Accumulation of Tritiated Thyrotropin Releasing Hormone in Different Organs, Especially in the Thyroid

Recently it has been shown that tritiated thyrotropin releasing hormone (TRH) is concentrated in the pituitary after i.v. injection[1-3] and that the radioactivity is concentrated in the liver only after a certain delay. On the other hand, we demonstrated that TRH has a clear effect on the thyroid in hypophysectomized rats[4]. It was therefore considered worth-while to investigate whether tritiated TRH is accumulated in the thyroid also and whether intracarotic injection has the same effect as i.v. injection. Some data from studies of the competition between TRH-^{3}H and cold TRH in several tissues are also presented.

Material and methods. 1. *Tritiated TRH* (1-[N-(5-Oxo-L-prolyl)-L-histidyl-2,5-$^3H_2$]-L-prolinamide hydrochloride). A solution of 65 mg of iodinated TRH (1-[2,5-Diiodo-N-(5-oxo-L-prolyl)-L-histidyl]-L-prolinamide)[5] in 1 ml of methanol and 0.04 ml of triethylamine was stirred in an atmosphere of tritium (2 ml, 5 Ci) at room temperature for 5 h with 40 mg of 10% Pd/C. After filtration of the reaction mixture and evaporation of the filtrate, crude tritiated TRH was obtained. Purification of the crude product was achieved by means of carrier-free electrophoresis at pH 1.9 in a buffer system of acetic acid and formic acid. Subsequently, the corresponding fractions were extracted several times with 20 ml portions of phenol. After partition of the combined phenol fractions in water/ether, the separated aqueous layer was evaporated to dryness. Repeated lyophilization, first from diluted HCl and then from H_2O, provided 16.5 mg (39%) of pure 1-[N-(5-Oxo-L-prolyl)-L-histidyl-2,5-$^3H_2$]-L-prolinamide hydrochloride (tritiated TRH hydrochloride) with a specific activity of 34.8 Ci/m*M*. For the distribution study, another batch with a specific activity of 9.15 Ci/m*M* was taken.

2. *Animals.* For the experiments, randomized male rats with an average weight of 200 g derived from the Holtzman line of the Sprague-Dawley strain were used. The animals were kept on a normal laboratory diet and water ad libitum.

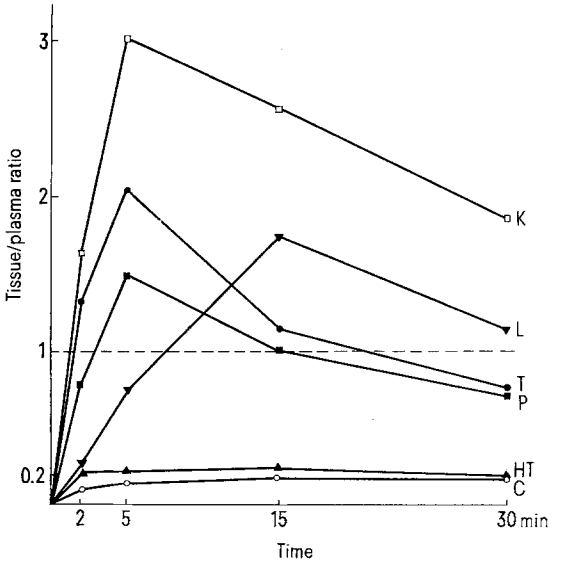

Fig. 1. Tissue/plasma ratio (T/P) after injection of TRH-^{3}H (spec. activity 9.15 Ci/m*M*, 1.2 μg/animal i.c. ,2ml saline pH 7.0) K, kidney; L, liver; T, thyroid; P, pituitary; HT, hypothalamus; C, cortex.

3. *Experimental procedure.* The animals were anaesthetized with Nembutal® (3–3.5 mg/100 mg of body weight), the left carotid artery was exposed surgically and 1.2 μg of the radioactive substance was rapidly injected in a volume of 0.2 ml of saline at pH 7.0 .The animals were decapitated 2, 5, 15 and 30 min after the injection and the blood was collected. Small pieces of liver, kidney, and frontal cortex, as well as basal hypothalamus, pituitary, and thyroid were removed. After blotting, rinsing, and weighing, they were transferred into scintillation vials.

The experiments were performed over a period of 6 weeks. A stock solution of TRH-^{3}H in saline was kept in deep frozen state and diluted for the individual experiments. This storage had no effect on the tissue/plasma (T/P) ratio during this period.

4. *Counting of radioactivity.* The tissues and the serum were digested with 0.2 ml of Soluene (Packard Instruments) and, after incubation at 60 °C overnight, the liquids were decolourized with H_2O_2. Then 20 ml of Insta-Gel (Packard Instruments) were added and the radioactivity was measured in a Packard liquid scintillation spectrometer after the disappearance of the autoluminescence. The quenching was corrected by the use of an external standard and the radioactivity was expressed as dpm per mg of tissue or per μl of blood.

5. *Competition studies.* The availability of TRH-^{3}H with a high specific activity of 34.8 Ci/m*M* enabled us also to study the competition of TRH-^{3}H with cold TRH and TRH-analogues. The procedure was slightly different. The anaesthetized rats were given an intracarotic injection 30 min after the injection of the anaesthetic, and 20 min later the serum and organs were collected. Bray's solution was used as a scintillation liquid. In all experiments the TRH-^{3}H was injected at a dose of 200 ng (= 500 p*M*) per animal either alone or together with the second compound in a volume of 0.2 ml.

Results. The distribution of the radioactivity in various tissues at various times after the intracarotic injection of 1.2 μg (~24 μCi) of tritiated TRH is illustrated in the Table. There was a rapid accumulation of radioactivity in kidney, pituitary and thyroid, whereas in the liver there was a delayed uptake of radioactivity. Only a minimal accumulation of radioactivity was noted in the hypothalamus and in the tissue of the frontal lobe cortex. The time dependency of the tissue to plasma ratio (T/P) is illustrated in Figure 1. The T/P ratios for pituitary, thyroid, liver, and kidney are greater than 1, thus indicating accumulation of radioactivity in these organs. For the kidney, thyroid, and pituitary, the T/P ratios reached a maximum after 5 min, for the liver after 15 min, and thereafter they gradually decreased. The T/P ratio for the hypothalamus and the tissue of the frontal lobe cortex always remained below 1.

Figure 2 shows the radioactivity accumulated in the pituitary, thyroid, and hypothalamus 20 min after injection of either tritiated TRH (500 p*M*) alone (100%

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The distribution of radioactivity (dpm/mg tissue) after intracarotic injection of 1.2 μg (~ 24 μCi) of tritiated TRH in various tissues at various times

Time (min)	2	5	15	30
No. of experiments	3	3	3	2
Cortex	44.95 ± 11.64	32.81 ± 3.72	23.99 ± 1.06	29.89 ± 5.54
Hypothalamus	101.26 ± 28.11	50.97 ± 12.63	33.93 ± 1.58	35.76 ± 2.01
Pituitary	369.88 ± 146.02	330.89 ± 14.93	142.38 ± 38.94	122.78 ± 12.52
Liver	127.94 ± 29.37	162.34 ± 38.67	236.03 ± 15.13	192.86 ± 25.56
Kidney	769.96 ± 89.84	663.57 ± 32.06	353.37 ± 100.03	317.76 ± 147.65
Thyroid	620.45 ± 203.46	449.89 ± 149.11	156.72 ± 16.76	132.27 ± 18.46
Plasma	469.02 ± 195.91	219.61 ± 8.16	135.43 ± 11.85	171.08 ± 17.86

dpm/mg tissue) or in combination with 5 or 50 n*M* of cold TRH. There was a clear decrease of the uptake of radioactivity in the 3 organs with simultaneous injection of TRH and tritiated TRH, indicating a competition at the site of accumulation, while plasma radioactivity was practically identical in all groups. Equimolar amounts of pyroglutamic acid, histidine, and proline, the 3 amino acids constituting TRH, together with tritiated TRH, had no effect on the accumulation of radioactivity in the 3 organs, nor had mono-iodo-L-tyrosine. On the other hand, the tripeptides L-pyroglutamyl-L-histidyl-L-tryptophan and L-prolyl-L-histidyl-L-proline-amide exerted a similar competitive effect as TRH[6].

Discussion. These experiments confirm results obtained already by others in that tritiated TRH is preferentially accumulated in the pituitary, kidney, and liver[1-3]. In addition, however, TRH-^{3}H is also concentrated in the thyroid. This is an interesting finding in view of our earlier observation that in hypophysectomized rats[4,5] TRH did clearly inhibit the thyroid activity.

The radioactivity in the plasma decreases very rapidly to 47% after 5 min and to 29% after 15 min to remain practically constant thereafter. The accumulation of the radioactivity in kidney and pituitary proceeds with remarkable rapidity, whereas the uptake in the liver is somewhat delayed. Contrary to MORIN et al.[2] and REDDING et al.[1], only in the liver and the kidney does the radioactivity remain above the serum level after 30 min. These authors have observed a longer lasting accumulation (> 2 h) in the pituitary as well. Both groups of workers administered the labelled substance i.v., whereas in our experiments the intracarotic injection was chosen in order to have a shorter contact with the blood until the radioactive material arrives at the pituitary. At present it is not clear whether these differences in accumulation are due to the different ways of injection or to other methodological differences. It is well known that TRH is rapidly degraded[7,8] by the plasma, and it may be speculated that the long lasting accumulation may reflect the uptake of degraded products.

In addition to the observations of other groups, we find a clear accumulation of radioactivity in the thyroid after intracarotic injection of tritiated TRH. Despite the fact that accumulation of a substance in tissues does not always correspond to a specific biological activity at this place[9], we think, considering our earlier data on the direct effect of TRH on the thyroid activity[4,5], that this preferential uptake of TRH by the thyroid may play a physiological role. A further indication in this direction is

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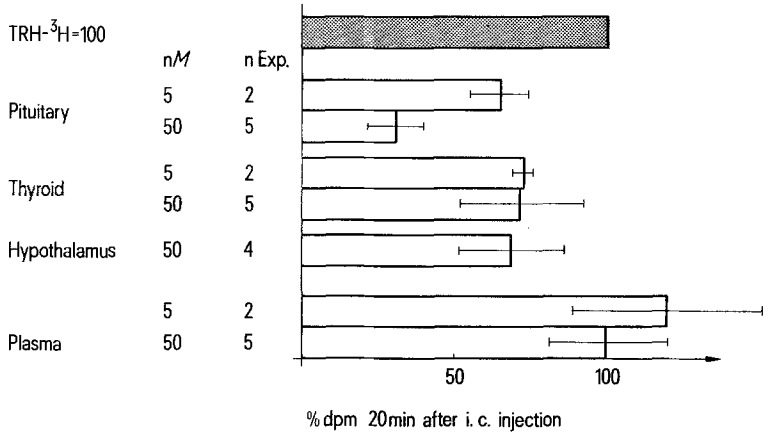

Fig. 2. Competition of TRH-^{3}H (500 p*M*) with cold TRH (5 or 50 n*M*) in vivo.

the reduced accumulation of radioactivity after simultaneous injection of tritiated TRH and cold TRH. Therefore, future studies on the feed-back mechanism in the hypothalamo-hypophyseo-thyroid axis should consider the existence of such a peripheral effect. The decrease of radioactivity by simultaneous injection of cold TRH is also shown for the pituitary and hypothalamus. For the pituitary, the prime site of action of TRH, this effect is expected. For the hypothalamus some reservations on the significance of these data are justified, because the dpm are always below the counts in the plasma. However, in view of the observation of TRH-sensible neurons in the hypothalamus[10], a hypothalamic effect may also be considered.

Résumé. La TSH-releasing hormone (TRH) tritiée a été produite. La substance radioactive est accumulée non seulement dans l'hypophyse et le rein, mais également dans la thyroïde après injection par voie endocarotidienne. La signification physiologique de l'action directe de la TRH sur la thyroïde est discutée.

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Supression of Fetal Hematopoeitic Development by Prednisone Treatment of Pregnant Rats

Some authors have reported stimulation of the bone marrow in adult animals following cortisone treatment[1–4]. Other investigators have found that cortisone depresses, in different animal species, the maturation and proliferation of hematopoietic cells[5–8]. Inhibition of nucleic acid synthesis in the bone marrow by cortisone has also been observed[9], and Betz[10] reported that cortisone impeded recovery of rat hematopoietic organs after X-irradiation.

We found previously that daily prednisone administration produced, at certain doses, bone marrow aplasia in rats previously irradiated with subletal doses of ^{60}CO[11]. In this work we have studied the influence of 16β-methyl prednisone administration to pregnant rats on the development of hematopoietic organs in their offspring.

24 inbred pregnant hooded rats were used. 12 animals were treated daily during the last week of pregnancy by s.c. injection with a suspension of 16β-methyl prednisone (Deltisona B, 8 mg, Lepetit) at a dose of 2 mg/kg/day (4 rats) and 4 mg/kg/day (8 rats). The rest of the animals were used as controls. Duration of pregnancy was checked in every case by daily inspection of vaginal contents, for cycle stage and presence of spermatozoids. As first day of pregnancy we took the last day in estrous with spermatozoids present followed by a prolonged diestrus and detectable fetuses. In total 181 animals were killed within the first 24 h of life. Body, thymus and spleen weights were taken. Spleen, thymus, liver and the right femur were embedded in 10% formol (the bone was also decalcified in 6% nitric acid), imbedded in paraffin, serial sections at 6 μm prepared and then stained with hematoxylineosin.

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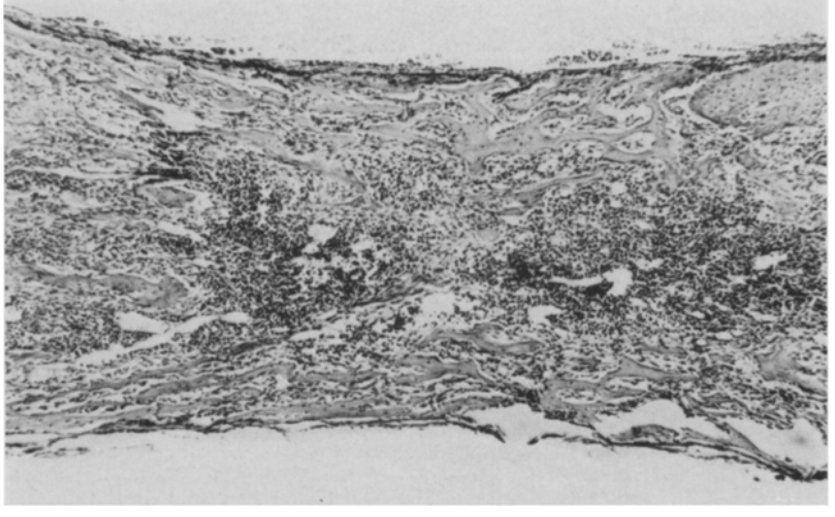

Fig. 1. Femur section of newborn rat born from untreated mother.