juvenile hormone, but the onset of vitellogenin synthesis is probably related to the 1st burst of juvenile hormone. Additional experiments such as juvenile hormone applications and allatectomies will be performed at various times; they should provide further information on the role of the peaks of juvenile hormone. In any case, the synthesis of yolk proteins cannot be initiated by ecdysteroids in *Acheta*, since previous work[30] has shown that ecdysteroid titres begin to rise in hemolymph after the appearance of vitellogenins in the blood.

Thus, we can conclude that there are strong temporal relations between ovarian physiological features and juvenile hormone levels which would appear to play a prominent role in the onset of vitellogenin synthesis and in initiating yolk deposition in the oocytes.

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Influence of the route of administration on thyrotropin-releasing hormone concentration in the mouse brain[1]

T. Mitsuma and T. Nogimori

The 4th Department of Internal Medicine, Aichi Medical University, Nagakute, Aichi 480-11 (Japan), August 3, 1982

Summary. TRH penetrates the mouse brain after i.v., i.p., i.m., oral or rectal administration. Except when TRH is administered orally, the penetration rate is about 0.2% of the total dose administered. Higher TRH contents are reached after rectal or i.m. administration.

Thyrotropin-releasing hormone (TRH) is administered in treatment of several neurological and psychiatric disorders and is usually given i.v[2–5]. By this method of administration, low concentrations in the brain are achieved, but these decrease rapidly[6–8]. Until now, the influence of different routes of administration on the brain penetration rate of TRH in the mouse, with possible consequences for therapeutic use in humans, has not been investigated. We examine in this paper, therefore, the influence of the route of administration on TRH brain concentrations in mice.

Material and methods. Animals. Male (dd strain) mice weighing 20 g were used. They were housed in temperature (22 °C)- and humidity (60%)-controlled quarters and fed a diet of laboratory chow and water ad libitum.

Drugs. Synthetic TRH was obtained from Takeda Co., Ltd (Japan). For rectal administration of TRH, a suppository containing 5 mg of TRH was provided by Takeda Co., Ltd (Japan)[1].

Experimental procedure. 1 mg of TRH dissolved with 0.2 ml of saline was injected i.v., i.p., or i.m. 2 mg of TRH were administered through gastric tubes. Suppositories containing 5 mg TRH were administered. Saline was used in the control animals. The mice were serially decapitated by guillotine under ether anesthesia, after TRH or saline administration and trunk blood was collected. The brain tissue was obtained by the previously described method[9] and washed with cold saline (4 °C) to eliminate the blood. TRH contents in the brain and blood were measured by specific radioimmunoassay[10].

Statistics. Mean and SE of samples were calculated for each group. Student's t-test was used to evaluate the differences between control and experimental groups.

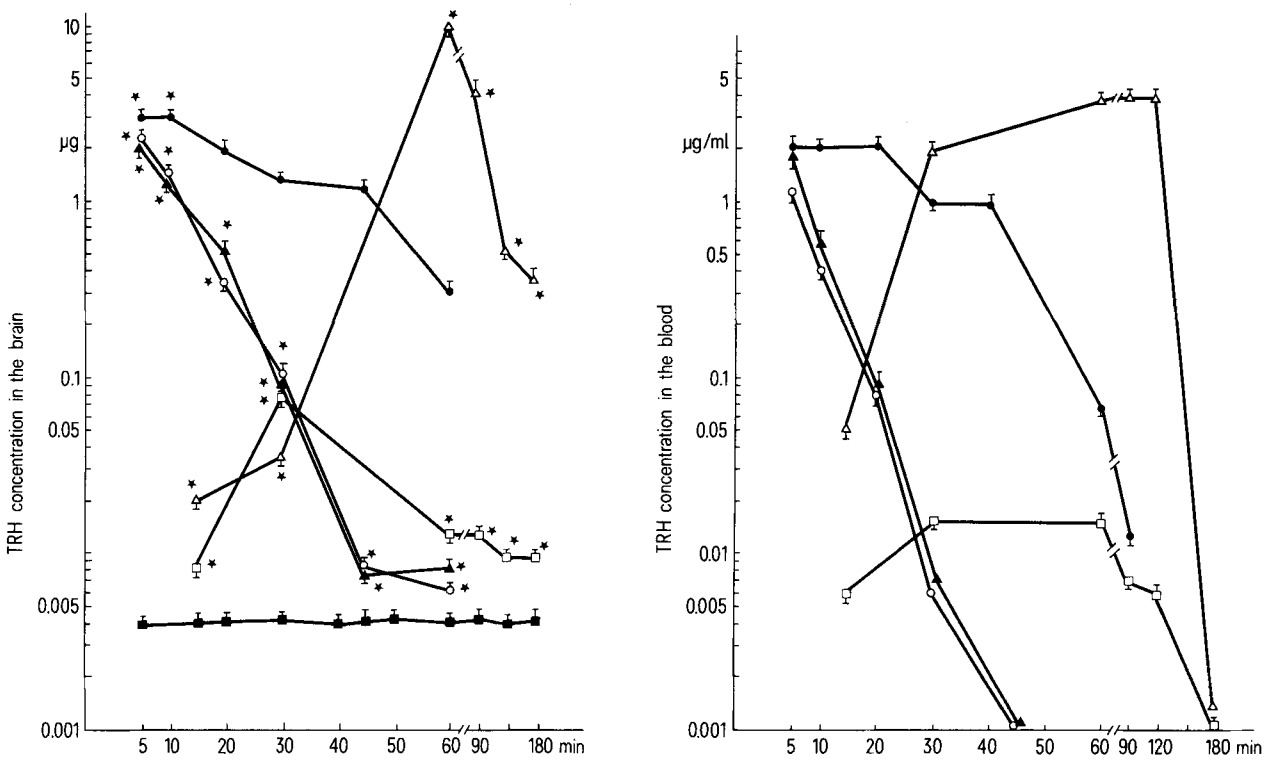

Figure 1. Changes in TRH concentrations in the brain and in the blood. Values are expressed as the mean ± SE in a group of 6 mice each. i.p. (▲——▲), i.v. (○——○), i.m. (●——●), p.o. (□——□), rectal (△——△), control (■——■). Differences from the control are indicated by * $p < 0.001$.

Results. TRH brain concentrations were significantly increased after TRH administration in each group. Peak levels of TRH in the brain were reached 5 min after i.v. or i.p. administration, 10 min after i.m., 30 min after oral, and 60 min after rectal administration (fig. 1). The TRH concentration at this time corresponded to 0.02-0.20% of the total doses administered in each group (fig. 2). The concentration in the brain decreased rapidly after i.v. or i.p. administration, but fell more slowly after i.m., oral or rectal administration (fig. 1). Blood TRH levels increased immediately, but decreased rapidly after peaking following i.v. or i.p. administration; after oral or rectal administration, the blood levels diminished more slowly.

Discussion. The present experiments clearly demonstrated that the TRH concentration in the brain increased following TRH administration by all routes. The brain penetration rate was approximately 0.2% of the total doses administered with the exception of the oral route. The findings confirm previous reports concerning i.v. administration[6-8]. TRH brain concentration decreased rapidly after i.v. or i.p. administration, and more slowly after i.m., oral or rectal administration. Therefore, changes in TRH concentrations in the brain might reflect the blood TRH levels.

The question can be raised as to whether the TRH measured in the brain might be partly derived from cerebral blood. However, the blood was eliminated from the brain tissue as far as possible. The cerebral blood volume is about 0.015 ml/g in brain tissues of rats[11-13]. On this basis it was calculated that 0.7% of the measured TRH was derived from cerebral flow. This amount is within the assay variation[10], so approximately 100% of the TRH measured in the present experiments was derived from brain tissue. The present findings, therefore, suggest that i.m. or rectal administration methods are to be favored when TRH is used therapeutically.

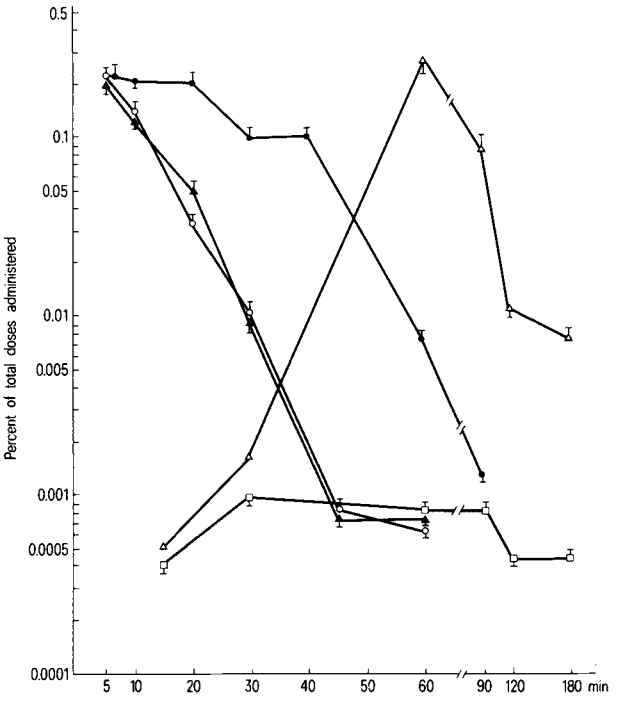

Figure 2. Changes of the ratio of TRH concentrations in the brain to total doses administered. Values are expressed as the mean ± SE in a group of 6 mice each, i.p. (▲——▲), i.v. (○——○), i.m. (●——●), p.o. (□——□), rectal (△——△).

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Intramural distribution of neuron specific enolase (NSE) in the human gastrointestinal tract

G.-L. Ferri[1], P.J. Marangos, S.R. Bloom and J.M. Polak[2]

Departments of Histochemistry and Medicine, Royal Postgraduate Medical School, Hammersmith Hospital, London W12 (England), and Laboratory of Clinical Science, National Institute of Mental Health, Bethesda (Maryland 20014, USA), January 10, 1983

Summary. NSE concentrations were measured by radioimmunoassay in the main separated layers of the human gastrointestinal tract. At all levels, a similar pattern of distribution of this protein was found, primarily parallel to that of nerve elements. Lower amounts of NSE were detected in the separated mucosal epithelium, containing the endocrine cells.

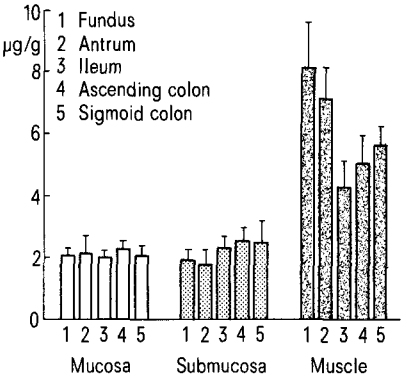
NSE is the γ-γ form of the glycolytic enzyme enolase[3-5], exclusively localized in neurons in the CNS[6]. More recently, NSE was also demonstrated in peptide- and/or amine-producing endocrine cells[7], including all endocrine cells types of the gut and pancreas[8], and in gut neurons[8,9]. In this study we have examined the quantitative intramural distribution of NSE immunoreactivity at various levels of the human gastrointestinal tract.

Materials and methods. Specimens of histologically normal stomach (fundus and antrum, n = 5), small and large bowel (ileum, n = 4, ascending colon, n = 4, and sigmoid colon, n = 7) were obtained fresh at surgery (from resections of carcinomas) and processed as previously described[10]. In brief, a sample from each specimen was taken as full-thickness control and another sample was microdissected into mucosa, submucosa and external muscle layer. A 3rd sample was injected with a 5 mmoles/l solution of sodium EDTA in the submucosa, incubated in the same solution (1 h) and vigorously shaken (20 sec). An epithelial pellet was collected by centrifugation, while the lamina propria was removed from the remainder of the wall by microdissection. This procedure proved to be highly effective in removing the epithelium from ileum and colon, but not from gastric samples. Its use in the stomach was therefore abandoned. Samples were extracted in 5 vols of 10 mmoles/l tris-PO_4, pH 7.3, containing 1 mmole/l $MgSO_4$. The polytroned homogenates were centrifuged at 100,000 × g for 1 h. NSE concentrations were measured by radioimmunoassay, as previously described[11]. The sensitivity was 100 pg per assay tube and the effective detection limit was 0.5–1 ng/ml. Samples of the separated layers were taken for routine histology, in order to check the quality of the separation obtained.

Results. In all cases, histology showed a clean separation between mucosa and submucosa, which took place at the level of the muscularis mucosae. After removal of the muscularis externa, only a few muscle fibers occasionally remained attached to the submucosa. No detectable contamination by structures from the other layers was found in the epithelial preparations.

The total recovery of NSE (sum of recoveries from the 3 separated layers) compared to the full-thickness samples was 101 ± 5% (mean ± SEM, n = 25). Of the total content of NSE, the largest part was recovered from the muscle layer (71.0 ± 2.4%, mean ± SEM, n = 25), while smaller amounts were found in the mucosa and submucosa (16.4 ± 1.6% and 12.6 ± 1.4%, respectively). When the mucosa was separated into epithelium and lamina propria, these 2 layers were found to contain 1.6 ± 0.2% and 15.0 ± 4.3%, respectively, of the NSE content (mean ± SEM, ileum and colon only, n = 15). The concentrations of NSE in the various layers are summarized in the figure.

Discussion. The separation procedure used in this study proved to be accurate and reproducible, as confirmed by histology, while the excellent recovery of NSE from the separated layers confirms its suitability for a quantitative study.



NSE concentration (µg/g of wet tissue weight) in the 3 separated layers at the various levels of the gastrointestinal tract. Bars represent means ± SEM; fundus and antrum; n = 5, ileum and ascending colon: n = 4, sigmoid colon: n = 7.