Reprod. Nutr. Dévelop., 1987, **27** (2 B), 501-510.

Effects of TRH and GRF administration on GH, TSH, T4 and T3 secretion in the lamb

Chantal WRUTNIAK, G. CABELLO, J. CHARRIER (*), J.-P. DULOR (*), Monique BLANCHARD (*), B. BARENTON (*)

With the technical collaboration of Christiane FOUCHER and Veronique GARANDEL (*)

Laboratoire des Maladies Métaboliques, I.N.R.A., Theix, 63122 Ceyrat, France.
(*) *Station de Physiologie Animale, I.N.R.A.-E.N.S.A., 34060 Montpellier cedex, France.*

Summary. The effects of various amounts of thyrotropin-releasing hormone (TRH) injected subcutaneously or intravenously (alone or in combination with growth hormone-releasing factor : GRF 1-44) on growth hormone (GH), thyroid-stimulating hormone (TSH), thyroxine (T_4) and triiodothyronine (T_3) were studied in the plasma of 2-week, 2-month and 3-month old lambs.

1. After subcutaneous TRH administration, increases in plasma TSH, T_4 and T_3 levels were equivalent, whatever the amount of TRH used (1, 2, 5 or 10 µg/kg). These responses lasted longer after 5 and 10 µg/kg.
2. After intravenous TRH administration in 2-week old lambs, the maximal increase in plasma TSH levels occurred after the injection of 0.25 µg/kg. However, plasma T_4 and T_3 responses were not different, whatever the amount used. As previously, the amount of TRH affected the duration of these responses more than the magnitude of the pituitary-thyroid axis response.
3. Whatever the injection route, amount used or animal age, TRH alone did not increase GH secretion in lambs. However, it slightly delayed the GH response to GRF.
4. GRF did not affect the response of TSH and T_4 to TRH ; however it could inhibit T_3 increase.

In conclusion, in contrast to results obtained in calves by Hodate *et al.* (1985), TRH did not enhance GH secretion in lambs but, as expected, induced sharp increases in plasma thyroid hormone levels. Its classification as a « growth factor » is therefore questionable, at least in lambs.

Introduction.

The effects of thyrotropin-releasing hormone (TRH) on pituitary growth hormone (GH) release is still unclear in ruminants. A great number of data have been obtained in cattle. If bovine GH release from pituitary tissue in response to TRH *in vitro* is not constant (La Bella and Vivian, 1971 ; Smith and Convey, 1975), a constant elevation of plasma GH levels after TRH administration has been demonstrated *in vivo* in cattle (Convey *et al.*, 1973 ; Kesner *et al.*, 1977 ; Johke, 1978). More recently, Hodate *et al.* (1985) have confirmed these results in six-month old calves by additional data showing that simultaneous administration of growth hormone-releasing factor (GRF-44) and TRH has a synergistic effect on GH release.

However, studies on the influence of TRH on GH secretion in sheep are inconclusive (Davis, 1975 ; Davis *et al.*, 1976, 1977). The aim of the present study

was to observe the effects of various amounts of TRH injected subcutaneously or intravenously (alone or in combination with GRF-44) on plasma GH, thyroid-stimulating hormone (TSH), thyroxine (T_4) and triiodothyronine (T_3) levels in two-week, two-month and three-month old lambs.

Material and methods.

All the animals used in this work were Limousin × Romanov lambs. The effects of subcutaneous TRH injections were studied in 6 three-month old animals

TABLE 1

Experimental schedule of SC TRH injections in six Limousin × Romanov lambs (three-month old).

Day of treatment	Febr. 24th	Febr. 26th	Febr. 28th	March 3rd
Amount of TRH injected (μg/kg)	1	2	5	10
Mean Bodyweight (kg)	27.3 ± 1.2	28.5 ± 1.4	28.5 ± 1.5	29.2 ± 1.8

TABLE 2

Experimental schedule (Latin Square) *of IV TRH* (μg/kg) *administration in ten Limousin × Romanov lambs* (two-week old).

Day of treatment / N° animals		April 16th	April 18th	April 21st	April 23rd	April 25th
243	253	0	0.1	0.25	0.5	1
248	259	0.1	0.25	0.5	1	0
255	263	0.25	0.5	1	0	0.1
267	258	0.5	1	0	0.1	0.25
272	256	1	0	0.1	0.25	0.5
Mean bodyweight (kg)		4.48 ± 0.32	4.96 ± 0.31	5.93 ± 0.35	6.45 ± 0.36	6.87 ± 0.39

TABLE 3

Experimental schedule of IV injections in five Limousin × Romanov lambs (two-month old).

Day of Treatment	Jan. 22nd	Jan. 24th	Jan. 27th	Jan. 29th	Jan. 31st	Febr. 4th
Injections	TRH 1 μg/kg	TRH 1 μg/kg + GRF 0.2 μg/kg	GRF 0.2 μg/kg	GRF 1 μg/kg	TRH 1 μg/kg + GRF 1 μg/kg	Saline
Mean bodyweight (kg)	17.8 ± 0.9	17.8 ± 0.9	19.2 ± 0.8	19.6 ± 0.8	20.4 ± 0.8	21.4 ± 0.7

(table 1). Increasing amounts of TRH were administered intravenously in 10 (5 ♂ and 5 ♀) two-week old lambs (table 2), whereas TRH, GRF-44 and TRH + GRF-44 were injected intravenously in 5 two-month old animals (table 3).

The TRH (Protireline) was provided by « Laboratoires Roche » (Neuilly-sur-Seine, France) and the human GRF-44 was a gift of « Sanofi Recherche » (Montpellier, France).

Blood was taken by venipuncture into heparinized test tubes 10 min before, just prior to and 10, 20, 30, 45, 60, 90, 120, 180, 240 and 360 min after injection. The plasma was separated by centrifugation and stored at − 20 °C until assay.

The thyroxine and triiodothyronine were measured by specific radioimmunoassay as previously described (Cabello and Levieux, 1980). Ovine GH and TSH were also measured by RIA using a double antibody separation method ; reagents (antisera and cold hormones) were supplied by the National Hormone and Pituitary Program (NIADDK, Bethesda). The sensitivity of these assays was 0.5 ng/ml for GH and 0.05 ng/ml for TSH ; intra-assay variation was 5 and 8 %, respectively.

Statistical comparisons were made using the paired t-test.

Results.

Subcutaneous TRH injection in three-month old lambs (fig. 1). — As expected, plasma TSH, T_4 and T_3 levels rose significantly ($P < 0.001$) after s.c. TRH injection. Neither the maximal value of these hormones nor their increase (maximal value - basal value) was significantly affected by the amount of TRH given.

Whereas plasma TSH levels were unchanged from 30 to 180 min post-injection when amounts of 5 and 10 µg of TRH/kg were used, they decreased significantly over this period when 1 and 2 µg of TRH/kg were injected ($P < 0.001$).

The increase in plasma T_4 levels was significantly longer for amounts of 5 and 10 µg of TRH/kg (5.3 ± 0.4 h and 5.2 ± 0.5 h, respectively) than for amounts of 1 and 2 µg of TRH/kg (3.4 ± 0.4 h and 3.8 ± 0.2 h, respectively ; $P < 0.005$).

The increase in plasma T_3 levels was also significantly longer for amounts of 2, 5 and 10 µg of TRH/kg (2.7 ± 0.3 h, 3.0 ± 0.3 h and 3.2 ± 0.3 h, respectively) than for 1 µg of TRH/kg (1.7 ± 0.2 h ; $P < 0.005$).

Whatever the amount injected, TRH did not significantly increase plasma GH levels in three-month old lambs.

Intravenous TRH injection in two-week old lambs (fig. 2). — Intravenous saline injection did not significantly affect plasma TSH, T_4, T_3 and GH levels.

Plasma TSH levels rose significantly after TRH injection ($P < 0.001$). However, the maximal value and increase recorded were significantly lower for 0.1 µg of TRH/kg than for all higher amounts ($P < 0.005$). Moreover, at 60 min post-injection, plasma TSH levels were positively related to the amount of TRH injected ($P < 1$ %).

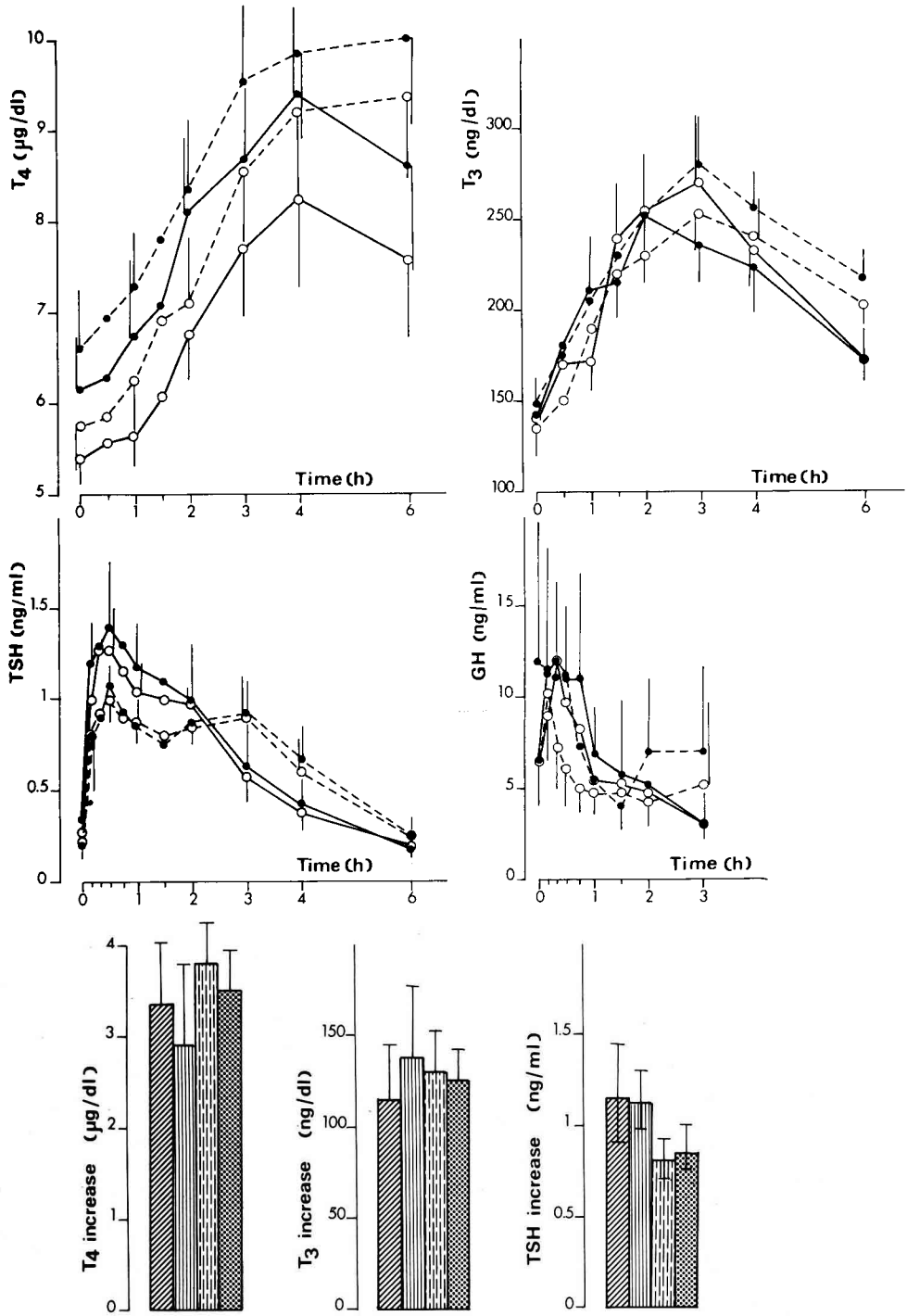


FIG. 1. — *Changes and increases in plasma T4, T3, TSH and GH levels after SC TRH administration three-month old lambs* (T4, T3, GH : Mean ± SEM ; TSH : Algebric conversion of mean of the log and mean of the log ± SEM of the log).
1 µg of TRH/kg : ●—● ▨ ; 2 µg of TRH/kg : ○—○ ▥ ; 5 µg of TRH/kg : ○----○ ▥ :
10µg of TRH/kg : ●---● ▩.

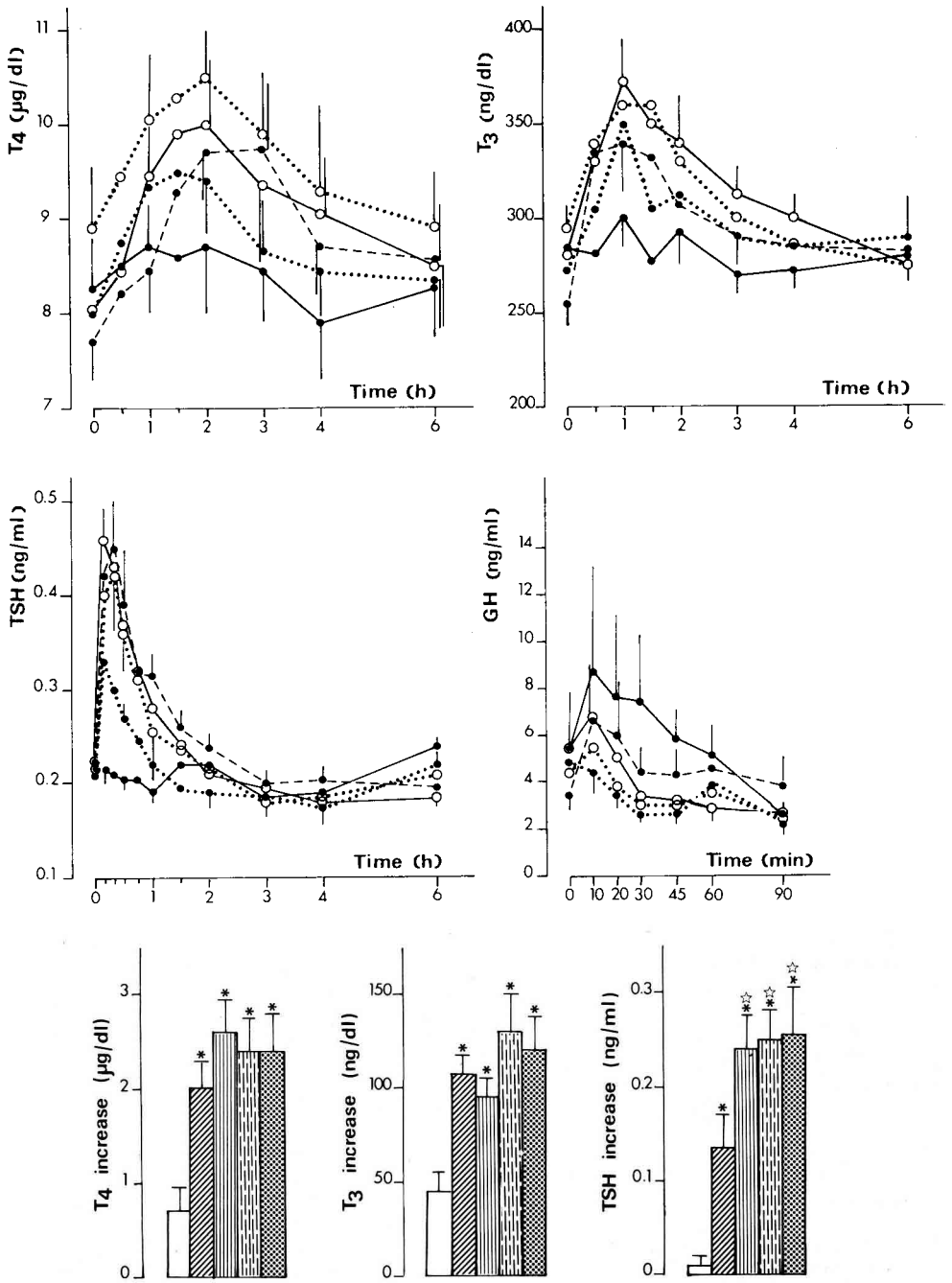


FIG. 2. — *Changes and increases in plasma T4, T3, TSH and GH levels after IV TRH administration in two-week old lambs* (Mean ± SEM ; * $P < 0.001$ with controls ; ☆ $P < 0.001$ with 0.1 µg TRH/kg).

Saline : ●—● □ ; 0.1 µg of TRH/kg : ●.....● ▨ ; 0,25 µg of TRH/kg : ○.....○ ▥ ; 0,5 µg of TRH/kg : ○—○ ▥ ; 1 µg of TRH/kg : ●---● ▩.

Plasma T_4 and T_3 levels rose significantly after TRH injection ($P < 0.001$). The maximal values were recorded after 2.2 ± 0.1 h and 1.2 ± 0.1 h, respectively for T_4 and T_3. The amount of TRH used did not significantly affect the increase in the plasma levels of these hormones. However, the differences between T_4 values at 3 h post-injection or T_3 values at 90 min post-injection and the basal levels were positively related to the amount of TRH given ($P < 5$ %).

Whatever the amount injected, TRH did not significantly affect plasma GH levels in two-week old lambs.

Intravenous TRH, GRF and TRH + GRF injections in two-month old lambs (fig. 3). — Intravenous saline or GRF (0.2 and 1 μg/kg) injection did not significantly affect plasma TSH, T_4 and T_3 levels.

After TRH administration plasma TSH levels rose significantly ($P < 0.001$). Neither the maximal value nor the increase was influenced by simultaneous GRF injection (0.2 and 1 μg/kg).

As in the preceding experiment, maximal plasma T_4 levels were recorded 2.3 ± 0.1 h after TRH administration. The addition of GRF (0.2 and 1 μg/kg) in the injection medium did not significantly affect the increase in thyroxinemia observed.

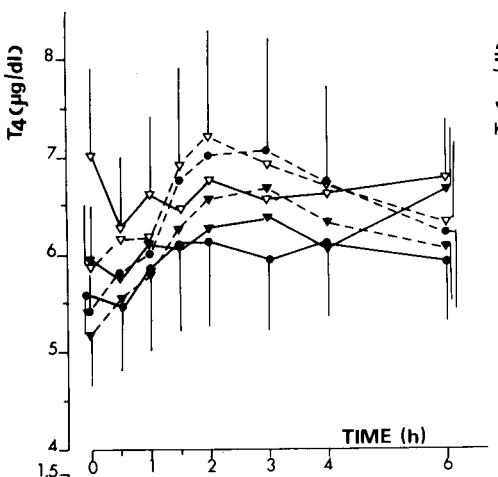
After TRH administration plasma T_3 levels rose significantly ($P < 0.001$) until 1.3 ± 0.1 h post-injection. This increase, significantly inhibited by the addition of GRF in the injection medium (0.2 μg/kg ; $P < 0.001$; 1 μg/kg ; $P < 0.05$), did not differ from that recorded after saline or GRF alone was administered.

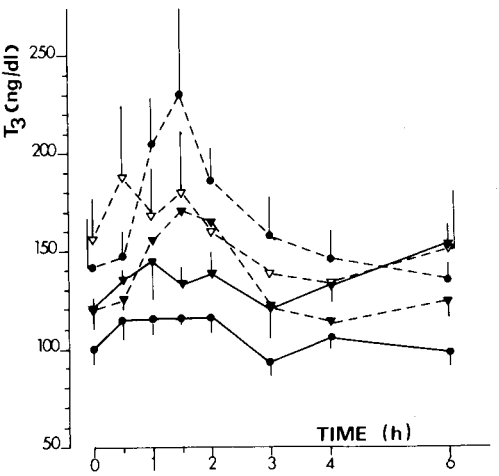
As in the other experiments, TRH injection did not significantly affect plasma GH levels in two-month old lambs. As expected, GRF-44 administration sharply increased plasma GH levels ($P < 0.001$) in a dose-dependent manner ; the addition of TRH did not influence the maximal value or the increase recorded. However, the profiles of the GH levels were slightly different with GRF alone or GRF + TRH, as follows :

— maximal values were observed significantly later after GRF + TRH than after GRF injection alone (0.2 μg/kg of GRF : 16 ± 2 min *vs* 10 ± 0 min, $P < 0.001$; 1 μg/kg of GRF : 20 ± 0 min *vs* 10 ± 0 min, $P < 0.001$) ;

— therefore, plasma GH levels were significantly lower at 10 min post-injection ($P < 0.05$) and higher at 20 and 45 min post-injection ($P < 0.05$) with 1 μg of GRF/kg + TRH than with 1 μg of GRF/kg. At 30 min post-injection, plasma GH levels were also higher with 0.2 μg of GRF/kg + TRH than with 0.2 μg of GRF/kg ($P < 0.05$).

FIG. 3. — *Changes and increases in plasma T4, T3, TSH and GH levels after IV TRH, TRH + GRF or GRF administration in two-month old lambs* (T4, T3, GH : Mean ± SEM ; TSH : Algebric conversion of the mean of the log and mean of the log ± SEM of the log ; ** $P < 0.001$ with controls ; ☆ $P < 0.05$ with TRH : ☆☆ $P < 0.001$ with TRH).
Saline : •—• ; GRF (0.2 μg/kg) : ∇—∇ ; GRF (1 μg/kg) : ▼—▼ ; TRH (1 μg/kg) •----• ; TRH (1 μg/kg) + GRF (0.2 μg/kg) : ∇----∇ ; TRH (1 μg/kg) + GRF (1 μg/kg) : ▼----▼.


T4 (µg/dl)
TIME (h)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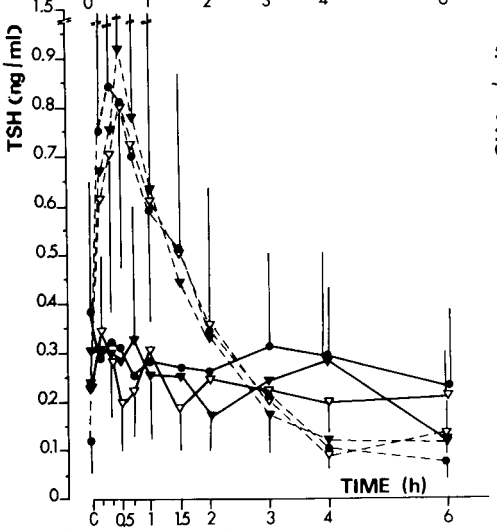
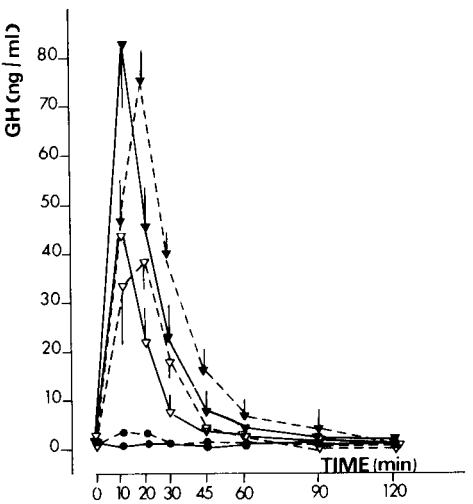
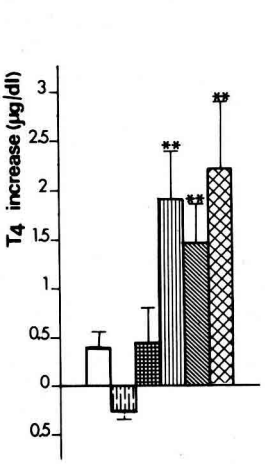
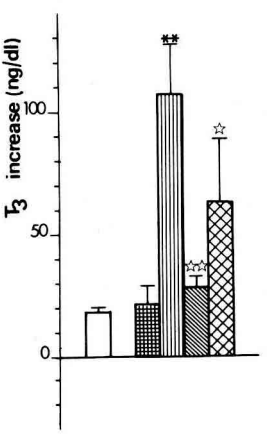
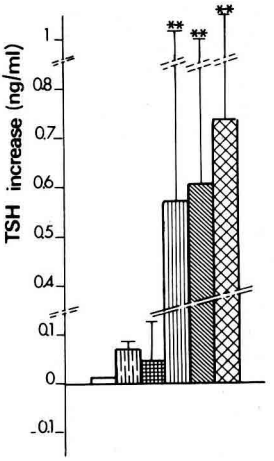
TIME (h)


TSH (ng/ml)
TIME (h)


GH (ng/ml)
TIME (min)


T4 increase (µg/dl)


T3 increase (ng/dl)


TSH increase (ng/ml)

Discussion.

To our knowledge, very few data have been reported about the response of plasma TSH, T_4 and T_3 to various amounts of TRH in lambs.

From our results it appears that when TRH was injected subcutaneously the increases in plasma TSH, T_4 and T_3 levels were equivalent, whatever the amount used. Therefore, it can be assumed that 1 µg of TRH/kg already induced maximal pituitary and thyroid responses. Higher amounts of TRH increased only the duration of TSH, T_4 and T_3 responses, probably because TRH persisted longer in the blood.

When TRH was injected intravenously, the increases in plasma TSH, T_4 and T_3 levels were equivalent for amounts of 0.25, 0.5 and 1 µg/kg ; Slebodzinski and Wallace (1977) reported the same phenomenon in cattle with 1, 2.5 and 5 µg of TRH/kg. As 0.1 µg of TRH/kg induced a significantly lower TSH response, it appears that 0.25 µg/kg would be the minimal amount of TRH associated with a maximal pituitary-thyroid axis response. T_4 and T_3 increases were maximal with 0.1 µg of TRH/kg, despite a lower TSH response, suggesting that a maximal thyroid response does not need maximal pituitary TSH release. As in subcutaneous injections, the relationships observed between plasma TSH levels at 60 min post-injection, plasma T_3 levels at 90 min post-injection or plasma T_4 levels at 3 h post-injection and the amounts of TRH given strongly suggest that the quantity of TRH injected affects the duration of pituitary-thyroid axis response more than the amplitude of that response.

When 1 µg of TRH/kg was given, the duration of T_4 and T_3 response was shorter in two-week or two-month old lambs than that previously reported in 24-hour old animals (T_4 : 2.3 h *vs* 4 h ; T_3 : 1.3 h *vs* 2 h) (Wrutniak and Cabello, 1985). This suggests that the utilization of TRH, TSH and/or T_4 and T_3 would be more important in the older lambs.

In agreement with the results of Hodate *et al.* (1985) obtained in calves, no TSH release was observed after GRF administration. The magnitudes of TSH increase after simultaneous injections of GRF and TRH were not different from those obtained by TRH injection alone.

Moreover, the present results suggest that GRF administration inhibits T_3 response to TRH without affecting T_4 response. Whether this effect was direct or mediated by the increase in plasma GH levels, there are several possible explanations :

— overall thyroid secretion would be reduced by GRF administration. However, this does not agree with the fact that T_4 increase was unchanged ;

— GRF would inhibit cell 5′-deiodination and therefore T_4 conversion to T_3 or it would increase cellular T_3 uptake. However, GRF did not decrease basal T_3 levels ;

— preferential T_3 secretion induced by TSH stimulation (Greer *et al.*, 1968) would be abolished by GRF administration. Work is in progress to verify this effect in a greater number of animals.

This study provides evidence that whatever the injection route, amount

injected or animal age, TRH alone does not increase GH secretion in lambs. These results contrast with those obtained in calves by Hodate *et al.* (1985) and suggest a strong species difference. As underlined by these workers, GH release in response to TRH in cattle is probably a phenomenon unique in ruminants : in addition to action on the pituitary (La Bella and Vivian, 1971 ; Smith and Convey, 1975), TRH may also act on the hypothalamus, in cattle *in vivo,* to stimulate the release of endogenous GRF and/or to inhibit the secretion of somatostatin.

Moreover, in contrast with the results *in vivo* in calves (Hodate *et al.,* 1985) or *in vitro* in chickens (Leung and Taylor, 1983), the simultaneous administration of GRF and TRH does not have a synergistic effect on GH release in lambs. Although unclear, the delayed GH response to GRF, after simultaneous TRH administration, probably has minimal physiological implications.

In the absence of its influence on GH release, the only evident effect of TRH is (as expected) a sharp rise in plasma TSH and thyroid hormone levels, which is not anabolic. Therefore, the use of TRH to increase body growth in lambs, as proposed by Davis *et al.* (1976), is not justified at this time.

12e Réunion du groupe Développement I.N.R.A. Montpellier, 28-30 mai 1986.

Acknowledgements. — This work was supported in part by « Sanofi Recherche » grant N° 9532-B. We wish to thank the NIADDK for supplying the oTSH and oGH RIA kits.

Résumé. *Influence de l'administration de TRH et de GRF sur la sécrétion de GH, TSH, T4 et T3 chez l'agneau.*

Les effets de l'injection SC ou IV de doses différentes de TRH (seule ou avec du GRF 1-44) sur les taux plasmatiques de GH, TSH, T4 et T3 ont été étudiés chez des agneaux âgés de 2 semaines, de 2 mois et de 3 mois.

1) Par voie sous-cutanée, la réponse maximale de la TSH, de la T4 et de la T3 n'est pas différente selon la dose utilisée (1, 2, 5 et 10 µg/kg). Cependant, cette réponse est prolongée pour les doses de 5 et 10 µg/kg.

2) Par voie intra-veineuse, à l'âge de 2 semaines, l'élévation maximale de la TSH plasmatique est observée dès la dose de 0,25 µg/kg. Cependant, la réponse des hormones thyroïdiennes n'est pas influencée par la dose utilisée (0,1, 0,25, 0,5 et 1 µg/kg). Comme précédemment, la durée de la réponse de la TSH, de la T4 et de la T3 est prolongée par l'augmentation des doses de TRH.

3) Quel que soit l'âge des animaux, les doses et la voie d'administration utilisées, la TRH seule n'augmente pas la sécrétion de GH chez l'Agneau. Cependant, elle retarde légèrement la réponse de la GH au GRF.

4) Le GRF ne modifie pas la réponse de la TSH et de la T4 à la TRH ; cependant, il pourrait inhiber l'augmentation de la T3.

En conclusion, contrairement aux résultats obtenus chez le Veau (Hodate *et al.,* 1985), la TRH ne modifie pas la sécrétion de GH chez l'Agneau mais induit des augmentations importantes des taux plasmatiques d'hormones thyroïdiennes. Ces arguments ne sont pas en faveur de l'utilisation de la TRH comme facteur de croissance dans l'espèce ovine.

References

CABELLO G., LEVIEUX D., 1980. Neonatal changes in the concentrations of thyrotropin, triiodothyronine, thyroxine and cortisol in the plasma of pre-term and full-term lambs. *J. develop. Physiol.*, **2**, 59-69.

CONVEY E. N., TUCKER H. A., SMITH V. G., ZOLMAN J., 1973. Bovine prolactin, growth hormone, thyroxine and corticoid response to thyrotropin-releasing hormone. *Endocrinology,* **92**, 471-476.

DAVIS S. L., 1975. Somatostatin : its influence on plasma levels of growth hormone, prolactin and thyrotropin in sheep. *J. anim. Sci.*, **40**, 911-916.

DAVIS S. L., HILL K. M., OHLSON D. L., JACOBS J. A., 1976. Influence of chronic thyrotropin-releasing hormone injections on secretion of prolactin, thyrotropin and growth hormone and on growth rate in wether lambs. *J. anim. Sci.*, **42**, 1244-1250.

DAVIS S. L., OHLSON D. L., KLINDT J., ANFINSON M. S., 1977. Episodic growth hormone secretory patterns in sheep : relationship to gonadal steroid hormones. *Amer. J. Physiol.*, **233**, E519-523.

GREER M. A., GRIMM Y., STUDER H., 1968. Qualitative changes in the secretion of the thyroid hormones induced by iodine deficiency. *Endocrinology,* **83**, 1193-1196.

HODATE K., JOHKE T., OHASHI S., 1985. Growth hormone, thyrotropin and prolactin responses to simultaneous administration of human growth hormone-releasing factor and thyrotropin-releasing hormone in the bovine. *Endocrinol. japon.*, **32**, 375-383.

JOHKE T., 1978. Effects of TRH on circulating growth hormone, prolactin and triiodothyronine levels in the bovine. *Endocrinol. japon.*, **25**, 19-26.

KESNER J. S., CONVEY E. M., DAVIS S. L., 1977. Bovine serum hormone concentrations after thyroprotein and thyrotropin-releasing hormone. *J. anim. Sci.*, **44**, 784-790.

LA BELLA F. S., VIVIAN S. R., 1971. Effect of synthetic TRF on hormone release from bovine anterior pituitary *in vitro. Endocrinology,* **88**, 787-789.

LEUNG F. C., TAYLOR J. E., 1983. *In vivo* and *in vitro* stimulation of growth hormone release in chickens by synthetic human pancreatic growth hormone releasing factor (hpGRFs). *Endocrinology,* **113**, 1913-1915.

SLEBODZINSKI A. B., WALLACE A. L. C., 1977. Pituitary and thyroidal responses to synthetic thyrotropin-releasing hormone in two breeds of cattle. *J. Endocr.*, **75**, 1-13.

SMITH V. G., CONVEY E. M., 1975. TRH-stimulation of prolactin release from bovine pituitary cells. *Proc. Soc. exp. Biol. Med.*, **149**, 70-74.

WRUTNIAK C., CABELLO G., 1985. Endocrine activity in preterm and full-term lambs. 1-Adrenal response to synacthen. 2-Thyoid response to ovine TSH or TRH. *Biol. Neonate,* **47**, 280-287.