An attractive proposal has been that each developing country, while fostering its own local language, should choose a major language as its "language of transfer".

UNISIST has evolved from a joint initiative of Unesco and ICSU and as such has stressed the natural sciences as the starting point, hoping to move progressively into the technological fields. Strong voices are insisting that from the beginning UNISIST should cover not only the pure but also the applied sciences, for from the point of view of information they cannot really be disentangled. Science and technology are being more and more affected by social and economic considerations as science policy and management become accepted as national imperatives. Therefore the separation of pure, applied and social sciences, especially from the point of view of information, is no longer realistic and efficient.

The third question is clearly controversial: what kind of organization can handle—coordinate and lead—so varied a conglomeration as governments, non-government organizations, learned societies, universities, industrial units? The draft report convincingly shows that UNISIST is feasible and necessary. Therefore the next steps must be to find the financial resources and create the administrative machinery for implementing and maintaining such a world-wide system of cooperation. To do this, Unesco, jointly with ICSU, is planning an international conference to be held probably in the last quarter of 1971 to bring together about 300 scientists, information specialists and government officials responsible for research and information policy. For this conference the final report on feasibility will be the working paper.

Administrative Structure

One suggested administrative structure, on the model of the very effective Intergovernmental Oceanographic Commission, is an International Commission for Scientific Information. Such a commission combining government, professional and user interests would indeed be a breakthrough. For the first time there would be a central steering body capable of evaluating, interpreting and then presenting to governments and others what can and must be done now, and furthermore with the executive strength to act. So far we have had no such overall and accepted body with the status and the power to do this. There are numerous international organizations each active in its own way: Unesco organizing documentation centres for developing countries, ISO promoting standards for documentation, WHO and FAO taking some responsibility for documentation in certain subject fields, OECD stimulating science information policy at the national level, ICSU coordinating abstracting services, FID furthering documentation at the professional level and so on. No organization has had the mandate and the resources to steer these multifarious activities and act on limited agreement. Nowhere are these problems seen and acted upon as a whole in all their intricate inter-relationships. By and large, the result has been an ineffectual Babel and therein lies our information crisis!

Characterization of Ovine Hypothalamic Hypophysiotropic TSH-releasing Factor

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Evidence is presented for the homogeneity of a preparation of hypothalamic TRF of ovine origin and its molecular structure has been established as 2-pyrrolidone-5-carboxylyl-histidyl-proline amide. Synthetic L-2-pyrrolidone-5-carboxylyl-L-histidyl-L-proline amide has physicochemical and biological characteristics which are quantitatively and qualitatively indistinguishable from those of the natural substance isolated in this laboratory.

THE existence of hypothalamic thyrotropin (TSH) releasing factor was demonstrated in 1962 (ref. 1). Recently[2] we obtained a highly purified preparation of hypothalamic TRF (acetate) of ovine origin according to the purification sequence summarized in Table 1 and demonstrated by analysis of a 6 M HCl hydrolysate that it contained as much as 81 per cent of its molecular weight in terms of the three amino-acids, glutamic acid, histidine and proline in equimolar ratios (theoretical contribution of the three amino-acids for a tripeptide acetate = 86 per cent). No free N-terminus could be demonstrated in this preparation of ovine TRF by dansylation. We subsequently reported that fully synthetic tripeptides of the generic structure PCA-His-Pro-R (PCA = 2-pyrrolidone-5-carboxyl) but not Glu-His-Pro-R, had TRF activity qualitatively indistinguishable from that of natural TRF. The specific activity of the tripeptides increases in the order $R = OH < OCH_3 < NH_2$, PCA-His-Pro-NH_2 having a biological activity not statistically different from that of natural TRF obtained as above[3–5]. Furthermore, the physicochemical characteristics of the various synthetic tripeptides, and particularly of PCA-His-Pro-NH_2, were closely related to those of natural ovine TRF. On the basis of low and high resolution mass spectrometric studies of highly purified ovine TRF and of a pure preparation of synthetic PCA-His-Pro-NH_2 (ref. 6), we have proposed that the molecular structure of hypothalamic ovine TRF is PCA-His-Pro-NH_2 (ref. 7).

We report here the detailed evidence on which this statement was based as well as information on (1) the nuclear magnetic resonance (NMR) spectra of pure ovine TRF and the synthetic tripeptide-amide; (2) quantitative amino-acid and ammonia analyses of natural ovine TRF and the two synthetic tripeptides PCA-His-Pro-OH and PCA-His-Pro-NH_2; (3) the biological activity of pure

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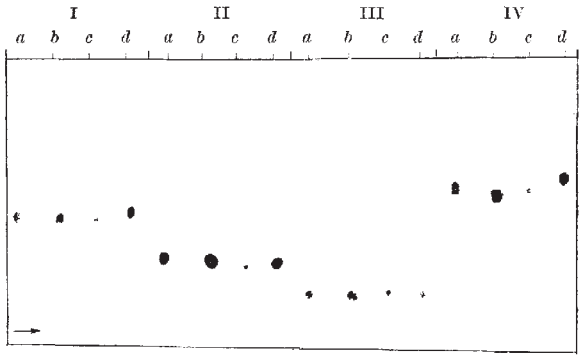


Fig. 1. Thin-layer chromatography of (*a*) 5 μg PCA-His-Pro-NH_2 prepared by total synthesis; (*b*) a mixture of 5 μg PCA-His-Pro-NH_2 prepared by total synthesis and 2·5 μg natural ovine TRF; (*c*) 2·5 μg natural ovine TRF; and (*d*) a mixture of 2·5 μg PCA-His-Pro-NH_2 prepared by total synthesis and 2·5 μg PCA-His-Pro-NH_2 prepared by ammonolysis of the methyl ester of PCA-Glu-His-Pro-OH. Thin-layer chromatography was performed on silica gel G (Eastman 'K 301 R2') in the systems: I, *n*-butanol–ethyl acetate–acetic acid–water (1 : 1 : 1 : 1); II, *n*-butanol–acetic acid–water (4 : 1 : 5); III, *n*-butanol–ethyl acetate–0·2 M aqueous NH_4OH (1 : 1 : 2); IV, collidine–ethanol–diethylamine–water (220 : 100 : 1 : 80). The upper phase was used in the case of two phase systems.

ovine TRF compared with synthetic PCA-His-Pro-NH_2. The new data support our earlier conclusions and prove that the tripeptide derivative PCA-His-Pro-NH_2 prepared by ammonolysis of the methyl-ester of PCA-His-Pro-OH[4,5] or prepared by total synthesis[6] has characteristics practically identical to those of natural ovine TRF and a biological activity which is not statistically different.

Physicochemical Characterization

Mixtures of 2·5–5·0 μg aliquots of natural ovine TRF and synthetic PCA-His-Pro-NH_2 move as a single spot as stained by the Pauly reagent in several chromatographic systems (Fig. 1).

For the examination of infrared spectra, 50 μg of natural ovine TRF (acetate) and of synthetic PCA-His-Pro-NH_2 (acetate) was deposited as thin films on the window of an 'Irtran' cell; spectra were observed in a Beckman 'IR–9' spectrometer. The spectra of the two materials (Fig. 2) are identical at all wavelengths with the exception of a peak at 1,110 ± 9 cm^{-1} (mean of six independent experiments) in the spectrum of synthetic PCA-His-Pro-NH_2 which is displaced to 1,121 cm^{-1} (mean of three independent experiments) in the spectrum of natural ovine TRF. This minor discrepancy, although observed with two different batches of TRF, is not statistically significant. Studies with a series of synthetic analogues of the generic structure PCA-His-Pro-R have indicated the possibility of producing such minor shifts by changing the *p*H of the solution(s) deposited on the 'Irtran' cell; the presence of traces of metal(s) chelated by either peptide may also be a possible explanation. We consider the similarities of the two spectra to be sufficient to indicate identity.

Table 1. SEQUENCE OF PURIFICATION FOR ISOLATION OF OVINE TRF

Stage	No. of fragments × 1,000*	Weight†	TRF units/mg‡
1. Lyophilized sheep hypothalamus	294	25 kg	
2. Alcohol–chloroform extract	294	294 g	1
3. Ultrafiltration 'UM-3'	294	71 g	3
4–5. Gel filtration, 'Sephadex *G*-25', 0·5 M acetic acid, 2X	286	16 g	16
6–7. Partition chromatography, 0·01 per cent acetic acid–*n*-butanol–pyridine (11 : 5 : 3), 2X	280	246 mg	800
8–9. Adsorption chromatography, Norit/H_2O–ethanol–phenol, 2X	275	4·2 mg	30,500 (1,100–200,000)
10. Partition chromatography, *n*-butanol–acetic acid–H_2O (4 : 1 : 5, upper phase)	273	2·0 mg	58,500 (12,500–150,000)
11. Repeat 10	270	1·0 mg	57,000 (12,000–124,000)

* Number of hypothalamic fragments available at each step in the purification sequence; the continuous decrease in number from step 4 to 11 results from the removal of aliquots for pilot studies, repeated bioassays, and so on.

† From step 3 to 11, the weights correspond to those of the TRF-active fractions at each step.

‡ Specific activity (TRF U/mg) with 95 per cent confidence limits in multiple four-point assays.

Low resolution mass spectrometry was performed on 20–40 μg samples of ovine TRF from stage 11 (Table 1) and of synthetic PCA-His-Pro-NH_2 which were methylated by treatment with an excess (50 μl.) of a solution of diazomethane in diethyl ether (3 × 5 min). Similar aliquots were also derivatized by treatment with 50 μl. of a 1 : 1 mixture of trifluoroacetic anhydride and trifluoroacetic acid (1 h, room temperature). Figs. 3 and 4 illustrate the low resolution spectra of the products. All samples were volatile in low resolution mass spectrometry (LKB 9000, direct probe, $\leq 10^{-6}$ torr) at probe temperatures ranging from 150°–200° C. Several mass spectra for each sample (seven in the case of TRF derivatives), recorded at various temperatures along the programmed thermal gradient, yielded identical fragmentation patterns corresponding to a single chemical entity. Combined with other evidence (quantitative amino-acid content[2], nuclear magnetic resonance spectra, thin-layer chromatographic data) these results allow us to claim the preparation, for the first time, of a hypothalamic hypophysiotropic releasing factor.

The mass spectra of the same derivatives (trifluoroacetylated or methylated) of natural ovine TRF and synthetic PCA-His-Pro-NH_2 are essentially identical (Figs. 3 and 4). No molecular ion was observed in any of these spectra; similar fragments were observed in the low resolution spectra of each derivative of natural TRF and synthetic PCA-His-Pro-NH_2. Ions were observed corresponding to the following structures: PCA, *m/e* 84; PCA + CH_2, *m/e* 98; His, *m/e* 94; His + CH_2 *m/e* 108; Pro minus 3H, *m/e* 93; Pro-NH_2 minus 3H, *m/e* 110 (unsaturated proline fragments such as pyrrole-2-carboxamide possibly produced by pyrolysis); $CONH_2$, *m/e* 44; PCA-His, *m/e* 221; His-Pro-NH_2 minus 2H, *m/e* 248. Other fragments, *m/e* 234, 235 and 249, could arise from PCA-His, His-Pro-NH_2 or also, as Schally *et al.* mentioned in the case of a preparation of porcine TRF[8], from the diketopiperazines of histidine and proline.

The elemental composition of all these fragments, except ion *m/e* 221 which was too weak to give a readable trace on the photoplate (Ilford 'Q2'), was confirmed on the methylated derivatives of TRF and synthetic PCA-His-Pro-NH_2, using high resolution mass spectroscopy ('CEC 21–110') (Table 2).

For nuclear magnetic resonance (NMR) spectrometry,

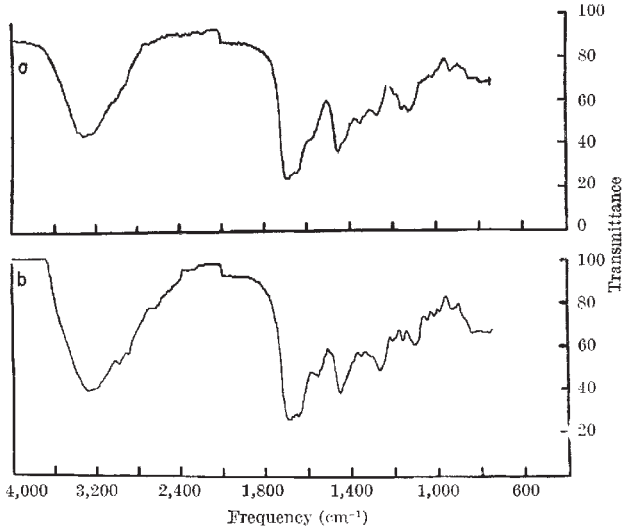


Fig. 2. Infrared spectra of (*a*) isolated ovine TRF and (*b*) synthetic PCA-His-Pro-NH_2.

0·8 mg of ovine TRF acetate (stage 11, Table 1) was dried in a 5 mm NMR tube and redissolved in 200–300 μl. ^2H_2O. The NMR spectrum of this preparation was obtained using a 100 MHz spectrometer (Varian 'HA–100') with time averaging by coupling to a computer of average transients ('C–1024'). Fig. 5*a* shows the spectrum, in comparison with a single sweep spectrum (Fig. 5*b*) of 25 mg synthetic PCA-His-Pro-NH_2 (acetate) as a solution in a similar volume of ^2H_2O using the same spectrometer. In the regions that can be compared, the spectra of the two compounds are practically identical. The singlet at 3·5 p.p.m. present in the TRF spectrum but absent in that of the synthetic compound may represent a minor impurity, but could also arise from a spinning side-band of the peak for water. The slight differences in the location of the maxima in the 7–9 p.p.m. region, presumably from the histidine ring protons, can be explained by the *p*H sensitivity of these bands in histidine NMR spectra[9].

Histidine and ammonia contents were determined in TRF and synthetic analogues, and the results are given in Table 3. Quantitative analyses of 6 M HCl hydrolysates of ovine TRF, synthetic PCA-His-Pro-OH, synthetic PCA-His-Pro-HN_2 and unhydrolysed TRF were obtained using a Beckman-Spinco modified model '120C'. The figures obtained for ovine TRF correspond to the presence of an amide group on the C-terminal proline.

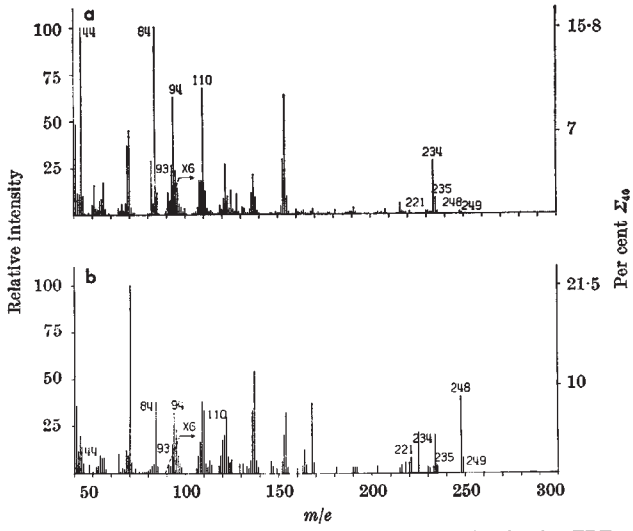


Fig. 3. Low resolution mass spectra of trifluoroacetylated ovine TRF (*a*) and synthetic PCA-His-Pro-NH_2 (*b*).

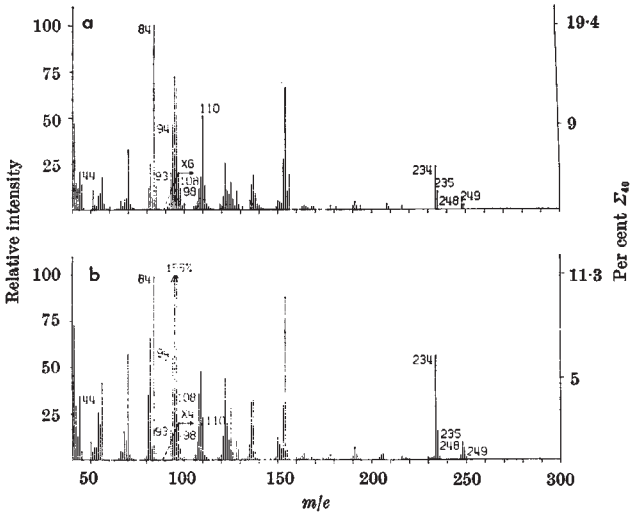


Fig. 4. Low resolution mass spectra of methylated ovine TRF (*a*) and synthetic PCA-His-Pro-NH_2 (*b*).

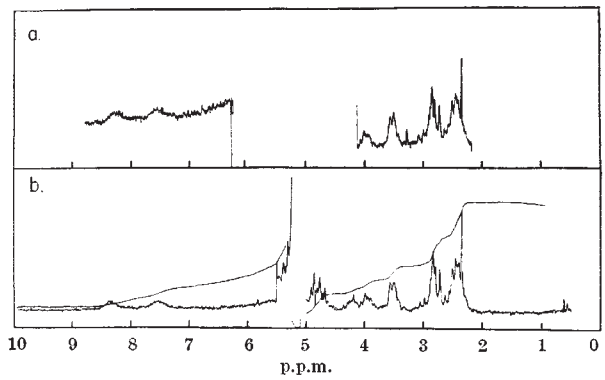
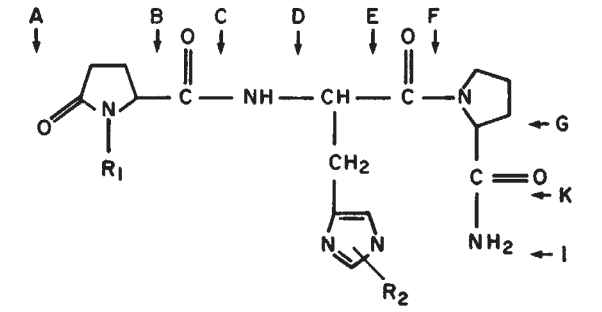


Fig. 5. Proton magnetic resonance spectra (100 MHz) of (*a*) ovine TRF acetate in ^2H_2O, time-averaged for 276 scans, 500 s/scan, 500 Hz sweep width in the regions shown, lock signal internal H^2HO (5·14 p.p.m. referenced to external capillary of tetramethylsilane in a separate experiment); (*b*) synthetic PCA-His-Pro-NH_2 (acetate) in ^2H_2O, single scan 1,000 s, sweep widths 1,000 Hz; referenced to external capillary of tetramethylsilane. Both spectra were obtained at 31° C.

Biological Activities

Using an *in vivo* mouse assay[5], six independent four-point assays of homogeneous natural ovine TRF gave a specific activity of 55,000 ± 9,900 U/mg (see ref. 10 for the definition of the TRF unit); five independent four-point assays of synthetic PCA-His-Pro-NH_2 gave a specific

Table 2. CHARACTERISTICS OF PRINCIPAL IONS OBTAINED BY FRAGMENTATION OF OVINE TRF (METHYLATED DERIVATIVE) IN HIGH RESOLUTION MASS SPECTROSCOPY



Fragmentation points	Measured mass	Error mMU	Elemental composition
A→B	84·0463	+1·5	C_4H_6NO
A→B, $R_1 = CH_3$	98·0576	−3·0	C_5H_8NO
D→E	94·0535	+0·4	$C_5H_6N_2$
D→E, $R_2 = CH_3$	108·0673	−1·4	$C_6H_8N_2$
F→K, minus 4H	93·0211	−0·4	C_5H_3NO
F→I, minus 3H	110·0480	0·0	$C_5H_6N_2O$
G→I	44·0120	−1·6	CH_2NO
A→E, R_1 or $R_2 = CH_3$, minus H or C→K or D→I, minus H	234·1112	−0·4	$C_{11}H_{14}N_4O_2$
C→I, minus 2H	248·1147	0·0	$C_{11}H_{14}N_5O_2$
A→E, $R_1 = R_2 = CH_3$ or D→I, $R_2 = CH_3$ or C→K, $R_2 = CH_2$, minus H	249·1327	−2·4	$C_{12}H_{17}N_4O_2$

$R_1 = R_2 = H$, except when indicated otherwise in the table. Arrows and letters A–K indicate fragmentation points in mass spectroscopy. In non-derivatized ovine TRF, $R_1 = R_2 = H$.

Table 3. AMMONIA AND HISTIDINE CONTENTS IN 6 M HCl HYDROLYSATES OF ISOLATED OVINE TRF AND SYNTHETIC PEPTIDES

	No. of determinations	nmoles His	nmoles NH_3*	NH_3/His
Synthetic PCA-His-Pro-NH_2	6	38·4 ± 4·6†	40·3 ± 6·1	1·07
Synthetic PCA-His-Pro-OH	5	39·1 ± 3·3	0·4 ± 3·8	0·01
Ovine TRF	2	20·5 ± 2·1	22·1 ± 1·0	1·08
Ovine TRF unhydrolysed	1	—	−2·0	—

* Corrected for exogenous NH_3 in blank hydrolysed with each determination; average blank = 15·0 ± 5·7 nmoles NH_3 for seven determinations.

† Standard error.

activity of 44,000 ± 1,100 U/mg; the two figures for the respective specific activities are not statistically different. Natural ovine TRF as well as synthetic PCA-His-Pro-NH_2 are regularly active ($P \leq 0.05$) in stimulating release of TSH *in vivo* (mouse TRF assay) in doses of 5 ng/animal, as a single intravenous injection; occasionally animals give a statistically significant response to a dose of 1 ng. Synthetic PCA-His-Pro-HN_2 stimulates release of TSH when administered orally to the assay animals in a dose of ≤ 1.0 μg (ref. 11). *In vitro*, hypothalamic ovine TRF or synthetic PCA-His-Pro-NH_2 stimulate release of TSH ($P \leq 0.05$ in complete four-point assays with factorial analysis) at doses of ≥ 50 pg/ml. incubation fluid; this corresponds to about 1×10^{-10} mole TRF/ml./mg pituitary tissue. As is the case with natural TRF[12], pretreatment of the *in vitro* incubated pituitary with tri-iodotyronine (T_3) inhibits the response to the synthetic tripeptide amide (Table 4). The *in vivo* pituitary response as a function of time measured by plasma TSH concentrations, following intravenous injection of synthetic PCA-His-Pro-NH_2 (Table 5), is not statistically different from that observed for natural ovine TRF[13]. Pretreatment of the assay animals with a large dose of T_3 (0·25 μg) 2 h before injection of three different doses of synthetic tripeptide amide shows (Table 6) the same type of "competitive inhibition" as reported earlier for natural ovine TRF[14].

Table 4. BIOLOGICAL ACTIVITY OF PCA-His-Pro-NH_2 (X): *in vitro* RELEASE OF TSH; INHIBITION BY T_4

	mU TSH released/ pituitary/h	Potency ratio (95 per cent confidence limits)
C: Saline	1·1	
T: 5×10^{-11} g (X)/ml.	3·4	T/C = 2·5 (1·8–3·7)
C: Saline	0·8	
T: 5×10^{-10} g (X)/ml.	5·2	T/C = 7·2 (5·2–11·1)
C: 5×10^{-10} g (X)/ml.	5·1	
T: 5 μg T_4 pretreatment; 5×10^{-10} g (X)/ml.	2·8	T/C = 0·51 (0·35–0·72)

In vitro incubation of rat pituitary tissues as described in ref. 12; mU/TSH released/h calculated for all fluids in a single multiple four-point assay against TSH international standard. Potency ratio with confidence limits calculated by factorial analysis; see ref 23 for a complete mathematical description of these calculations for TSH/TRF assay. C, Control hemi-pituitaries; T, treated hemi-pituitaries.

Table 5. ACTIVITY OF PCA-His-Pro-NH_2 (X): ELEVATION OF PLASMA TSH IN NORMAL RATS

Treatment	Time (min)	μU TSH/ml. plasma (95 per cent confidence limits)	*P*
Saline	0	159 (82–248)	
Saline	+3	153 (76–243)	—
Saline	+10	115 (47–189)	—
Saline	+30	145 (71–226)	—
15×10^{-11} g (X)	0	131 (60–205)	
15×10^{-11} g (X)	+3	585 (360–1,570)	*
15×10^{-11} g (X)	+10	879 (500–3,170)	*
15×10^{-11} g (X)	+30	370 (238–736)	*

Plasma samples are aliquots from pools of six rats each receiving treatments as indicated, all animals anaesthetized with 'Nembutal' at time of injection (intrajugular); all injection volumes, 1·0 ml. *P*, Significance level by the multiple comparison test of Duncan; —, not different from control; * = 99 per cent; all calculations and statistical analyses as in ref. 23.

Table 6. ACTIVITY OF PCA-His-Pro-NH_2 (X): INTERACTION BETWEEN T_3 AND (X) ON THE RELEASE OF TSH *in vivo*

Treatment	*N*	TRF assay adj. $\log_{10}$ c.p.m. ± *S.E.*	*P*
Saline	3	3·1279 ± 0·0617	
5×10^{-12} g (X)	4	3·4657 ± 0·0534	*
2×10^{-11} g (X)	5	3·6132 ± 0·0493	*
8×10^{-11} g (X)	6	3·8218 ± 0·0437	*
T_3, saline	6	3·1244 ± 0·0436	
T_3, 5×10^{-12} g (X)	6	3·1065 ± 0·0437	—
T_3, 2×10^{-11} g (X)	7	3·1605 ± 0·0404	—
T_3, 8×10^{-11} g (X)	6	3·3022 ± 0·0437	—
T_3, 1×10^{-9} g (X)	6	3·4803 ± 0·0436	*

Experimental design and mathematical analysis as in ref. 14; *N* = number of assay animals/treatment; *P* as in Table 5; T_3: 0·25 μg intraperitoneal injection of tri-iodothyronine 2 h before TRF injection.

Synthetic PCA-His-Pro-NH_2, like natural ovine TRF[15], elicits a highly specific response in the release of TSH: PCA-His-Pro-NH_2 does not stimulate secretion of adrenocorticotropin or luteinizing hormone according to measurements performed concomitantly with TSH in the fluids of *in vitro* pituitary incubations; similarly, PCA-His-Pro-NH_2 does not stimulate secretion of luteinizing hormone *in vivo* in the rat (plasma levels measured by solid-phase radioimmunoassay) whereas it does stimulate secretion of TSH. Pure TRF has no oxytocic activity (rat uterus *in vitro*), no smooth muscle stimulating activity (guinea-pig ileum *in vitro*), no vasopressor activity (blood pressure recording of the pithed rat), no MSH activity (*in vitro* assay of Shizume *et al.*[16]). The biological activity of synthetic PCA-His-Pro-NH_2 is destroyed by incubation with plasma like that of hypothalamic ovine TRF (ref. 17 and W. V., R. B., T. F. D. and R. G., manuscript submitted). Furthermore, incubation of natural ovine TRF with a microbial pyrrolidone-carboxylyl peptidase (R. E. Fellows and A. Mudge, manuscript in preparation) destroys the biological activity; at the same time it liberates a ninhydrin-positive peptide which, as the free peptide or its dansyl-derivative, has chromatographic mobilities similar to those of a fragment—presumably His-Pro-NH_2—obtained by incubation of synthetic PCA-His-Pro-NH_2 in the same conditions. These results also favour the presence of an N-terminal PCA- in isolated ovine TRF. Plasma inactivation of TRF by a PCA-peptidase is an obvious possibility.

Conclusions in the Context of Other Work

Results from low and high resolution mass spectrometry, NMR and infrared spectrometry, thin-layer chromatographic data, amino-acid analyses and enzymatic degradation all favour the structure 2-pyrrolidone-5-carboxylyl-histidyl-proline amide (Fig. 6) for the ovine hypothalamic hormone TRF isolated in this laboratory. L-2-Pyrrolidone-5-carboxylyl-L-histidyl-L-proline amide prepared by synthesis has physical characteristics indistinguishable from those of natural ovine TRF; it also has similar biological activity.

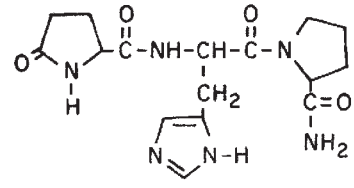


Fig. 6. Molecular structure of isolated hypothalamic ovine TRF.

Recently, Folkers *et al.*[18] and Bowers *et al.* (paper presented at a meeting of the American Thyroid Association, Chicago, November 1969) have confirmed that mixtures of synthetic peptides obtained by ammonolysis of the esterified derivatives of the sequence Glu-His-Pro have considerable TRF activity, in agreement with our earlier reports. Their statement that the synthetic peptides are not active orally is probably best explained by the fact that they administered doses of the materials which were too low. More recently, the same group[19] has reported on the similarity in properties of a preparation of porcine TRF[20] and a synthetic preparation "presumably (pyro)-Glu-His-Pro-NH_2" in a variety of thin-layer chromatographic systems and bioassays; characterization of the synthetic material was claimed on the basis of "its hydrolysis to the three amino-acids", its NMR spectrum and thin-layer chromatographic mobilities. Although other possibilities were considered, the authors concluded that the structure PCA-His-Pro-NH_2 was in agreement with the known properties of porcine TRF; these reports are reviewed in ref. 21. If the conclusions of Bøler *et al.*[19] are correct, then the structures of hypothalamic TRFs from two different species would be identical.

Our results, concerning the ovine TRF as it was isolated in our laboratory, do not exclude the possibility that the

native molecule might exist as Gln-His-Pro-NH_2, the N-terminal glutamine having been cyclized to PCA-during the extraction procedure; there are precedents for this in the chemistry of the eledoisins[22]. The possible activity of synthetic Gln-His-Pro-NH_2 has not yet been studied.

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[1] Guillemin, R., Yamazaki, E., Jutisz, M., and Sakiz, E., *CR Acad. Sci.*, **255**, 1018 (1962).
[2] Burgus, R., and Guillemin, R., *Proc. Conf. on Hypothalamic Hypophysiotropic Hormones, Tucson* (in the press).
[3] Burgus, R., Dunn, T. F., Ward, D. N., Vale, W., Amoss, A., and Guillemin, R., *CR Acad. Sci.*, **268**, 2116 (1969).
[4] Burgus, R., Dunn, T. F., Desiderio, D., Vale, W., and Guillemin, R., *CR Acad. Sci.*, **269**, 226 (1969).
[5] Burgus, R., Dunn, T. F., Desiderio, D. M., Ward, D. N., Vale, R., Guillemin, R., Felix, A., Gillessen, D., and Studer, R., *Endocrinology*, **86** (in the press).
[6] Gillessen, D., Felix, A. M., Lergier, W., and Studer, R. O., *Helv. Chim. Acta*, **74** (in the press).
[7] Burgus, R., Dunn, T. F., Desiderio, D., and Guillemin, R., *CR Acad. Sci.*, **269**, 1870 (1969).
[8] Schally, A. V., Arimura, A., Bowers, C. Y., Kastin, A. J., Sawano, S., and Redding, T. W., *Rec. Prog. Hormone Res.*, **24**, 297 (1968).
[9] Meadows, D. H., Zardetzky, O., Epand, R. M., Ruterjans, H. H., and Sheraga, H. A., *Proc. US Nat. Acad. Sci.*, **60**, 766 (1968).
[10] Guillemin, R., and Sakiz, E., *Nature*, **207**, 297 (1965).
[11] Vale, W., Burgus, R., Dunn, T. F., and Guillemin, R., *J. Clin. Endocrinol. Metab.*, **30**, 148 (1970).
[12] Guillemin, R., Yamazaki, E., Gard, D. A., Jutisz, M., and Sakiz, E., *Endocrinology*, **73**, 564 (1963).
[13] Ducommun, P., Sakiz, E., and Guillemin, R., *Endocrinology*, **77**, 792 (1965).
[14] Vale, W., Burgus, R., and Guillemin, R., *Proc. Soc. Exp. Biol. Med.*, **125**, 210 (1967).
[15] Guillemin, R., and Vale, W., *Endocrinol. Experimentalis*, **1**, 137 (1967).
[16] Shizume, K., Lerner, A. B., and Fitzpatrick, T. B., *Endocrinology*, **54**, 533 (1954).
[17] Guillemin, R., *Ann. Rev. Physiol.*, **29**, 313 (1967).
[18] Folkers, K., Enzmann, F., Bøler, J., Bowers, C. Y., and Schally, A. V., *Biochem. Biophys. Res. Commun.*, **37**, 123 (1969); Bowers, C. Y., Schally, A. V., Enzmann, F., Bøler, J., and Folkers, K., Program of 45th Meet. American Thyroid Assoc., Chicago, Nov. 13–15, 1969, p. 15.
[19] Bøler, J., Enzmann, F., Folkers, K., Bowers, C. Y., and Schally, A. V., *Biochem. Biophys. Res. Commun.*, **37**, 705 (1969).
[20] Schally, A. V., Redding, T. W., Bowers, C. Y., and Barrett, J. F., *J. Biol. Chem.*, **244**, 4077 (1969).
[21] Burgus, R., and Guillemin, R., *Ann. Rev. Biochem.*, **39** (1970) (in the press).
[22] Schröder, E., and Lübke, K., in *The Peptides*, **1**, 194; **2**, 133 (Academic Press, New York, 1965, 1966).
[23] Sakiz, E., and Guillemin, R., *Proc. Soc. Exp. Biol. Med.*, **115**, 856 (1964).

Defective Viral Particles and Viral Disease Processes

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Preparations of many different types of viruses contain defective particles which consist of viral structural proteins and a part of the viral genome. Such particles are capable of specifically interfering with the growth of homologous, standard virus and may play a major part in the evolution of viral diseases.

Biologically active defective viral particles have been described with increasing frequency in animal virus systems, suggesting that they may be more common than had been suspected. We propose that such defective particles have a role in determining the evolution of viral diseases. Many host defence mechanisms are known which combat viral infections[1,2]. These involve antiviral antibodies and lack of attachment sites on host cells. General factors such as fever, localized acidity, heredity and interferon have also been implicated but, except for some tentative suggestions[3–5], the defective virus has been overlooked as a possible important determinant in the outcome of natural viral infections.

To support our hypothesis that defective viral particles play a part in the course of viral infections, the present knowledge of defective particles will be summarized. Some *in vitro* studies will be presented which relate to known disease processes, and an explanation at the molecular level will be offered for the appearance of defective particles in viral preparations and for the biological activity of defective particles.

The defective particles which are pertinent here have the following properties: (1) they contain normal viral structural protein; (2) they contain a part of the viral genome; (3) they can reproduce in the presence of helper virus; (4) they interfere specifically with the intracellular replication of non-defective homologous virus. When defective particles are added to infected cells, the yield of non-defective virus is reduced. This interference can occur in the absence of detectable interferon and is dependent on the nucleic acid contained in defective particles. As will be seen, this specific interference by defective particles may be an important mechanism in virus–host interactions.

Because defective virus with the properties mentioned above occur in many and possibly all animal virus systems, we propose to call them DI particles (DI = defective interfering). The non-defective homologous virions which are capable of independent replication are called standard virus.

Animal Viruses which form DI Particles

Von Magnus[6] first described "incomplete" or DI particles, produced after inoculation of fertilized eggs with high multiplicities of influenza virus. The DI particles are antigenically identical to standard virions[7,8] but are lacking the largest of the RNA species found in standard virions and contain small, unidentified pieces of RNA[9].

The synthesis of similar DI particles by cells or animal tissues on infection with high multiplicities or "undiluted passage virus" has now been reported for Rift Valley fever virus[10], vesicular stomatitis virus[11], fowl plague virus[12], simian virus 40 (ref. 13), polyoma virus[14], lymphocytic choriomeningitis virus[15], Sendai virus[16],