Preferential release of tri-iodothyronine following stimulation by thyrotrophin or thyrotrophin-releasing hormone in sheep of different ages

R. Peeters, N. Buys†, D. Vanmontfort, J. Van Isterdael*, E. Decuyper and E. R. Kühn†

Laboratory for Physiology of Domestic Animals, Kardinaal Mercierlaan 92, B-3001 Heverlee, Catholic University of Leuven, Leuven, Belgium
*Zootechnical Centre, Bijzondere weg 12, B-3042 Lovenjoel, Catholic University of Leuven, Leuven, Belgium
†Laboratory of Comparative Endocrinology, Naamsestraat 61, B-3000 Leuven, Catholic University of Leuven, Leuven, Belgium

(Requests for offprints should be addressed to E. R. Kühn)

REVISED MANUSCRIPT RECEIVED 9 September 1991

ABSTRACT

The influence of TRH and TSH injections on plasma concentrations of tri-iodothyronine (T3) and thyroxine (T4) was investigated in neonatal (injection within 0.5 h after delivery) and growing lambs and in normal, pregnant and lactating adult ewes (all 2 years old and originating from Suffolk, Milksheep and Texal crossbreeds). Neonatal lambs had higher levels of T3, T4 and GH compared with all other groups, whereas prolactin and TSH were higher in lactating ewes. In all animals, injections of TRH increased plasma concentrations of prolactin and TSH after 15 min but not of GH at any time. Small increases in T3 and T4 were observed in neonatal lambs, without any effect on the T3 and T4 ratio, after prolactin administration, whereas prolactin did not influence plasma concentrations of T3 or T4 in all other experimental groups. Similar results for thyroid hormones were obtained after TRH or TSH injections. It was therefore concluded that the effects observed after TRH challenge were mediated by the release of TSH. With the possible exception of neonatal lambs, plasma concentrations of T3 after administration of TRH or TSH were always increased before those of T4; the increase in T3 occurred within 0.5–1 h compared with 2–4 h for T4 in all experimental groups. This resulted in an increased ratio of plasma T3 to T4 up to 4 h after injection. It is concluded that, in sheep, TRH and TSH preferentially release T3 from the thyroid gland probably by a stimulatory effect of TSH on the intrathyroidal conversion of T3 to T4.

Journal of Endocrinology (1992) 132, 93–100

INTRODUCTION

Peripheral deiodination of thyroxine (T4) to tri-iodothyronine (T3) is considered to be the last step in thyroid hormone synthesis in all vertebrates, whereas the thyroid gland is mainly involved in the production and secretion of T4 (Kühn, 1990). Thus, injection of thyrotrophin (TSH) in fish (Fontaine, 1969; Chan & Eales, 1976; Kühn, Huybrechts, Govaerts et al. 1986a; Byamungu, Corneillie, Mol et al. 1990), amphibians (Kühn, Darras & Verlinden, 1985; Jacobs, Govaerts, Vandorpe et al. 1988) and adult chickens (Kühn, Decuyper, Iqbal et al. 1988; Kühn, Herremans, Dewil et al. 1991) increases plasma concentrations of T4, without affecting circulating levels of T3. In quail (McNabb, Stanton, Weirich & Hughes, 1984) and chick embryos (Kühn et al., 1988), however, TSH increases plasma concentrations of both T3 and T4. In quail there is little change in the plasma T3 to T4 ratio after TSH stimulation, whereas in the chicken this ratio is decreased.

Mammals may differ in this regard, since it has been demonstrated in rats (Erickson, Cavaliere & Rosenberg, 1982), dogs (Wu, 1983), mice (Wu, Reggio & Florsheim, 1985) and even man (Ishii, Inada, Tanaka et al. 1983) that TSH stimulates the intrathyroidal conversion of T4 to T3.
Though it is firmly established that TSH (and TSH-releasing hormone; TRH) increases plasma concentrations of T₃ and T₄ in all mammalian species studied, evidence for a preferential release of T₃ is scarce. The aim of the present study was to investigate the influence of TRH and TSH on such preferential release of T₃ in sheep. Since thyroid physiology is continually changing between fetal and neonatal life (Klein, Oddie & Fisher, 1978) and lactation (Fraser & McNeilly, 1982) but apparently is unaffected by gestation (Buys, Peeters, De Clerck et al. 1990), this study was performed during different periods of a sheep's life span.

MATERIALS AND METHODS

All experiments were carried out on sheep originating from Suffolk, Milk sheep and Texel cross-breeds. Neonatal lambs were injected within 30 min of parturition. Experiments on growing lambs were performed on ±2-month-old animals and as adult sheep on 2-year-old ones. Injections into pregnant sheep were performed at 4 months of gestation. All hormones were dissolved in 0.5 ml saline (0.9% w/v NaCl) and injected into a jugular vein after an initial blood sample had been taken. Seven to ten animals were used in each experiment, and consecutive blood samples were taken at 15 and 30 min (only pregnant ewes) and 1, 2 and 4 h after injection.

The following hormones were used: TRH (UCB-Bioproducts, Brussels, Belgium), TSH (Sigma Chemical Co. Ltd, St Louis, MO, U.S.A.) of bovine origin and ovine GH and ovine prolactin (oGH; NIAMDD-oGH-14 and oPRL; NIAMDD-o-PRL-17, Bethesda, MD, U.S.A.). Blood samples were collected in heparinized tubes, centrifuged and stored at −20°C for radioimmunoassay (RIA). Assays of T₃ and T₄ were performed using kits from Wellcome (Beckenham, Kent, U.K.) and Abbott, Diagnostic Division (Antwerp, Belgium), except for pregnant ewes where the T₃ and T₄ concentrations in plasma were assayed using tracer obtained from Amersham International plc (Amersham, Bucks, U.K.), rabbit T₃ antiserum from Mallinckrodt (Dietzenbach, Germany) and a laboratory-raised rabbit T₄ antiserum. This T₄ antiserum had 0.16% cross-reactivity with T₃. All RIAs had good parallelism with plasma dilution curves, an intra-assay variability of <5% and an interassay variability of <10%.

The RIA of oPRL, oGH and oTSH were performed using the immunoreagents of the NIAMDD.

Statistical analyses of the results were performed by a t-test for paired data with a repeated measures analysis of variation (SAS, 1985) and the general linear models procedure.

RESULTS

Basal levels of hormones

Neonatal lambs had higher levels of T₃, T₄ and GH compared with growing lambs and adult ewes. The GH level declined rapidly during the experiment in neonatal lambs and reached, after 4 h, values which were comparable with those in growing and adult animals. Prolactin concentrations in lactating ewes were much higher (150 µg/l) compared with all other groups (10 µg/l). In lactating ewes the TSH levels (6 µg/l) were also higher than in other groups (1–2 µg/l).

In all experiments, injection of saline did not influence plasma hormone concentrations.

Effect of TRH in neonatal, growing and adult sheep

Injections of TRH increased plasma concentrations of prolactin and TSH after 15 min in neonatal and growing lambs and adult non-pregnant ewes (Fig. 1). In particular in neonatal and growing lambs, this was followed by a sharp fall in plasma prolactin but values remained elevated above the controls for the remainder of the 4 h sampling period, except for growing lambs where the increase lasted only up to 2 h. GH levels were not affected. In neonatal lambs, both T₃ and T₄ increased after 2 h and remained elevated after 4 h (Fig. 2). In growing animals this increase for both hormones was already present after 1 h. In adult ewes, T₃ concentrations were increased after 1 h, and T₄ concentrations after 2 h.

In neonatal and growing lambs the TRH challenge raised the T₃ to T₄ ratio after 1 h and 2–4 h respectively, but this increase was not very pronounced (P<0.05) (Fig. 2). In adult sheep this ratio was increased after 1 h (P<0.001) and remained elevated up to 4 h (P<0.05).

Effect of TSH in neonatal, growing and adult sheep

Injection of TSH into neonatal and growing lambs and adult ewes increased plasma T₃ levels after 1 h, and values remained elevated up to 4 h (Fig. 3). Increases in T₄, however, were found after 2 h in neonatal and adult animals, whereas in growing lambs a significantly higher T₄ concentration was present only after 4 h. A small (P<0.05) increase in the T₃ to T₄ ratio was seen after 1 h in neonatal lambs. This increase was more pronounced in growing and adult animals and lasted up to 2 h.

Effect of prolactin in neonatal, growing and adult sheep

Injection of 500 µg prolactin increased T₃ and T₄ after 1, 2 and 4 h in neonatal lambs (Fig. 4). Concentrations of T₄ remained unaffected after injection of 500 µg in growing lambs or of 750 µg in adult ewes. Only small
The effects of TRH and TSH injections on plasma concentrations of T₃ and T₄ are summarized in Tables 1 and 2.

In pregnant ewes, injection of 200μg TRH and 400μg TSH increased plasma concentrations of T₄ after 2 and 4 h respectively. Plasma T₃, however, was increased after 30 min and remained elevated up to 4 h with TRH and 2 h with TSH. The T₃ to T₄ ratio was increased 2 h after injection of TRH (P<0.001) and 15 and 30 min (P<0.05) and 1 h (P<0.01) and 2 h (P<0.05) after injection of TSH (Table 1).

In lactating ewes similar results to pregnant ewes were obtained with regard to the influence of 200μg of
TRH on plasma concentrations of T₃ and T₄. T₃ concentrations were increased after 1 h, and T₄ after 4 h. This resulted in an increased T₃ to T₄ ratio 1, 2 and 4 h after TRH injection. The TRH injections increased TSH and prolactin after 15 min and this rise was maintained up to 4 h (Table 2). GH, however, was not affected significantly by TRH but levels were variable compared with the levels of other hormones and there was nevertheless a two- to three-fold increase in mean value of GH level after TRH injections (Table 2).

Injections of GH (750 µg) (results not shown) and prolactin (750 µg) in lactating ewes did not influence circulating levels of T₃ and T₄.

**Effect of T₄ in pregnant ewes**

Injection of 400 µg T₄ in pregnant ewes increased plasma levels of T₄ significantly after 0·25, 0·5 and 1 h, but had no effect on plasma T₃ (Table 1).
<table>
<thead>
<tr>
<th>Treatment</th>
<th>T&lt;sub&gt;3&lt;/sub&gt; (nmol/l)</th>
<th>T&lt;sub&gt;4&lt;/sub&gt; (nmol/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Basal</td>
<td>15 min</td>
</tr>
<tr>
<td>Saline</td>
<td>1·607</td>
<td>1·613</td>
</tr>
<tr>
<td></td>
<td>±0·075</td>
<td>±0·061</td>
</tr>
<tr>
<td>T&lt;sub&gt;3&lt;/sub&gt;</td>
<td>1·753</td>
<td>1·662</td>
</tr>
<tr>
<td></td>
<td>±0·223</td>
<td>±0·083</td>
</tr>
<tr>
<td>TRH</td>
<td>1·960</td>
<td>2·120</td>
</tr>
<tr>
<td></td>
<td>±0·132</td>
<td>±0·412</td>
</tr>
<tr>
<td>TSH</td>
<td>1·67</td>
<td>1·869</td>
</tr>
<tr>
<td></td>
<td>±0·112</td>
<td>±0·131</td>
</tr>
</tbody>
</table>

*P < 0·05, **P < 0·01, ***P < 0·001, compared with preinjection values (paired data) (n = 7).
TABLE 2. Influence of an injection into lactating ewes of saline and 200 μg TRH on plasma concentrations of GH, prolactin, TSH (μg/l; means ± S.E.M.) and T4 and T3 (nmol/l; means ± S.E.M.)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Preinjection values</th>
<th>Postinjection values</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>15 min</td>
<td>30 min</td>
</tr>
<tr>
<td>GH</td>
<td>32±2 ±11±6</td>
<td>42±2 ±15±1</td>
</tr>
<tr>
<td>Prolactin</td>
<td>152±9 ±17±2</td>
<td>691±3 ±41±6**</td>
</tr>
<tr>
<td>TSH</td>
<td>6±60 ±0±54</td>
<td>13±69 ±1±83*</td>
</tr>
<tr>
<td>T3</td>
<td>54±18 ±6±56</td>
<td>54±18 ±5±79</td>
</tr>
<tr>
<td>T4</td>
<td>1±084 ±0±081</td>
<td>1±075 ±0±089</td>
</tr>
</tbody>
</table>

*P < 0.05, **P < 0.01 compared with preinjection values (paired data) (n = 10).

DISCUSSION

The present data indicate that plasma concentrations of T3 are increased in growing, adult pregnant, non-pregnant and lactating sheep between 0.5 and 1 h after injection of TRH or TSH, whereas a significant rise in T4 occurs only after 2–4 h. This results in a higher T3 to T4 ratio.

This preferential increase in T3 was not present in neonatal lambs, where both T3 and T4 were increased 2 h after TRH or TSH administration. This simultaneous release of both thyroid hormones has been observed previously in neonatal lambs after stimulation with TRH (Wallace, Nancarrow, Evison & Radford, 1979) or TSH (Davicco, Lefaivre & Barlet, 1982). The observed fall in plasma concentrations of T4 and increase in T3 which is observed before parturition (Fisher, Dussault, Sack & Chopra, 1977; Klein et al. 1978) therefore is probably not due to a stimulated intrathyroidal conversion of T4 into T3 but to an increased peripheral production. It is known that the T4 to T3 converting activity in liver and kidney homogenates increases progressively from fetus to the neonate (Wu, Klein, Chopra & Fisher, 1978).

The preferential increase in plasma concentrations of T3 in growing and adult sheep probably reflects the preferential secretion of T3 from the thyroid gland for the following reasons.

Firstly, TRH increased prolactin concentrations in the plasma of all the experimental groups, confirming the results from several other studies (Thomas, Jack, Manns & Nathanielsz, 1975; Fitzgerald, Evis & Cunningham, 1981). In the present study injection of prolactin may increase T3 and T4 but mainly in neonatal lambs and has no influence on the T3 to T4 ratio. It should be noted that this contradicts a previous observation where injection of 500 μg prolactin did not influence plasma concentration of thyroid hormones (Kühn, Van Osselaer, Siau et al. 1986c).

Secondly, it is possible that all T4 released from the thyroid by TRH or TSH is converted immediately into T3, resulting in a preferential increase in plasma concentration of T3. An injection of T4 in pregnant ewes, however, does not influence plasma levels of T3, which contradicts this suggestion.

Thirdly, in man it has been demonstrated that thyroidal secretion of T3 is greater in patients with hyperthyroidism than in euthyroid individuals. Injection of TSH in man preferentially increases plasma concentrations of T3 (Carpi, Bianchi, Zuchelli et al. 1979).

Fourthly, numerous reports indicate that, in several mammalian species, TRH stimulates the intrathyroidal conversion of T4 to T3 (see Introduction). TRH and TSH had similar effects on plasma concentrations of thyroid hormones in the present experiments. The effect of TRH is apparently mediated by TSH which is released within 15 min of injection.

Finally, it is unlikely that the observed effects are the result of a peripheral (hepatic) stimulated conversion of T4 to T3 by GH as demonstrated in several mammals, e.g. the rat (Kühn, Peeters & Pauwels, 1986b), cow (Peel, Frank, Bauman & Gorewit, 1983), neonatal lamb (Kühn et al. 1986c), goat (Iqbal, Cheema & Kühn, 1990) and man (Sato, Suzuki, Taketani et al. 1977). In our study, GH was not released after the TRH challenge, confirming results of Thomsett, Marti-Henneberg, Gluckman et al. 1980), with a possible exception of lactating ewes, although the two- to threefold rise in GH after TRH injection was not significant. The variation coefficient of the GH values 1 and 2 h after TRH, however, is greatly enhanced and may be an indication of a higher pulsatility. This may mask an increase being statistically significant by classical ANOVA, but nevertheless may be meaningful biologically since an increased pulsatility of GH often results in a higher biological activity. Therefore, a possible additional stimulatory effect of GH after a TRH challenge in lactating ewes may not yet be excluded.

Our results on sheep therefore suggest that the observed increases in plasma concentrations of T3 before T4, after a TSH or TRH challenge, are the
direct result of a stimulated intrathyroidal 5'-
monodeiodinase activity and increased T₃ secretion from the thyroid gland.

These results also suggest that in sheep, with the exception of the neonatal period, the thyroid may contribute to changes in circulating levels of T₃. It is difficult to speculate on mechanisms within the thyroid to achieve enhanced production of T₃. Since in-vitro experiments on dogs have indicated that the intrathyroidal 5'-monodeiodinase activity is stimulated by TSH, with increases in the maximum velocity (Vₘₐₓ) but no demonstrable change in apparent Km (Wu, 1983), it may be argued that T₃ is coming from but derived from preexisting T₄, rather than from newly synthesized poorly iodinated thyroglobulin. A preferential release of T₃ from the thyroid follicle cells into the circulation is another possibility which cannot yet be excluded.

ACKNOWLEDGEMENTS

The valuable technical assistance of F. Voets, L. Noterdaeme and W. Van Ham is gratefully appreciated.

REFERENCES


