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DEGRADATION OF TRH AND ITS ANALOGUES BY RAT SERUM AND BRAIN HOMOGENATE

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SUMMARY

Synthetic TRH and thirty TRH analogues were subjected to enzymic degradation by rat serum or brain homogenate. Synthetic TRH and thirteen TRH analogues were similarly inactivated in both serum and brain extracts. Four TRH analogues were more stable than synthetic TRH in both serum and brain homogenate. Eight TRH analogues modified at the level of the pyroglutamic acid or histidyl residue were more stable in serum than in brain tissue. Five TRH analogues modified at the level of the prolineamide end were less degraded in brain homogenate than in serum.

Thyrotropin-releasing hormone, pGlu-His-Pro-NH_2 (TRH) is rapidly inactivated during in vitro incubation in rat serum or tissue homogenates including the brain (1,2). The mechanisms of TRH inactivation are not fully understood although it has been proposed that in serum there is a cleavage of the amide group at the prolyl end (3) and also an opening of the lactam ring in the pyroglutamyl residue (4). The administration of TRH to humans or rats is followed by short-lasting changes in the activity of the central nervous system (CNS). The short duration of the effects of TRH on CNS has been attributed to the rapid degradation of TRH by brain tissue (5). Thus the development of TRH analaogues resistant to the enzymic inactivation may be of interest. The following experiment has been designed in order to determine whether some TRH analogues are stable during the incubation in rat serum and/or brain tissue.

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MATERIALS AND METHODS

TRH and analogues: Synthetic TRH and 30 synthetic TRH analogues were tested. D amino-acids were substituted for natural amino-acids in four analogues. The other analogues were modified at the level of the pyroglutamic acid (5 cases) or histidyl (8 cases) or prolineamide (13 cases) residue.

Preparation of rat serum and brain homogenate: Ten adult male rats of the Sprague-Dawley strain were killed by decapitation between 8 and 9 a.m. Trunk blood was collected, then serum was separated and frozen until use. The brain was quickly removed, immersed in phosphate buffer 0.01 M, NaCl 0.14 M (PBS, pH 7.0) (1: 10 w/vol) and homogenized using a Potter apparatus. Then the homogenate was centrifuged at 900xg for 10 minutes; the pellet was discarded and the protein concentration in the supernatant was determined according to Lowry et al. (6). The protein concentration in the 900xg supernatant was adjusted at 10 mg/ml and the sample was frozen until use.

Degradation reaction: 10 µg of synthetic TRH or TRH analogue in 0.15 ml PBS were incubated with 100 µl of serum or the 900xg supernatant containing 1 mg protein. Control incubations were set up similarly except that the serum and the 900xg supernatant had been previously heated at 56°C for 40 min. The reaction was stopped after 10, 30 or 60 min by the addition of 400 µl 90% methanol. After centrifugation at 2500xg for 15 min., the supernatant was separated and evaporated under air pressure at room temperature. Then, the dry residue was suspended in one ml PBS bovine serum albumin (BSA) 1%, pH 7.0.

Determination of TRH and its analogues: The radioimmunoassay system for TRH has been previously described (7). Separate standard curves were constructed for each analogue. All the samples were assayed in duplicate and at three different dilutions. Experimental results are expressed as the percentage of TRH or TRH analogue remaining, taking the amount in the control incubations as 100%.

RESULTS

LLL-, LLD- and DDD-TRH are rapidly and similarly inactivated in rat serum or brain homogenate. LDL-TRH is also quickly degraded in serum, but more slowly inactivated than synthetic TRH in brain homogenate. DLL-TRH is stable in serum and only slightly degraded in brain homogenate (Table 1).

The degradation of TRH analogues which have been modified on the pyroglutamic acid residue is rapid when they are incubated in brain tissue. In opposition, after one hour incubation in serum, two of them (Cyclopentoyl-His-Pro-NH_2 and Cyclobutoyl-His-Pro-NH_2) are stable and two others (Trp-His-Pro-NH_2 and Ac Ala-His-Pro-NH_2) are only slightly inactivated (Table 2).

Table 1. Degradation of synthetic TRH and TRH analogues substituted with one or three D amino-acids by the serum or 900xg supernatant of brain homogenate from adult male rats.

Analogues	Incubation in serum; TRH recovered (%)			Incubation in brain homogenate; TRH recovered (%)		
	10 min	30 min	60 min	10 min	30 min	60 min
LLL-TRH	43.0±4.0	18.9±1.2	13.0±0.8	33.4±4.0	13.9±1.3	9.5±3.4
DLL-TRH	114.7±7.7	107.7±11.3	81.2±16.4	88.1±3.9	73.3±3.9	63.9±5.4
LDL-TRH	8.7±1.5	< 3	< 3	84.7±11.0	49.8±3.1	24.1±5.1
LLD-TRH	51.8±1.2	22.5±8.8	2.1±0.4	63.5±13.6	21.3±3.0	24.7±3.3
DDD-TRH	23.2±3.3	7.4±0.8	6.4±1.0	47.9±1.3	27.8±4.9	< 2

Each number represents the mean±S.E.M. of four determinations. The results are expressed as the percentage of immunoreactivity remaining after the incubation periods (10, 30 or 60 min), taking the amount in the control incubations as 100%.

Table 2. Degradation of TRH analogues bearing a modification on the pyroglutamic acid residue.

Analogues	Incubation in serum; TRH recovered(%)			Incubation in brain homogenate; TRH recovered(%)		
	10 min	30 min	60 min	10 min	30 min	60 min
Cyclopentoyl-His-Pro-NH_2	107.3±7.04	109.7±6.2	108.5±11.9	54.2±0.6	9.4±0.9	1.6±0.3
Cyclobutoyl-His-Pro-NH_2	110.4±15.7	108.1±11.2	112.9±2.9	67.6±0.4	15.2±2.9	5.2±1.3
Trp-His-Pro-NH_2	74.2±5.0	56.6±1.2	56.2±8.7	29.4±1.5	19.8±2.1	11.9±1.4
Ac Ala-His-Pro-NH_2	105.1±2.5	91.4±6.7	44.3±4.7	82.0±16.4	51.3±10.2	34.7±0.5
Ac Gln-His-Pro-NH_2	26.2±2.4	15.4±1.9	16.2±1.6	96.2±26.6	21.6±0.7	2.9±0.6

See the legend on Table 1 for the explanations on the experimental procedure and the expression of the results.

The substitution of the histidyl group by four different amino-acids (ornithine, arginine, asparagine or lysine) results in no or weak inactivation by serum, but in a high rate of degradation by brain homogenate. pGlu-[3 Me-His]-Pro-NH_2 is rapidly destroyed during in vitro incubation in serum or brain tissue. In opposition pGlu-[1 Me-His]-Pro-NH_2 is stable in both serum and

Table 3. Degradation of TRH analogues bearing a modification on the histidyl residue.

Analogues	Incubation in serum;TRH recovered(%)			Incubation in brain homogenate;TRH recovered(%)		
	10 min	30 min	60 min	10 min	30 min	60 min
pGlu-Orn-Pro-NH_2	84.8±3.8	85.8±4.6	94.8±14.7	92.4±0.9	69.8±1.8	32.1±2.8
pGlu-Arg-Pro-NH_2	90.8±10.4	64.6±3.8	34.9±1.5	23.7±1.0	4.4±1.1	8.8±2.9
pGlu-Asn-Pro-NH_2	103.2±17.9	72.7±6.2	68.5±2.3	41.6±1.7	29.7±1.0	16.5±2.7
pGlu-Lys-Pro-NH_2	83.3±0.9	53.1±4.2	54.9±1.9	28.9±6.9	8.9±1.2	7.2±2.0
pGlu-[1 Me-His]-Pro-NH_2	90.3±5.0	92.9±8.9	101.4±11.9	82.4±8.8	87.7±7.4	90.2±8.2
pGlu-[3 Me-His]-Pro-NH_2	66.1±6.5	54.7±12.0	12.7±2.7	43.6±8.4	27.9±2.8	16.9±3.7
pGlu-Ser-Pro-NH_2	16.6±3.2	6.7±0.7	4.0±1.2	34.7±5.2	22.8±5.5	9.3±0.9
pGlu-Phe-Pro-NH_2	43.0±3.1	12.7±3.1	5.6±0.8	41.0±2.0	7.3±1.3	3.5±0.9

See the legend on Table 1 for the explanations on the experimental procedure and the expression of the results.

brain homogenate. Two other analogues where serine and phenylalanine have been introduced instead of histidine are rapidly inactivated in both plasma and brain tissue.

When changes are made at the level of the prolineamide residue, the rate of degradation of the first five analogues listed in Table 4 is different in serum or brain homogenate. Indeed, these TRH analogues are degraded in serum but stable or only slightly inactivated in brain tissue. Among these analogues, pGlu-His-Piperidineamide and pGlu-His-Pyrolidineamide are degraded in serum more slowly than synthetic TRH; however the rate of their degradation in serum is much faster than in brain homogenate. Two other analogues, pGlu-His-Pro-OH and pGlu-His-Hyp-NH_2 are stable in brain homogenate and only slightly inactivated in serum. The six last analogues listed in Table 4 are inactivated similarly by both serum and brain enzymes.

DISCUSSION

These data show that synthetic TRH and several TRH analogues are inactivated similarly in rat serum or brain homogenate. However, some TRH analogues are stable (pGlu-[1 Me-His]-

Table 4. Degradation of TRH analogues bearing a modification on the prolineamide residue.

Analogues	Incubation in serum TRH recovered (%)			Incubation in brain homogenate TRH recovered (%)		
	10 min	30 min	60 min	10 min	30 min	60 min
pGlu-His-Sarcosineamide	47.9±26.7	17.5±2.3	13.4±4.2	112.6±3.3	84.7±11.5	63.4±3.7
pGlu-His-Pro-OEt	26.9±0.8	7.9±0.2	2.9±0.4	92.1±0.9	71.4±0.6	36.3±0.2
pGlu-His-Pyrolidineamide	95.5±6.9	58.7±15.4	29.4±1.0	65.5±13.0	62.7±6.3	68.9±3.6
pGlu-His-Piperidineamide	99.8±5.1	53.7±0.9	47.2±5.9	100.7±1.7	104.9±15.0	88.9±3.1
pGlu-His-Pro-NH-CH_2-CH_2-OH	62.7±6.8	6.0±2.3	<2	110.8±11.3	106.8±10.6	85.1±8.3
pGlu-His-Pro-OH	89.8±3.5	75.1±2.4	62.7±3.7	86.1±6.4	86.5±3.7	84.8±2.3
pGlu-His-Hyp-NH_2	104.4±2.5	94.6±7.0	83.6±3.3	94.7±20.7	84.9±3.7	111.0±11.0
pGlu-His-Azotidine-Carboxamide	51.8±1.2	22.5±8.8	2.1±0.4	63.5±13.6	21.3±3.0	24.7±3.3
pGlu-His-Hexamethyleneimide	62.7±1.3	32.2±5.1	13.3±1.9	67.5±11.4	48.2±9.6	23.5±9.9
pGlu-His-Pro-NH-CH_3	84.6±48.9	52.9±2.5	22.8±2.5	56.1±1.3	36.1±4.8	32.7±2.7
pGlu-His-CH_3-Ala-NH_2	83.2±7.4	66.1±0.8	43.0±7.0	79.5±1.5	46.5±3.4	22.8±3.5
pGlu-His-Pro-N $(Et)_2$	41.3±4.8	19.0±1.9	3.9±0.3	38.0±3.9	13.3±0.8	7.5±0.5
pGlu-His-Pro-NH Et	101.5±11.0	20.4±11.4	<3	62.3±6.6	19.4±2.3	5.2±1.8

See the legend on Table 1 for the explanations on the experimental procedure and the expression of the results.

Pro-NH_2, pGlu-His-Hyp-NH_2) or only slightly degraded (DLL-TRH, pGlu-His-Pro-OH) during in vitro incubation in both mediums. The rate of degradation of several TRH analogues which are modified on the pyroglutamic acid or histidyl residue is much lower in serum than in brain extracts. Three of them are even quite stable in serum: Cyclopentoyl-His-Pro-NH_2, Cyclobutoyl-His-Pro-NH_2, pGlu-Orn-Pro-NH_2. In opposition, five TRH analogues that are modified on the prolineamide residue are resistant to brain enzymes, but degraded in serum. They are: pGlu-His-Sarcosineamide, pGlu-His-NH-CH_2-CH_2OH, pGlu-His-Pro-OEt, pGlu-His-Pyrolidineamide and pGlu-His-Piperidineamide.

These data suggest that different mechanisms are involved in the inactivation of TRH. Besides deamidation of the prolineamide end, serum enzymes may act at the level of the pGlu-Histidyl residue. Indeed the degradation of TRH analogues by serum has been prevented by modifications on the pyroglutamic acid and/or histidyl

residue. Brain enzymes may be acting mainly on the prolineamide end; they have been inactive on some TRH analogues where the prolineamide residue has been modified. Other examples of differences in the TRH-degrading system present in serum and brain have been reported. The evolution of the TRH- degrading activities in the serum and brain of developping rats is different. During the first three weeks of life, the enzymic activity is high in the brain and low in the serum. Then, the enzymic activity decreases progressively in the brain and increases in the serum (8). After incubation of tritiated TRH with serum, only proline and intact TRH are found; when the incubation is carried out in hypothalamic homogenates, proline, prolineamide, deamido-TRH and intact TRH are detected (9).

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