Study on the Existence of TRH in the Cerebrospinal Fluid in Humans

Hiroshi Ishikawa

Department of Anatomy, School of Medicine
Showa University, Hatanodai,
Shinagawa-ku, Tokyo 142

Received August 21, 1973

Summary: TRH(TSH-releasing hormone) activity can be verified by withdrawing CSF(cerebrospinal fluid) from a cadaver two to five hours after death by suboccipital puncture or inserting a needle directly into the third ventricle after removing the top of the skull in cases of pathological examination. The fluid collected is concentrated down to 1,000 molecular weight or less, and TRH activity is determined by the in vitro bioassay method. Where this TRH is synthesized, or by what route it enters into CSF, or why it must enter into CSF are yet unanswered questions.

Löfgren originally suggested in 1960 that hypophysiotrophic hormones can perhaps be transported from its origin in the brain to the pituitary(1). Thereafter Knowles and Kumar(2), Kendall et al. (3), Poter et al.(4), Scott and Knigge(5) and others have speculated that CSF seems to transport hypophysiotrophic hormones. Also Matsui (6)(7) and Braak(8) observed morphologically that the endings of the neurons, which contain amine granules, come into contact with CSF in the third ventricle through the gaps between the ependymal cells. Heller et al.(9) found that anti-diuretic hormones exists in the CSF of rabbits; and Halász(10) speculated that releasing hormones(one kind of hypophysiotrophic hormones) secrete into the third ventricle after observing that the basophilic cells expanded from the transplantation of anterior pituitary cells into the infundiblum. Yoshimura, et al.(11) reported that isolated ungranulated chromophobes of rat anterior pituitaries were differentiated into acidophils or basophils after transplantation into third ventricles. Ohtsuka, et al.(12) demonstrated that

isolated ungranulated chromophobes from rats were differentiated into ACTH secreting acidophils by the action of CRF in vitro.

Bluni et al.(13), after observing the surface of the third ventricle under a scanning electron microscope, speculated that the ependymal cells have two functions: secretion and absorbtion. His report, however, did not consider the question of whether these functions are simultaneous or consecutive.

All of the above seems to suggest that posterior pituitary hormones, releasing hormones, and amine may secrete into the CSF of the third ventricle but, up to the present, direct evidence that CSF containes releasing hormones has never been presented. This study attempts to answer the question of whether TRH, one kind of releasing hormone, does indeed exist in CSF.

Materials and Methods: CSF was gathered by suboccipital puncture or direct a needle insertion into the third ventricle of three hundred male and female autopsy cases and cadavers within two to five hours after death(cases of death by brain disease or damage were excluded), and kept at -20°C. When, in withdrawing the CSF, any blood was detected to be mixed therein, it was discarded. By means of a diafilter(Type MC-4; manufactured by the Nihon Shinku Gijutsu Corporation), the stock of about 4,700 ml of CSF was concentrated at 4° C until 15 ml of concentrated solution which held less than 1,000 molecular weight was obtained. This was used as the sample and stored at -20°C.

The in vitro bioassay method of TRH: The synthetic TRH used in this study was made in the Yamanouchi Seiyaku Company by the solid phase technique. The synthesis of l-pyroglutamyl-l-histidyl-l-prolinamide is described by many investigators, including Folkers, et al.(14); Burgus et al.(15); Baugh et al.(16).

By cubing one hundred mg of fresh anterior pituitaries taken from twenty-five year old males into cubic millimeters and culturing the pieces as explants at 37°C under 95 % oxygen - 5 % carbon dioxide for 30 days in flasks containing a 2 ml medium of NCTC-109 (both the medium and the gases were changed daily), the explants became perfect monolayers. These monolayer cells secreted TSH at a rate of 200 ng/ml/day. When, next, various doses(in 0.001, 0.01, 0.1, 1, 10, 100, 1000 μg/ml) of synthetic TRH was added to the medium and, after 30 minutes, the amount of TSH released was radioimmunoassayed, the results occurred as shown in Figure 1. The

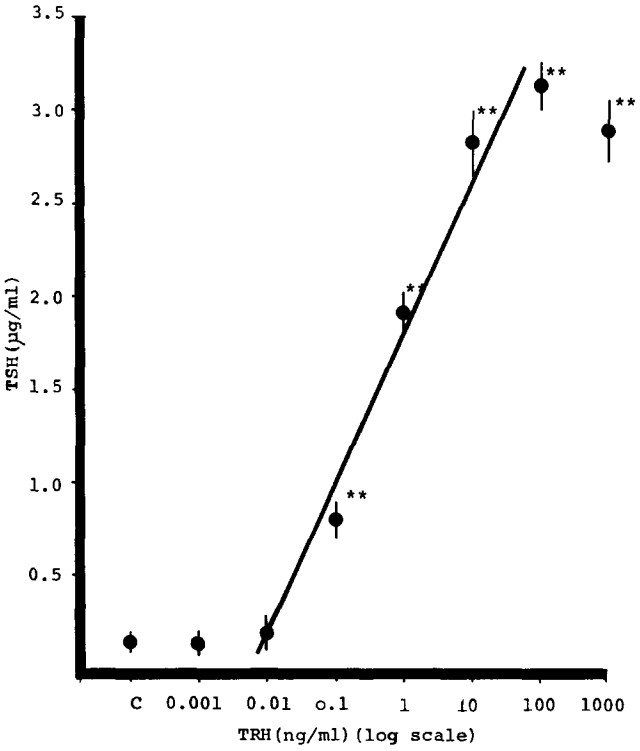

Fig. 1 Effect of synthetic TRH on the release of TSH from 100 mg human anterior pituitary tissue in vitro. TSH in the incubation medium were estimated by radioimmunoassay; each point represents the Mean±SE of 6 observations. Asterisks indicate levels of significance in comparison with control **$p < 0.01$

in vitro assay of TRH was carried out according to the graph in Figure 1.

Immunoassay of TSH: Measurements were performed in duplicate, using highly purified human TSH prepared according to the method of Hartree, et al.(17). 2.0 μg of highly purified TSH was mixed with 1.5 mCi of ^{131}I and iodinated by the chloramine-T technique of Greenwood et al.(18) to specific antibodies of 450 - 600 μCi ^{131}I/μg TSH. Details of radioimmunoassay procedure was carried out according to Patel et al.(19). Sensitivity was calculated at 0.06 μU of TSH. The standard curve(Fig. 2) was obtained using 250 μl TSH-free human serum.

Results: Samples of CSF which had been concentrated to under 1,000 molecular weight were placed in the amounts of 0.1, 0.2, 0.4, and 0.8 ml into NCTC-109 medium, forming, in each case, 2 ml of culture medium. Into this medium was put monolayer cells which were formed by anterior pituitary cells after 30 days in culture. After 30 minutes, the amount of TSH released in the medium was measured by the radioimmunoassay method(Figure 2). When these amounts of TSH are located on the vertical axis of the graph in Figure 1, the amount of TRH can be immediately found. Table 1 shows the amounts of TRH thus gotten in the cultures containing the varying amounts of concentrated CSF.

Discussion: This experiment clearly shows that TRH is included in CSF. However, since the CSF was gotten from human bodies two to five hours after death, the TRH may have been released some time after death. In a live body, if TRH does exist in CSF, what kind of nucleus or nuclei would be synthesized and by which route does it get into CSF ? Perhaps, as Vigh et al.(20), Leonhardt(21), Rodriguez(22), Wittkowski(23), Kobayashi(24) speculated, the

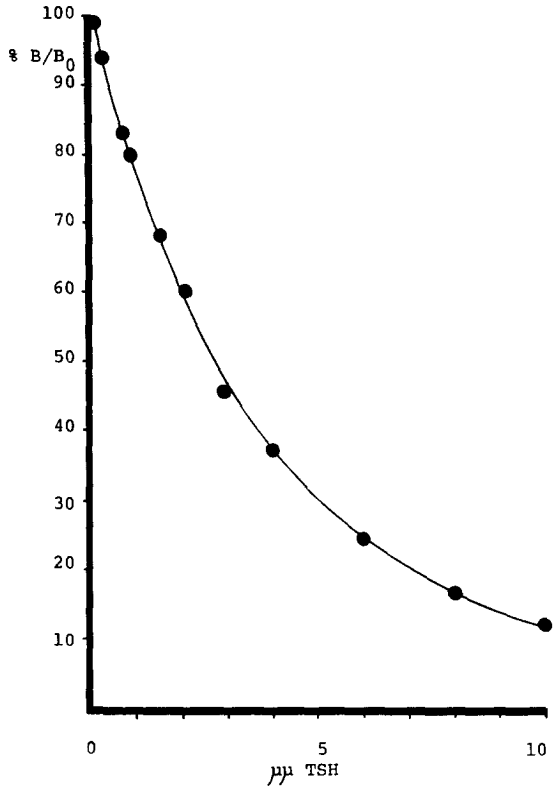

Fig. 2 The standard curve obtained by using a tracer mass of 25 pg and antiserum dilution to 1 : 300,000

Table 1.

Concentrated CSF (ml)	TRH (μg)
0.1	0.22 ± 0.04*
0.2	0.41 ± 0.06*
0.4	0.75 ± 0.10*
0.8	1.23 ± 0.24*

The amount of TRH in varing doses of concentrated CSF.
Asterisks indicate the Mean ± SE of six observations

ependymal cells either synthesize or release hormones by secretion, or both.

When we read the report of Kendall et al.(3), that when TRH was injected into both the third ventricle and the lateral ventricles in the brains of rats, the amount of TSH released in the third ventricle was greater, we might suspect that, in humans as well, there is a strong possibility that TRH would be secreted in the third ventricle. In any case, even though the reason why TRH is secreted in CSF remains a mystery at present, the determination that it is secreted in CSF seems to indicate that the secretion is somehow or other one part of the regulation mechanism of synthesis and release of TSH from the anterior pituitary.

References

1. Löfgren,F., Kungl. Fysiograf. Sallskapet I Lund Forh.30,115 (1960)
2. Knowles, F., Phil. Trans. roy.Soc., Lond.,B. 256,357 (1969)
3. Kendall,J.W.,Rees,L.H.,and Kramer,R., Endocrinology 88,1503 (1971)
4. Porter,J.C.,Mical,R.S.,Tippit,P.R.,and Drane,J.W., Endocrinology 86,590(1970)
5. Scott,D.E. and Knigge,K.M., Z.Zellforsch.microsk.Anat. 105, 1 (1970)
6. Matsui,T., J.Fac.Sci.Univ.Tokyo.Sec.IV. 11,49 (1966)
7. Matsui,T., J.Fac.Sci.Univ.Tokyo.Sec.IV. 11,71 (1966)
8. Braak,H.,Z.Zellforsh.microsk.Anat.83, 398 (1967)
9. Heller,H.,Hasan,S.H. and Saifi,A.Q.,J.Endocr. 41,273 (1968)
10. Halász,B.,Pupp,L. and Uhlarik,S., J.Endocr. 25, 147 (1962)
11. Yoshimura,F.,Harumiya,K.,Ishikawa,H. and Ohtsuka,Y., Endocrinol. Japon 16, 531 (1969)
12. Ohtsuka, Y.,Ishikawa,H.,Omoto,T.,Takasaki,Y. and Yoshimura,F., Endocrinol. Japon 18, 133 (1971)
13. Bluni,J.E.,Montemuro,D.G.,Clattenburg,R.E. and Singh,R.P., Anat.Rec., 174,407(1972)
14. Folkers,K.,Enzmann,F.,Boler,J.,Bowers,C.Y., and Schally, A.V.,Biochem.Biophys.Res.Commun.,37,123 (1969)
15. Burgus,R.,Dunn,T.F.,Desiderio,D. and Guillemin,R., C.R.Acad.Sci.(Paris) 269, 1870 (1969)
16. Baugh,C.M.,Kramdieck,C.L.,Hershman,J.M. and Pittman,J.A.Jr., Endocrinology 87, 1015 (1970)
17. Hartree,A.S.,Butt,W.R. and Kirkham,K.E., J.Endocr. 29, 61 (1964)

18. Greenwood,F.C.,Hunter,W.M. and Glover,J.S., Biochem.J. 89, 114 (1963)
19. Patel,Y.C.,Burger,H.G. and Hudson,B.,J.Clin.Endocr. 33, 768 (1971)
20. Vigh,B.,Aros,B.,Wenger,T.,Koritsansky,S. and Cegledi,G., Acta Biol.Hung. 13, 407 (1963)
21. Leonhardt,H.,Z.Zellforsch.microsk.Anat. 74, 1 (1966)
22. Rodríguez,E.M.,Z.Zellforsh.microsk.Anat. 102, 153 (1969)
23. Wittkowski,W.,Z.Zellforsch.microsk.Anat. 93, 530 (1969)
24. Kobayashi,H.,In: Brain-Endocrine Interaction. Median Eminence: K.M.Knigge,D.E.Scott, and A.Weindl,eds.Karger,Basel, pp 67 (1972)