Effects of triiodothyronine, triiodothyroacetic acid, iopanoic acid and iodide on the thyrotropin-releasing hormone-induced thyrotropin release from superfused rat pituitary fragments

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Abstract. The effects of triiodothyronine, triiodothyroacetic acid, iopanoic acid and potassium iodide were investigated on basal and stimulated thyrotropin release in an in vitro experimental model. Rat pituitary fragments were superfused by Medium-199 with or without T₃ (10⁻⁷ mol/l), triiodothyroacetic acid (10⁻⁸-10⁻⁶ mol/l), iopanoic acid (10⁻⁷-10⁻³ mol/l) or potassium iodide (10⁻²-10⁻⁴ mol/l). This was followed by a 6-min pulse of thyrotropin-releasing hormone (10⁻⁸ mol/l). TSH was measured in 3-min fractions. The TRH-induced TSH release from the pituitary fragments was inhibited by T₃ (10⁻⁷ mol/l), by triiodothyroacetic acid (10⁻⁷-10⁻⁶ mol/l), and by high concentrations of iodide (10⁻⁴ or 10⁻³ mol/l). Iopanoic acid had no significant effect at the concentrations tested. It is assumed that in vitro, and at similar concentrations, the inhibitory effect of triiodothyroacetic acid on the TRH-induced TSH release is comparable to that of T₃, whereas iopanoic acid may have no direct detectable effect. In contrast, a direct inhibitory effect of inorganic iodide, at least in pharmacological concentrations in vivo, cannot be excluded.

Besides thyroxine and triiodothyronine, numerous compounds have been used to modulate thyrotropin secretion in both experimental and clinical investigations.

Triiodothyroacetic acid is now used to suppress TSH secretion in thyroid hormone resistance (1-3) as well as in the postoperative treatment of patients with differentiated thyroid carcinoma (4). Up till now, the in vitro TSH suppressive effect of triiodothyroacetic acid has only been studied in thyrotropic tumour cell cultures (5), but not in normal superfused rat pituitary fragments.

Iopanoic acid (IOP), as well as other iodine-containing contrast agents, blocks phenolic ring deiodinase enzyme activity, thereby decreasing the intrapituitary T₄-T₃ conversion rate. The effect of IOP on thyrotropic activity was widely studied from this aspect (6-11). In our experiment we investigated in thyroid hormone-free medium the direct effect of IOP on the thyrotropin-releasing hormone-mediated TSH release.

Inorganic iodide may have a dual but indirect effect on TSH secretion according to the dose and experimental or clinical conditions: 1. it blocks thyroid hormone synthesis, thus increasing the TSH response to TRH by decreasing thyroid hormone negative feed-back (12-14), and 2. in iodine deficiency, iodide supplementation leads to restoration of normal thyroid hormone release, thereby decreasing TSH secretion (15). Again, our question was to determine, whether iodide may have a direct in vitro effect on TSH release.

Thus, by using an in vitro experimental model, superfused rat anterior pituitary fragments, we tried to determine whether triiodothyroacetic acid, IOP and iodide used at different concentrations could have direct effects on TRH-induced TSH release.
Materials and Methods

Materials and perfusion procedure

Young adult (2-3 months old) male Wistar rats weighing 230-250 g were housed in a temperature regulated room (21 ± 1°C) and subjected to 12-h light and 12-h dark cycles. After decapitation, the neural lobe was discarded and one pituitary gland, cut into four fragments was transferred to a perfusion chamber. Usually eight chambers, connected with a constant speed roller pump (from Technicon Analyzer, USA), were run simultaneously, with a regular flow rate of 100 μl/min, according to the procedure used by Roussel et al. (16). The lag-time of the system due to the dead volume (400 μl) was 4 min, the biological lag-time varied from 2 to 5 min. The tissue was superfused with Medium-199 from GIBCO (Grand Island Biological Co, Grand Island, NY. Cat. No. 041-02950) containing BSA (0.25%), ascorbic acid (20 mg/l), and bacitracin (30 mg/l), pH 7.35-7.4, and was gassed with 96% O₂/4% CO₂ in a water bath at 37°C.

After a 90 min equilibration period, the superfusion was continued for 135 min with or without inclusion into the superfusion medium of T₃ 10⁻⁷ mol/l (Henning, Berlin, Germany), triiodothyroacetic acid (TRIAC) (Sigma, stock solution 10⁻⁴ mol/l in 20% methanol, final concentrations 10⁻⁸-10⁻⁶ mol/l), iopanoic acid (Sterling-Winthrop, stock solution 10⁻⁵ mol/l in 0.2 mol/l NaOH, final concentrations 10⁻²-10⁻¹ mol/l), or potassium iodide 10⁻²-10⁻¹ mol/l. The superfusion was followed by a 6-min pulse of TRH (Hoechst, Frankfurt am Main, Germany).

Fig. 1.
Effect of T₃ on TRH-induced TSH release.
Time course of in vitro TSH release from rat pituitary fragments superfused at a flow rate of 100 μl/min without (a) or with (b) addition of T₃ (10⁻⁷ mol/l) for 135 min followed by a 6-min pulse of TRH (10⁻⁸ mol/l). Each value is the mean ± S.E.M. of 5 experiments. Results are expressed in terms of NIH TSH RP-2 standard. ■-TRH.
Quantification of the effect of T₃ (10⁻⁷ mol/l) on the TRH-induced TSH release from superfused rat pituitary fragments (c). Results are expressed as the ratio of the TRH-induced TSH release, calculated over the first 15 min of the TSH response, to basal release over the same period of time. The columns show mean and 95% confidence intervals with common standard deviation. The asterisk indicates significant difference with the multiple comparison test (Tukey). ANOVA: F-value=17.10, p=0.003. *p<0.05.
(Reproduced with permission from Experimental Gerontology, vol 26, Szabolcs et al.: Age-related changes in the thyrotropin (TSH) responsiveness to thyrotropin-releasing-hormone (TRH) stimulation and to the inhibitory effect of triiodothyronine (T₃); in vitro study on superfused rat pituitaries. Copyright 1991, Pergamon Press).
Fractions were collected every 3 min for a period of 15 min before and 60 min after the TRH test. Perifusions with TRH alone and with TRH in the presence of various concentrations of T₃, TRIAC, IOP or iodide were run at the same time. TSH was measured in 3-min fractions during the first 45 min and in every second fraction during the following 30 min.

Samples were collected on ice and stored at −20°C until assayed for TSH. Fractions from a given experiment were analysed in the same assay to minimize inter-assay variation in the data analysis. Results are expressed either as TSH release (in nanogram per min) or as the ratio of the TRH-induced TSH secretory response (calculated over the first 15 min of the initial burst of release) to the spontaneous hormone release evaluated from each chamber over the same time. Since there was a small individual variation in the lag-time of the TRH effect, the calculation of the TRH-induced TSH response was started 9 min after addition of TRH to the system. In this way, the peak TSH levels were always included into the calculation. TSH response was calculated as the 15-min TSH release after TRH, since the extension of the calculation over a

![Graph](image)

**Fig. 2.**
Effect of TRIAC on TRH-induced TSH release.
Time course of in vitro TSH release as indicated in Fig. 1. a: without TRIAC (N=5); b: TRIAC (10⁻⁸ mol/l (N=4); c: TRIAC 10⁻⁷ mol/l (N=4); d: TRIAC 10⁻⁶ mol/l (N=5); e: Quantification of the effect of TRIAC. Results expressed as indicated in Fig. 1. ANOVA: F-value=6.23, p=0.006. **p<0.01 compared with the control.
longer period of time had been connected to a decrease of the dose-related differences in our study.

Statistics were calculated by the analysis of variance (ANOVA) followed by multiple comparison by the method of Tukey. Means, 95% confidence intervals as well as the F and p values are given in the figures and in the legends to figures.

**Radioimmunoassay**

Rat TSH was measured by application of materials kindly supplied by the National Institute of Arthritis, Diabetes, Digestive & Kidney Diseases (NIADDK) and elaborated by A. F. Parlow, Pituitary Hormones and Antisera Center, Torrance, CA.

Antiserum NIADDK-anti-rTSH-S5 (C21381) was used in a working dilution of 1:5000 and a final dilution of 1:10000 in phosphate buffer. As a standard, NIADDK-rTSH-RP-2 was used in a concentration range of 200 ng/l to 25 μg/l, 100 μl per tube; 25 μl superfusion fluid was added to the assay and completed by 75 μl of assay buffer. For tracer preparation highly purified rTSH (NIADDK-rTSH-I-8) was used; 2.5 μg of peptide were iodinated by

![Fig. 3. Effect of IOP on TRH-induced TSH release.](image)

Time course of in vitro TSH release as indicated in Fig. 1. a: without IOP (N=8); b: IOP $10^{-7}$ mol/l (N=5); c: IOP $10^{-6}$ mol/l (N=8); d: IOP $10^{-5}$ mol/l (N=4); e: Quantification of the effect of IOP. Results expressed as indicated in Fig. 1. ANOVA: not significant.
the addition of 0.5 μCi Na^{125}I and 2 mg Protac 125 (Baker) as oxidizing agent. Reaction time was 6 min. Purification was carried out on a PD-10 column; 1:2000 prediluted antisera bound more than 70% of the purified tracer.

Sensitivity of the assay was 100 ng/l. Intra-assay coefficient of variation was 5.6% in a sample of 6.4 μg/l (N=10). Inter-assay coefficient of variation was 6.8% for a sample of 6.4 μg/l in a total of 16 assays.

Results
After placing the pituitary fragments into the chambers, there was a release of TSH (not shown in the figures) which gradually fell to reach, after about 90 min, a constant level of basal secretion. Addition of the different test substances (T₃, TRIAC, IOP, iodide) at this time for 2 h did not affect the basal rate of TSH release from the pituitary fragments. The dynamic pattern of TSH release in response to TRH (10⁻⁸ mol/l) was characterized by a rapid increase in TSH release within 9 min after the addition of TRH, which then declined slowly to reach basal levels within about the next 30 min (Fig. 1-4).

By comparing the ratio of the TRH-induced secretory response (calculated over the first 15 min of

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Fig. 4.
Effect of iodide on TRH-induced TSH release.
Time course of in vitro TSH release as indicated in Fig. 1. a: without iodide (N=6); b: iodide 10⁻⁷ mol/l (N=4); c: iodide 10⁻⁶ mol/l (N=6); d: iodide 10⁻⁵ mol/l (N=4); e: iodide 10⁻⁴ mol/l (N=4); f: Quantification of the effect of iodide. Results expressed as indicated in Fig. 1. ANOVA: F-value=4.03, p=0.016.
* indicates p<0.05 compared with the control.
the initial burst of release) to the spontaneous hormone release, the following results were observed:

1. The TSH secretory response to TRH was significantly decreased by 10^-7 mol/l T3 (Fig. 1).
2. TRIAC at 10^-7-10^-6 mol/l concentrations caused a significant decrease of the TRH-induced TSH response (Fig. 2).

3. No significant effect of IOP (10^-7-10^-5 mol/l) could be detected (Fig. 3). In one experiment (not shown in the figures) IOP 10^-6 mol/l and T3 10^-7 mol/l were perfused simultaneously. No additive suppressive effect of IOP was seen.

4. A direct and suppressive effect of iodide on the TRH-induced TSH release could be demonstrated at a high iodide concentration (10^-5 mol/l) (Fig. 4).

Discussion

At the concentrations used here, neither T3 nor TRIAC, IOP or iodide could modify basal TSH secretion. Their inhibitory effect on the TRH-mediated TSH response, if any, was relatively small and did not exceed 25%. Whereas dose-response studies were carried out with TRIAC, IOP and iodide, T3 has only been tested in a single concentration, since its in vitro effect on the TRH-mediated TSH release is well established and we used T3 here only to show that our superfusion system provides similar experimental conditions compared with those used by other authors. In fact, the T3 concentration able to inhibit TRH-induced TSH release (10^-7 mol/l) was very close or identical to those used by others: 0.3x10^-7 mol/l (17) and 10^-7 mol/l (18) with a serum or bovine serum albumin enriched medium. An inhibitory effect could also be detected with low T3 concentrations but only by using serum-free medium in static incubation: 2x10^-8 mol/l in the experiment of Chopra et al. (19), or with even lower T3 concentrations: 1.5x10^-8 mol/l as described in a dynamic study by Shiota et al. (20). This means that in in vitro superfusion conditions, only supraphysiological T3 concentrations could inhibit TRH-induced TSH release. This may be valid for the other investigated compounds, too; thus, their inhibitory concentrations found here in vitro should be accepted with caution for comparison with in vivo conditions.

We found that TRIAC (10^-7-10^-6 mol/l) suppressed TRH-induced TSH release from the superfused rat pituitary fragments showing that, in vitro, its inhibitory effect is comparable to that of T3. These values are very similar to those described in the data obtained from experiments with thyrotropic tumour cell cultures (5). Although the nuclear receptor affinity for TRIAC is not lower than that for T3 (5,21,22), its biological effect on TSH secretion in vivo is much weaker, probably because of a much faster metabolism (23-25).

IOP seems to have no direct inhibitory effect on TRH-mediated TSH release, since it did not significantly modify the TSH response in our dynamic in vitro study even with concentrations 100-times higher (10^-5 mol/l) than that of T3. Higher concentrations used in in vitro studies decreased protein synthesis as a sign of toxicity (26). It is possible that the short time of IOP perfusion (135 min) before the TRH pulse is not sufficient for the action of IOP. It is now well accepted that as a consequence of the inhibition of T4 5’-monodeiodination due to IOP, there is a diminution of the intrapituitary T3 formation and a consecutive increase in TSH secretion (7,10,26-28). In an in vivo kinetic study Suzuki et al. (29) have shown that it takes about 32 hours after IOP administration to see an increase in plasma TSH in humans. The situation is somewhat different concerning inorganic iodide: at a concentration 100 times higher (10^-3 mol/l) than that used here for T3 (10^-7 mol/l), iodide effectively diminished the TRH-induced TSH release. At first sight it seems to be a high supraphysiological concentration, but the physiological range of serum inorganic iodide (10^-7 mol/l) is, similarly, about 100 times higher than that of T3 (10^-9 mol/l). Even more, oral iodide treatment, used either for goitre prevention or for therapy in iodine-deficient areas (15), may increase serum iodide concentration up to a level of 10^-6 mol/l. Thus, it seems reasonable to think that therapeutic doses of iodide may have a direct inhibitory effect on TRH-mediated TSH release in vivo, even though the mechanism of action remains to be determined. In hypothyroid patients with endemic cretinism, supplementary iodine decreased serum TSH without increasing thyroxine and triiodothyronine levels (30).

From our experimental data on superfused rat pituitary fragments it appears that under similar experimental conditions (about 3 hours superfusion) TRIAC and T3 have a comparable inhibitory effect on TRH-mediated TSH release, that IOP seems to have no detectable direct effect by itself.
whereas a direct and rather fast inhibitory effect of iodide, at least in pharmacological doses, cannot be excluded.

Acknowledgments

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