Vol. 146, No. 1, 1987 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
July 15, 1987 Pages 354-360

ONTOGENESIS OF TRH mRNA IN THE RAT PANCREAS

A. Dutour, P. Giraud, C. Kowalski, L'H. Ouafik, P. Salers, V. Strbak*, and C. Oliver

Laboratoire de Neuroendocrinologie Expérimentale, INSERM U 297, Faculté de Médecine Nord Marseille Bd Pierre Dramard, 13326 Marseille Cedex 15, FRANCE

Received June 4, 1987

SUMMARY : The rapid changes in TRH levels in the rat pancreas during the neonatal period make this organ an interesting model for the study of the regulation of TRH biosynthesis. Pancreatic RNAs were isolated by the guanidinium thiocyanate method and layered onto CsCl cushion. Northern blot preparations were hybridized with 32P labeled TRH cDNA probe. Pancreatic TRH mRNA was first detected in 19-day old fetuses and reached the highest level on day 0, then decreased, being barely detectable 14 days after birth. The neonatal injection of streptozotocin induced a dramatic drop of TRH mRNA levels 24 hours later. This result suggests that the peculiar evolution of TRH level in pancreas is partly due to the evolution of the expression of the TRH gene. © 1987 Academic Press, Inc.

Despite the early isolation of TRH, the mechanisms of its biosynthesis have remained obscure until recently. The hypothesis that TRH, like other neuropeptides, arises from the post-translational processing of a large precursor protein (1,2) has been confirmed by the characterization of the cDNA clone coding for the precursor of TRH in the rat hypothalamus (3). The cDNA sequence of the TRH precursor encodes a protein of 255 amino acids; this protein contains five copies of the sequence Gln-His-Pro-Gly flanked by paired basic aminoacids.

Originally isolated from mammalian hypothalami, TRH has been detected all over the brain, the gastrointestinal tract

* Present address : Institute of Experimental Endocrinology Centre of Physiological Sciences Vlarska 3, Bratislava, Tchecoslovaquia.

0006-291X/87 $1.50
Copyright © 1987 by Academic Press, Inc.
All rights of reproduction in any form reserved.

and in the pancreas where TRH has been localized in the beta cells of the islets of Langerhans . The ontogenetic pattern of TRH in the pancreas is peculiar reaching a peak two days after birth before decreasing rapidly towards low levels in the adults (4,5).

We have previously shown (6,7) that the peptidylglycine α-amidating monooxygenase (PAMase) (one of the enzymes involved in the post-translational processing of the TRH precursor) reaches high levels of activity in the rat pancreas during the neonatal period, following an evolution pattern similar to that of TRH in this tissue.

In this study, we have quantified TRH mRNA levels in the rat pancreas during ontogenesis using a cDNA clone kindly provided by Dr R.H. Goodman.

MATERIALS AND METHODS

- Animals :
Female rats of the Sprague Dawley strain were bred in our laboratory and their litters were used in the experiments. The day sperm was identified in vaginal smears was designated day O of pregnancy and the day of birth was designated day O of life. Pools of pancreas were collected at various pre and postnatal developmental stages.

- Streptozotocin treated rats :
On the day of birth (Do) half of a litter was injected i.p. with streptozotocin (Sigma) (70 mg/kg dissolved extemporaneously in 0.1M sodium citrate, pH 4.6). Control rats received the same volume (0.1 ml) of the vehicle 24 hours later. The animals were sacrified and their pancreas collected. The glycemia of all streptozotocin-treated pups was higher than 3 g/l.

- Isolation of RNA :
Immediately after the collection of the pancreas, the RNA was isolated by the guanidium thiocyanate method (8) and was further purified by sedimentation through a layer of 5.7 M CsCl (36000 k, 15 hours). The RNA was ethanol precipitated, recovered by centrifugation, dried in vacuo, and finally dissolved in water. Absorbance measurements were obtained at 260-280 nm. The 260/280 ratio was between 1.5 and 1.6. Poly (A+) mRNA from 1 day old rats was selected on Oligo(dt) cellulose according to Aviv and Leder (9).

- Northern blot analysis :
RNA was denaturated in 50% formamide, 18% formaldehyde

buffer at 65°C for 5 min and then electrophoresed through a 1% agarose gel containing 18% formaldehyde, 20 mM Morpholino-propanesulfonic acid, 50 mM Sodium Acetate, 1 mM EDTA. The RNAs were electrotransfered onto a nylon membrane (Genescreen, Dupont), in 25 mM phosphate buffer. The membrane was heated at 80°C for 2 hours. The transfer of RNA from the gel to the membrane filter was controlled in each case by ethidium bromide staining of the gel.

- Hybridization :

TRH cDNA insert (a gift from R.H. Goodman) was excised from PUC 12 by Eco RI digestion. This insert was labelled by nicktranslation using (32P) deoxycytidine 5 triphosphate (Amersham 110 TBq/mmole) (9). A Specific activity of 10^9 cpm/ug of DNA was obtained. Each blot was incubated overnight at 42°C with 10 ml of prehybridization buffer (4xSSC, 50% formamide, 5X Denhardt's, SDS 0.1%, sonicated sperm DNA 250 ug/ml, sodium phosphate 50 mM), 10% dextran sulfate. Hybridization was performed overnight at 42° with gentle mixing in 10 ml of the above solution containing the nicktranslated probe ($1x10^6$ cpm/ml). After the hybridization, filters were washed twice at 50°C in 0.2 x SSC, 0.2% SDS; then, the filters were exposed to autoradiography for 48 hours with an intensification screen at -70°C. The hybridization signal was quantified by scanning the autoradiograph with a laser densimeter equiped with an area integrator (Shimadzu).

RESULTS

Ontogenesis of TRH mRNA (Fig. 1, Fig. 2 and Fig. 3)

TRH mRNA signal was present in the pancreas of 19 day old fetuses and reached a maximum on the day of birth (day 0). After day 3, an important decrease of TRH mRNA was evidenced.

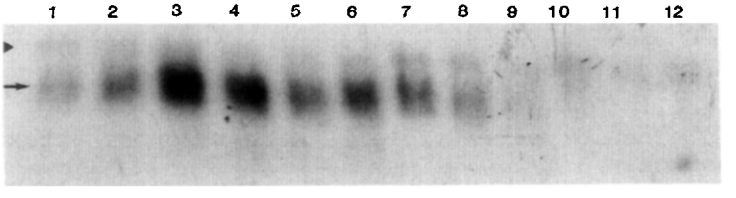


Fig. 1 . Northern blot analysis of pancreatic TRH mRNA during ontogenesis

After denaturation, RNA were electrophoresed through a 1% agarose, 18% formaldehyde gel, then electrotransfered to nylon membranes and hybridized with cDNA TRH probe. Then, the filter was exposed to autoradiography for 48 hours with an intensification screen at -70%C. The arrows corresponds to the TRH mRNA signal and the arrows head indicates the position of 18 S ribosomic RNA.

1=E 19, 2=E 20, 3=E 21, 4=D 0, 5=D 1, 7=D 3, 8=D 5, 9=D 9, 10=D14, 11, 12 = Adults

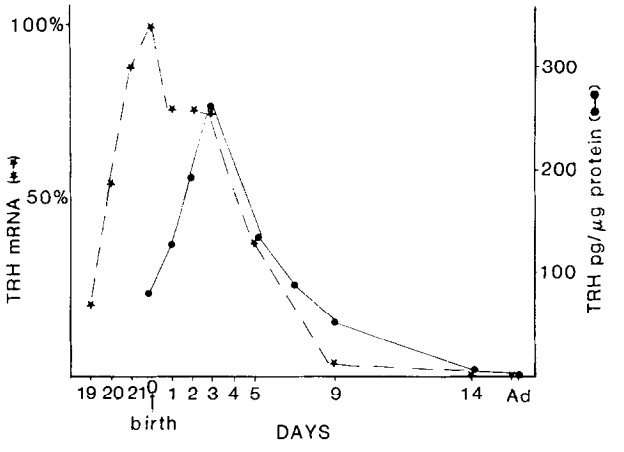


Fig. 2 . Ontogenesis of TRH mRNA (★--★) and TRH (●—●) concentrations in rat pancreas.

TRH mRNA is expressed as percentage of the level of TRH mRNA on day 0. TRH concentrations (pg/ug/prot) were previously determined (5,6) by specific radioimmunoassay in pancreatic extracts.
Ad = Adult.

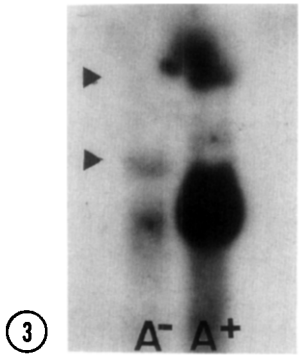
TRH mRNA was not detectable in adults. This study was performed with total RNA. The amount of mRNA run on the gels was thus limited (10 ug of total RNA run on the gels correspond to 0.1-0.3 ug of mRNA). Selection of polyadenylated RNA from 1 day old rat on Oligo(dt) cellulose column (Fig. 3) showed that TRH mRNA signal was polyadenylated with an estimated size of 1.9 kb corresponding to the size of TRH mRNA detected in rat hypothalamus (3).

Effect of streptozotocin (Fig. 4)

The administration of streptozotocin was followed 24 hours later by a dramatic drop in TRH mRNA levels.

DISCUSSION

The present study is the first demonstration of TRH mRNA in the rat pancreas. The presence of TRH mRNA in the rat pancreas is further evidence that TRH is synthesized in this organ although one cannot discard the possibility that it is also released by nerve endings at this level. The dramatic



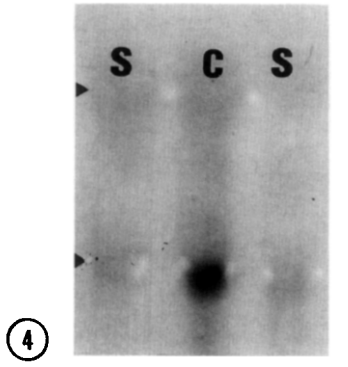


Fig. 3 . Northern blot analysis of Poly (A+) mRNA from pancreas of 1 day old rat

Poly (A+) mRNA from 1 day old rat was selected on Oligo(dt) cellulose according to Aviv and Leder (9). The Poly (A-) and Poly (A+) RNA was analysed by Northern blot as described in the legend of Fig. 1. The arrows head show 28 S and 18 S RNA.

Fig. 4 . Northern blot analysis of pancreatic TRH mRNA : effect of streptozotocin

Rats were injected with streptozotocin on day 0, pancreas were collected on day 1 and the pancratic RNA analysed by Northern blot as described in the legend of Fig. 1. The lane S correspond to treated rats, the lane C to controls. The arrows head show 28 S and 18 S RNA.

drop of TRH mRNA levels in streptozotocin-treated rats suggests that like Insulin, all TRH biosynthesis steps occur in the beta cells of the islets of Langerhans. Indeed, we have previously reported that half of PAMase, the enzymatic activity involved in the last step of the post-translational processing of TRH precursor, is also contained in the beta cells. As previously discussed, immunoreactive TRH concentrations in the rat pancreas reach a peak in 3-day old rats, then decrease rapidly toward the minute levels found in the adults. The present data indicate that the ontogenetic evolution of TRH mRNA parallels that of TRH. Indeed, a specific TRH mRNA signal is observed in the pancreas of 19-day old fetuses. The signal is highest during the 48 hours surrounding birth, then its intensity decreases rapidly. The

induction profile of TRH mRNA indicates that the rapid increase in this peptide is not associated with all events related to birth since it starts to occur before parturation. However, its subsequent decrease may be the consequence of changes in hormones levels, neuronal activity or nutritional states that are observed during the neonatal period. Using an indirect assay method for TRH precursor (by quantification of the immunoreactive TRH-OH generated after sequential enzymatic treatment of pancreatic extracts by trypsin and carboxypeptidase A) (2), L'H. Ouafik et al have shown that the evolution of this peptide follows the same ontogenetic profile (unpublished observations). Thus, it appears that the peak level of TRH mRNA and TRH precursor preceeds that of PAMase by one day and that of TRH by two days. It appears that the transcription of DNA coding for TRH precursor and the enzymatic PAMase activity involved in the post-translational processing of this precursor are similarly regulated in the rat pancreas during the perinatal period. The same coregulation has been observed in At-T20 cells which display a decreased in both Proopiomelanocorticotropin levels and PAMase activity, when incubated with glucocorticoids (12). The evolution pattern of insulin mRNA during pancreatic differentiation is quite different, as recently reported by Han et al (11). Insulin mRNA is first demonstrated in the pancreas of 12-day old fetuses and its level increase steadily until birth. The highest level is found in newborn rat where it is significantly higher than in adults.

The rapid changes in TRH levels in the rat pancreas during the neonatal period make this tissue a valuable model for studying the regulation of TRH biosynthesis. The availability of a method for measuring TRH mRNA in addition

to the determination of TRH, its precursor and PAMase activity should allow a precise evaluation of the mechanisms which are involved in TRH biosynthesis.

ACKNOWLEDGMENTS

The authors thank Dr R.H. Goodman (Boston, Ma) for his generous gift of TRH cDNA clone, Dr Dagorn (INSERM U 31) for valuable advice in the preparation of pancreatic RNA and Ms R. Querat for her excellent secretarial assistance. V. Strbak was supported by a grant from INSERM.

REFERENCES

1. Rupnow, J.H., Hinkle, P.M. & Dixon, J.E. (1979) Biochem. Biophys. Res. Commun. 89, 721-728
2. Ouafik, L'H., Dutour, A., Castanas, E., Boudouresque, F. & Oliver, C. (1985) Biochem. Biophys. Res. Commun. 128, 664-669
3. Lechan, R.M., Wu, P., Jackson, I.M.D., Wolf, H., Cooperman, S., Mandel, G. & Goodman, R.H. (1986) Science 231, 159-161
4. Martino, E., Lernmark, A., Hisao, S., Steiner, D.F. & Refetoff, S. (1978) Proc. Natl. Acad. Sci. U.S.A. 75, 4265-4267
5. Dutour, A., Ouafik, L'H., Castanas, E., Boudouresque, F. & Oliver, C. (1985) Life Sci. 37, 177-183
6. Ouafik, L'H., Dutour, A., Salers, P., Giraud, P., Boudouresque, F., Castanas, E. & Oliver, C. (1986) Biochem. Biophys. Res. Commun. 138, 179-184
7. Ouafik, L'H., Giraud, P., Salers, P., Dutour, A., Castanas, E., Boudouresque, F. & Oliver, C. (1986) Proc. Natl. Acad. Sci. U.S.A. 84, 261-264
8. Chiirgwin, J.M., Przybyla, A.E., Mac Donald, P.J. & Ruten, W.J. (1979) Biochemistry, 18, 5294-5399
9. Aviv, H. & Leder, P. (1972) Proc. Natl. Acad. Sci. USA 69, 1408-1412
10. Maniatis, T., Fritsch, E.F. & Sambrook, J. (1982) Molecular Cloning : A Laboratory Manual (Cold Spring Harbor, N.Y.).
11. Han, J.E., Rall, L. & Rutter W. (1986) Proc. Natl. Acad. Sci. U.S.A. 83, 110-114
12. Mains, R.E. & Eipper, B.A. (1984) Endocrinology 115, 1683-1690