. The *K_m* for rat and cat liver using rT₃ as substrate were markedly different, the *K_m* for cat liver using rT₃ as a substrate being at least 500-fold greater than that for rat liver. The *K_m* values using rT₃ in cat liver

homogenates could only be approximated, as it was impossible to obtain rT₃ in solution at concentrations greater than 50 μM. Thus only data points with substrate concentrations well below the *K_m* of the enzyme could be used. With T₄ as substrate, the *K_m* and *V_{max}* of the enzymes in rat and feline liver homogenates were very similar.

¹²⁵I-BrAc-rT₃ affinity labelling

In rat liver microsomes (*n*=4), affinity labelling with ¹²⁵I-BrAc-rT₃ demonstrated an ¹²⁵I-labelled band with molecular mass 29·1 ± 0·9 kDa. This labelled protein has previously been characterised as IDI in this tissue. The labelling of this protein was prevented in the presence of PTU and GTG (Figs 1, 2). Similarly, in feline liver and kidney microsomes, a ¹²⁵I-labelled band was found with molecular mass 26·2 ± 0·3 kDa (Fig. 1) and 27·2 ± 0·1 kDa (Fig. 2) respectively. As in rat liver, the labelling of this protein was prevented by the addition of PTU and GTG. The affinity-labelled proteins in cat liver and cat kidney showed a labelling intensity similar to that of the 29 kDa labelled protein found in rat liver.

TABLE 2. Kinetic data for rat and feline liver homogenates (*n*=3), with rT₃ or T₄ as substrate. Values are medians (range)

	rT ₃	T ₄
Rat		
<i>K_m</i> (μM)	0·20 (0·16–0·40)	2·25 (0·54–3·06)
<i>V_{max}</i> (pmol/mg per min)	100·00 (12·50–100·00)	0·625 (0·18–2·50)
Cat		
<i>K_m</i> (μM)	>100 (>100)	1·35 (0·44–4·20)
<i>V_{max}</i> (pmol/mg per min)	56·20 (25·00–142·80)	0·25 (0·07–0·34)

FIGURE 1. Affinity labelling of rat liver and cat liver (A) and kidney (B) microsomes with ^{125}I -BrAc- rT_3 in the presence of PTU and rT_3 . Lanes: 1, rat liver microsomes; 2, rat liver microsomes with 1 mM PTU; 3, rat liver microsomes with 1 mM PTU+6 nM rT_3 ; 4, 7, 10, cat liver/kidney microsomes; 5, 8, 11, cat liver/kidney microsomes with 1 mM PTU; 6, 9, 12, cat liver/kidney microsomes with 1 mM PTU+6 nM rT_3 .

Serum rT_3 concentration in euthyroid cats

The mean $\pm$ s.d. serum rT_3 concentration in cats was 164 ± 57 pM (range 86–244 pM). The mean serum rT_3 concentration in rats was 330 ± 40 pM (range 267–371 pM). The serum rT_3 concentration in cats was significantly lower than that in rats ($P=0.001$).

DISCUSSION

The iodothyronine deiodinases play a crucial role in regulating thyroid hormone metabolism in many tissues. The expression of the selenoenzyme IDI has been studied extensively in humans and rats, and such work has suggested that this enzyme

FIGURE 2. Affinity labelling of rat liver and cat liver (A) and kidney (B) microsomes with ^{125}I -BrAc- rT_3 in the presence of GTG. Lanes: 1, rat liver microsomes; 2, rat liver microsomes with 40 nM GTG; 3, 5, 7, cat liver/kidney microsomes; 4, 6, 8, cat liver/kidney microsomes with 40 nM GTG.

provides a major source of plasma T_3 . However, the observation that the preferred substrate for IDI in these species is rT_3 and not T_4 has led some to suggest that a major role of IDI may be to metabolise rT_3 (Visser *et al.* 1988). More limited studies of IDI have been reported in other species, but the use of affinity labelling with ^{125}I -BrAc- rT_3 has suggested that the hepatic expression of IDI is greatest in the rat and dog, with humans, mice, rabbits, cows, pigs, sheep, goats, chickens and ducks having a similar level of expression (Schoenmakers *et al.* 1992). Activity measurements of IDI with rT_3 as substrate have shown that there are also clear species differences in the turnover number of hepatic IDI. Rats, rabbits and goats have the greatest turnover number, whereas dogs have the lowest, which is approximately 10 times lower than that found in the rat. No studies on feline IDI have been reported.

We have shown that, although certain characteristics of hepatic and renal IDI are similar in the cat and rat, the substrate specificity of the enzyme between these species is clearly different. Affinity labelling of hepatic microsomes using ^{125}I -BrAc- rT_3 demonstrated that the feline enzyme was expressed at levels similar to those found in the rat, although the feline enzyme had a molecular mass that was approximately 3 kDa smaller than that of the rat enzyme. Such modest differences in the molecular mass of IDI have been reported previously between many species (Schoenmakers *et al.* 1992). The sensitivity of the rat and feline enzyme to PTU and GTG was also similar, in that both these agents prevented affinity labelling of IDI by ^{125}I -BrAc- rT_3 . The feline kidney also expressed levels of IDI similar to those found in feline liver, as assessed by affinity labelling.

When the activity of IDI was compared in rat and feline liver homogenates using T_4 as substrate, we found that the relative activities of the enzyme were not significantly different between the species (Table 1). More detailed kinetic analysis showed that the K_m for T_4 of the rat enzyme was similar to that of the feline enzyme. The $V_{\max}$ of the enzyme in cat and rat liver was also not significantly different, which was in agreement with the affinity labelling data, which also suggested that similar masses of enzyme were present in each of the tissues.

The use of rT_3 as substrate revealed marked differences in the ability of rat and feline IDI to metabolise this substrate. The rate at which feline liver homogenates metabolised rT_3 under the standard assay conditions using 6 nM rT_3 was approximately 0.2% of the reaction rate found in rat homogenate. Similar differences in the reaction rate

between the rat and the cat were also demonstrated when washed microsomes were used (data not shown), suggesting that there was no inhibitory component in the cytosolic fraction of the cat.

With rT_3 as substrate, it was difficult to determine a precise and accurate estimate of K_m in feline liver. This was because the K_m for this substrate was estimated to be much greater than the greatest concentration of rT_3 that could be obtained in solution. However, at a conservative estimate, the K_m of the feline enzyme was at least 500-fold greater than that of the enzyme in rat liver.

IDI in humans, dogs, mice, chicks and tilapia have been cloned, illustrating several important features (St Germain & Galton 1997). Site-directed mutagenesis studies have shown that the selenocysteine residue is critical for the catabolism of thyroid hormones (Berry *et al.* 1991b), and insertion of this selenocysteine requires the presence of a selenocysteine insertion sequence (SECIS) in the 3' untranslated region of the mRNA (Berry *et al.* 1991a). In addition to the selenocysteine residue, there are two essential histidines at amino acid residues 158 and 174 in rats and humans (Berry 1992) and the phenylalanine at residue 65 of the human IDI is of critical importance in the ability of the enzyme to catalyse the 5'-deiodination of rT_3 and di-iodothyronine (T_2) sulphate (Toyoda *et al.* 1997). The expression of this phenylalanine at residue 65 is highly conserved, except in the dog, in which a leucine is substituted. This substitution at amino acid 65 makes canine IDI very inefficient in catalysing 5'-deiodination of rT_3 and T_2 sulphate (St Germain & Galton 1997). Our observation that cat IDI was even less efficient than the dog at deiodinating rT_3 suggests that, in the cat, similar changes in amino acid sequence may be occurring. However, the fact that the ^{125}I -bromoacetyl derivative of rT_3 was able to bind to the feline enzyme suggests that any such changes in the amino acid sequence in the feline IDI have a greater effect on the ability of IDI to deiodinate rT_3 as compared with the ability of the enzyme to bind rT_3 . The IDI of tilapia is relatively resistant to inhibition by PTU and GTG, although the structural characteristics responsible for this difference are unknown (St Germain & Galton 1997). However, we have found that IDI in the cat can be inhibited by PTU and GTG, as has been found in other mammals.

Our data suggest that, in the cat as in the rat, a major source of circulating T_3 is likely to arise from hepatic deiodination of T_4 . Indeed, the mean $\pm$ s.d. concentrations of serum T_3 in the rat (1.5 ± 0.14 nM; Beckett *et al.* 1987) and the cat (0.69 ± 0.29 nM; Thoday *et al.* 1984) are similar. Our data further suggest that hepatic metabolism of rT_3

by IDI cannot proceed at a significant rate in the cat. However, as the serum concentrations of rT_3 were significantly lower in the cat than in the rat, this suggests either that the major fraction of serum rT_3 does not arise from the liver in the cat or, alternatively, that metabolism of rT_3 must occur by alternative pathways. A clear candidate for such a pathway is prior sulphation, with subsequent deiodination of sulphated rT_3 (Rutgers *et al.* 1991).

In rats and humans, sulphation of thyroid hormones is considered to be an important regulator of thyroid hormone metabolism. In these species, some sulphated iodothyronines are deiodinated at a faster rate by IDI than the corresponding non-sulphated iodothyronines (Rutgers *et al.* 1991). This has led to the suggestion that a balance between the expression of the sulphotransferases, sulphatases and deiodinases involved in thyroid hormone metabolism may be important in the regulation of thyroid hormone expression during foetal development (Richard *et al.* 1998). We have not been able to examine the ability of feline IDI to metabolise rT_3 sulphate or the ability of feline liver to synthesise rT_3 sulphate, but clearly this would be of interest. Another possibility is that rT_3 is metabolised in the cat by pathways that do not involve deiodination. Such pathways may involve metabolism of the side chain, ether-bond cleavage, glucuronidation (slow in the cat) or methylation (Beckett & Arthur 1994). Further studies are required to clarify these issues.

Using affinity labelling and activity assays, we have shown that IDI is not expressed in the thyroid of the cat. In this regard, the cat is similar to goat, cattle, sheep, rabbits, pigs, llama and deer. In contrast, thyroidal IDI is strongly expressed in humans, dogs, rats, mice and guinea pigs and, in these species, expression of the enzyme appears to be regulated through activation of the cAMP cascade, through changes in adenylate cyclase activity (Tang *et al.* 1994). When thyroidal IDI is expressed, it appears to provide a significant source of circulating T_3 , particularly when activation of the thyroid-stimulating hormone (TSH) receptor is initiated, such as occurs in hypothyroidism via TSH or in Graves' disease through thyroid-stimulating immunoglobulins. Clearly, therefore, in the cat, thyroidal deiodination cannot be involved in an adaptive response to hypothyroidism. Spontaneous adult-onset hypothyroidism in the cat is extremely rare (Rand *et al.* 1993). Similarly, thyroidal deiodination of T_4 cannot be a source of T_3 in the hyperthyroid cat, as we have shown that, in such animals, IDI is not expressed in the thyroid. In these hyperthyroid cats, the increased serum T_3

concentrations must arise from peripheral conversion of T_4 by IDI, and also possibly through increased *de novo* synthesis of T_3 on thyroglobulin in the thyroid.

In conclusion, our data suggest that, in the cat, plasma T_3 originates largely from the deiodination of T_4 in the liver and kidney, but clearance of rT_3 must proceed by a metabolic route that is not dependent on deiodination of the unsulphated molecule.

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