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TRH DEGRADATION RATES VARY WIDELY BETWEEN DIFFERENT ANIMAL SPECIES

DAVID BREWSTER and KENNETH WALTHAM
Department of Drug Metabolism, Reckitt and Colman Pharmaceutical Division, Hull, U.K.

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Abstract—A study of the degradation of thyrotropin releasing hormone (TRH) by tissues of a variety of animal species and man has been conducted. Rates of degradation of TRH varied widely between different species and also between different tissues. Except for brain, tissues of dogs appeared devoid of TRH degrading activity. Rates of hydrolysis of TRH by rat plasma were dependent on the animal strain. Constituent amino acids were the major TRH metabolites produced by plasma whereas deamidated TRH was a major product from liver tissues. The cyclic metabolite histidylproline diketopiperazine was not a prominent metabolite in these studies.

Thyrotropin releasing hormone (TRH, L-pyroglutamyl-L-histidyl-L-prolineamide) is known to provoke the secretion of thyrotropin (TSH) from the pituitary [1, 2]. The release of prolactin and growth hormone has also been reported [3, 4]. In addition to its endocrine functions, however, there have been reports to suggest that TRH exerts direct actions on the central nervous system (CNS). For example, TRH administered centrally or peripherally antagonises the sedation and hypothermia produced by CNS depressants such as reserpine, chlorpromazine and various barbiturates [5, 6]. It has also been suggested that TRH may have clinical utility as an antidepressant [7, 8].

Although clearly rich in biological activity, TRH is rapidly degraded by certain mammalian tissues [9, 10]. Intravenous studies in rats and humans has shown TRH to have a plasma half-life of only 4–5 min [11, 12]. Such results may explain why the claimed antidepressant activity of TRH is short lasting [7] and they have prompted a pursuit of analogues with not only increased intrinsic potency but improved protection against biological inactivation [13–15]. The literature is still unclear, however, as to the extent to which TRH degrading activity is common to various animal species. This is an important consideration since the pharmacological potency and duration of action of TRH or its analogues may be influenced by their relative rates of degradation. In the present work we compare the rates and products of degradation of TRH in plasma and other tissues of a variety of mammalian species including man.

MATERIALS AND METHODS

Materials. [2-^{14}C-His]-TRH (sp. act. 3.4 μCi/mg) was prepared according to previous methods [14] and [2,3,4,5-^{3}H(*N*)-Pro]TRH (specific activity 276 mCi/mg) was obtained from New England Nuclear, Boston, U.S.A. Authentic samples of TRH-acid (L-pyroglutamyl-L-histidyl-L-proline) and L-histidyl-L-proline diketopiperazine (His–Pro) were prepared in our own laboratories.

Animals. Unless otherwise stated, laboratory animals used in these studies were MFI mice, Golden Syrian hamsters, Sprague–Dawley rats, Duncan–Heartly guinea pigs, New Zealand White rabbits and Beagle dogs. Plasma from pigs, goats, donkeys and primates was supplied by the Huntingdon Research Centre, Cambridge, U.K.

Assay methods. Samples of fresh plasma (pooled from between 2–5 individual animals) were diluted with an equal volume of 100 mM phosphate buffer pH 7.4. For the *in vitro* studies with other tissues, 10% (w/v) homogenates in buffer were prepared using a Silverson laboratory homogeniser. Incubations (37°) were begun by the addition of [^{14}C]-TRH to afford an initial concentration of 10 μg/ml. For studies at 10 ng/ml the [^{3}H]-TRH material was used. During incubation, aliquots (100 μl) were removed into small tubes containing methanol (100 μl) and chilled rapidly in a cardice–acetone freezing mixture. Tubes were centrifuged and the supernatants applied to the origins of 0.25 mm thick silica t.l.c. plates (Merck, Kieselgel F_{254}, 5 × 20 cm) then eluted in a solvent system comprising chloroform/methanol/NH_4OH (Sp. Gr. 0.88) 5:3:1 (by vol.). Silica from the developed chromatograms was scraped as 1 cm bands into scintillation vials and suspended in 3 ml water and 7.5 ml ES299 scintillant (Packard) for radioassay. Positions of authentic standard reference compounds were located by brief exposure to iodine vapour. Radioactive zones corresponding to unchanged TRH were expressed as a percentage of the total radioactivity recovered from the plates.

To obtain kinetic parameters for the hydrolyses, semilogarithmic plots of the decay curves were performed and lines of best fit obtained by linear regression analysis. Degradation half-life values ($t_{\frac{1}{2}}$) obtained from regression lines were converted to first order rate constants (K) using the standard first order rate law.

RESULTS

Initial studies in pooled plasma of female rabbits

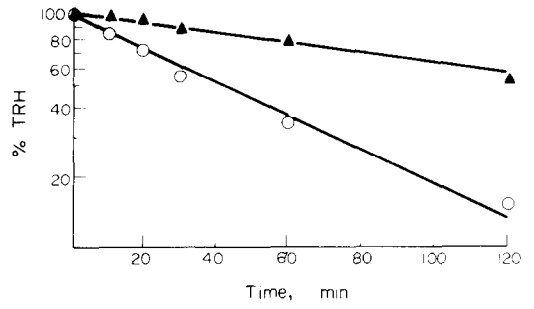

Fig. 1. Degradation of [^{14}C-His]-TRH by plasma of female rabbits (▲) or female adult humans (○). Results obtained from a pooled plasma sample of two individuals in each case.

or female humans showed plasma to degrade TRH (initial concentration 10 μg/ml) the kinetics of which were first order (see Fig. 1). The rates of degradation were very different however, the half-life in rabbit being over 2 hr but only 39 min in human plasma. The degradation of TRH was first order in all other species studied, rates of hydrolysis varied widely however (Fig. 2). TRH degradation was 40 times faster in pig than in guinea pig. Surprisingly, in dog plasma little if any degradation was observed (<5% within 6 hr). Sex did not appear to have a great bearing on plasma degrading activity as judged by studies in rats and humans (Fig. 2). No degradation was observed in either male or female dog plasma. Since the original concentrations (10 μg/ml) of TRH

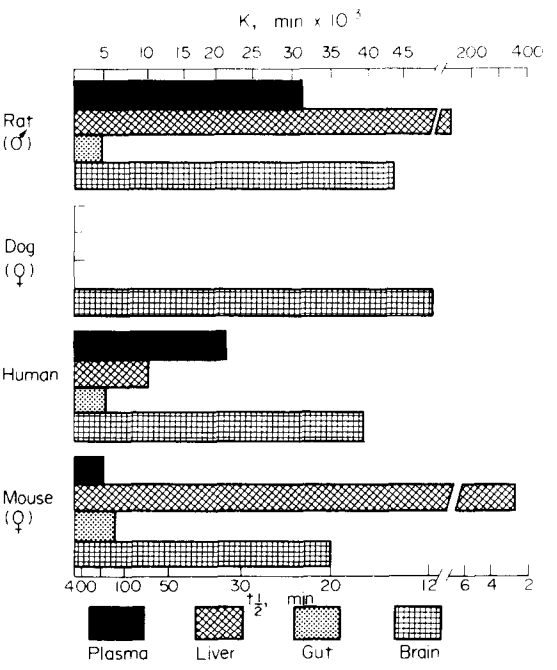

Fig. 3. Rates of degradation of [^{14}C-His]-TRH in various animal tissues. Buffered plasma or 10% (w/v) homogenates of tissues in phosphate buffer pH 7.4 were prepared as described under Methods. Human liver of one adult woman was obtained by needle biopsy and a lateral section of human duodenum by biopsy of one adult male. Human brain (cortex) was obtained at post-mortem of one adult male.

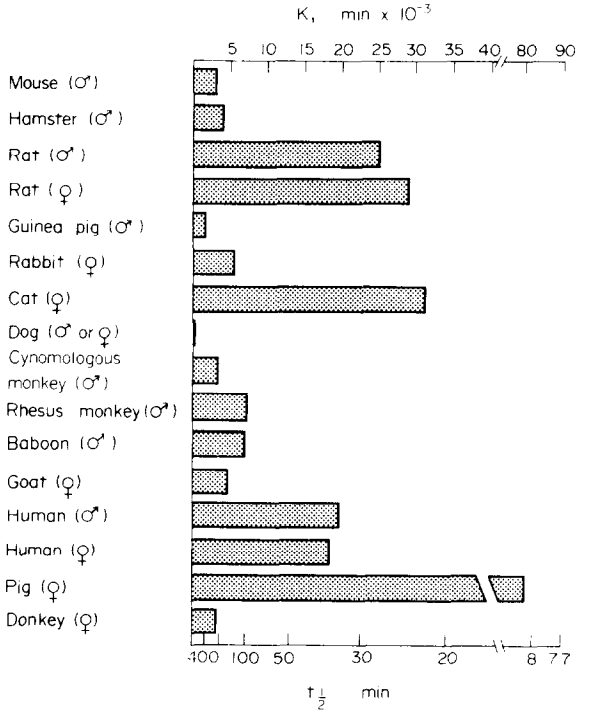

Fig. 2. Rates of degradation of [^{14}C-His]-TRH by plasma of various species. Values represent those obtained from pooled plasma samples of 2–5 individuals of the same sex. K, first order rate constant; $t_{\frac{1}{2}}$, first order half life. All incubations were carried out at an initial concentration of 10 μg/ml.

Table 1. Degradation of [^{14}C-His]-TRH by plasma of different strains of rat

Strain	% TRH Remaining After 20 min	Metabolites Detected
Sprague–Dawley	62.8 ± 1.5	His only
New Wistar	51.9 ± 0.8	His, trace TRH acid, trace His–Pro
Listar Hooded	39.8 ± 2.5	His only

Plasma was diluted with an equal volume of phosphate buffer pH 7.4 (see methods). Results represent mean values ± S.E.M. for plasma samples of 5 individual male animals.

in these studies were high, relative to physiological concentrations, studies at initial concentrations of 10 ng/ml were conducted. The 1000-fold dilution did not profoundly affect degradation rates. In male Sprague–Dawley rat plasma $t_{\frac{1}{2}}$ values were reduced from 26 min to 14 min and in male human plasma from 33 min to 23 min. No significant degradation of TRH was observed in dog plasma at this concentration.

In view of the wide species variations in plasma degrading activity the effect of animal strain was investigated. In three different strains of male rats significant differences in TRH degrading activity were observed (Table 1). Inter-individual variation in activity within the same strain was small (Table 1).

Species differences in TRH degrading activity were also observed in other tissues. Thus, liver was an active tissue for rat and mouse but less active in man whereas dog liver was essentially devoid of activity (<5% change with 6 hr) (see Fig. 3). TRH degrading activity was relatively low in all gut tissues and again absent in dog. In all four species examined brain appeared consistently rich in degrading activity. Of the dog tissues examined, brain was the only tissue with such activity (Fig. 3).

The metabolic patterns observed in the studies with the above tissues were variable but in general products were dependent on the tissue type rather than the species. Thus, following incubation of [^{14}C-His]-TRH in plasma, histidine was the only significant product whereas in liver and gut both

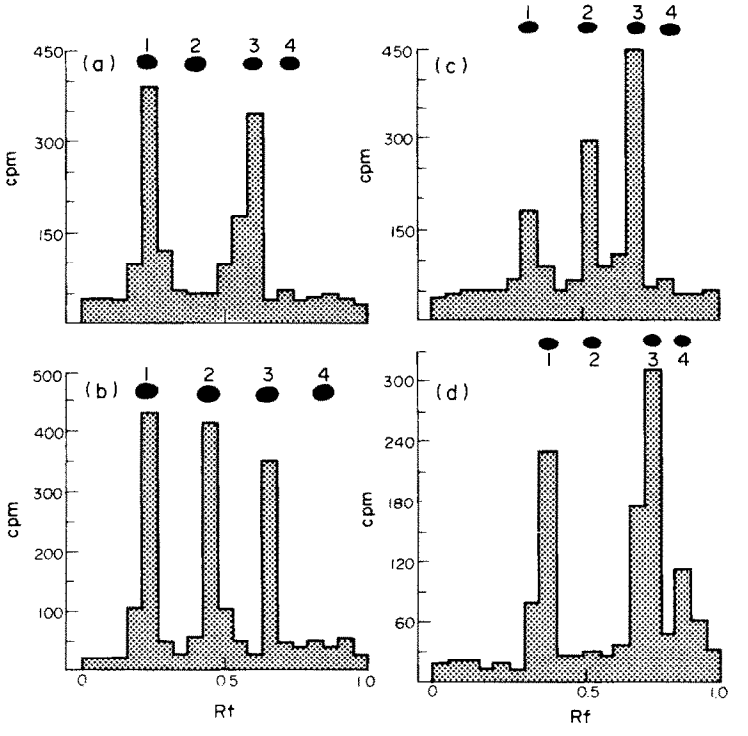

Fig. 4. Metabolic patterns resulting from the incubation of [^{14}C-His]-TRH in plasma or tissue homogenates. (A) male rat plasma after 20 min; (B) male rat liver homogenate after 10 min; (C) human gut homogenate after 120 min; (D) human brain (cortex) homogenate after 15 min. Positions of authentic reference standards are shown as (1) L-histidine; (2) TRH-acid; (3) TRH; (4) His–Pro.

TRH-acid and histidine were observed. In brain tissue histidine was the predominant metabolite but His–Pro was often observed, especially in man. Typical chromatograms for these tissues are shown in Fig. 4. In the New Wistar strain of rats small amounts (<5%) of TRH-acid and His–Pro were observed in plasma (see Table 1).

DISCUSSION

It is clear that the degradation of TRH varies widely between different species. It is also apparent that variations may exist between different animal strains. Such variations could be the basis for some of the differing literature reports concerning TRH inactivation rates. In serum for example, TRH inactivation has been reported to be 5-fold faster for rat than for man [16] whereas in the present study little difference in rates was observed. Such variations are unlikely to be due to differences in TRH concentrations between studies.

It has been suggested that serum enzymes play an important role in the regulation of hypothalamic hormones and therefore thyrotropin secretion [17]. This hypothesis is strengthened by reports that, in rat, thyroid hormones modulate serum TRH degrading activity [16, 18]. Results from the present studies would suggest that some adjustment to this model may be necessary. In guinea pig plasma the half-life of TRH was over seven hours and therefore in terms of TRH regulation it is likely that urinary excretion by glomerular filtration would be the most important mechanism of elimination *in vivo*. Certainly, serum control of TRH availability is unlikely to apply in a species such as dog which appears devoid of TRH degrading activity.

Variability in TRH degrading activity may be an important consideration in both the endocrinology and pharmacology of the hormone since potency and possibly duration of action may in influenced by the hydrolytic activity of both peripheral and CNS tissues. In the extrapolation of animal pharmacology to man, choice of species may be important. We have demonstrated that the rate of degradation of TRH in mouse plasma was approximately 10-fold slower than in rat. This is interesting since in reversing chlorpromazine induced hypothermia, TRH is 20 times more potent in mouse than rat [19]. The nature of TRH degradation products in different species is also of interest since the metabolite His–Pro is claimed to possess pharmacological activity [20–22]. This metabolite although produced by human brain was not a major metabolite in the present *in vitro* studies.

Suggestions that TRH may have clinical utility as an antidepressant have prompted studies on the CNS activities of the hormone [5, 6]. With the objective of increasing pharmacological potency the design of TRH analogues with increased resistance to biological inactivation has been approached [13–15]. From the present work, however, it can be seen that a further advantage of stabilised TRH analogues would be a reduction in the variability to which TRH degradation is subject. This could provide extra confidence in the extrapolation of animal data to the clinical situation.

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