and since so much nitrogen is sequestered in canavanine, the bruchid larva is probably using canavanine as a food resource.

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2. The canavanine content of *D. megacarpa* seeds was calculated from the data of Table 1. The seed testa was not included in this determination.
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6. Some canavanine is sequestered in the adult but the amount is insignificant compared to that in the seed or frass.
7. Arginase assays were conducted as described in Table 3 except for the use of 250 μmole of L-arginine containing 0.25 μc of [^{14}C-guanidino]-L-arginine, 5 mg of commercially prepared urease (1250 unit/mg), and buffer at *p*H 9.5.
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11. The deproteinized assay mixture of Table 3 was analyzed for canaline production by the colorimetric assay method of G. A. Rosenthal [*Anal. Biochem.* **51**, 354 (1973)]. This assay revealed formation of at least 2.87 μmole of L-canaline. Similar analysis of the frass was not successful because of the presence of interfering substances. Reaction of the frass with Nessler reagent produced a strong positive reaction for ammonia.
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16. This work was supported by NSF grants GB-40198, GB-35032X, and BMS75-14268; by NIH biomedical support grant 5-SO5-RR07114-08; and by the Research Committee of the University of Kentucky. Critical reading of the manuscript of this report by S. F. Conti is also gratefully acknowledged. This is publication No. 76-7-152 of the Kentucky Agricultural Experiment Station, Lexington.

3 September 1976; revised 12 November 1976

Thyrotropin-Releasing Hormone: Stimulation of Colonic Activity Following Intracerebroventricular Administration

Abstract. *Intraventricularly administered thyrotropin-releasing hormone in rabbits elicited an increase in intraluminal pressure changes, a response commonly associated with muscular activity of the colon. The response appears to be central in origin with peripheral expression relying primarily on cholinergic receptors.*

For many years thyrotropin-releasing hormone (TRH) was considered to play a very specific role in the hypothalamicohypophyseal axis, as evidenced by the name. Recently, however, it has been found capable of producing physiological actions apparently independent of its pituitary influences. Clinical trials with parenterally administered TRH have led some researchers to attribute antidepressant capabilities to this compound (*1*), although others have questioned this conclusion (*2*). The results have been more consistent in animals than in humans, and they indicate stimulation of the central nervous system by TRH with or without the influence of anesthesia (*3*). Identification of TRH accumulations in various brain areas (*4*) has lent greater credence to the possibility that TRH and other neuropeptides actively participate in the modulation of various central integration processes.

Recent reports describing the clinical side effects of intravenously administered TRH often mention symptoms that are commonly associated with actions on the gastrointestinal tract, that is, nausea, vomiting, hunger, and poorly defined epigastric sensations (*5*, *6*). Although it has been proposed that these effects are not physiological but reflect a direct pharmacological action of TRH on smooth muscle (*5*), we believe that central mechanisms may be involved since we have been able to demonstrate an increase in gastrointestinal activity in rabbits injected intraventricularly with TRH. This phenomenon might represent an example of central modulation of a peripheral event by a neuropeptide; therefore, we felt that a more precise characterization of the response was warranted.

In this study we used male New Zealand rabbits (2.3 to 3.0 kg) that had been fasted for at least 24 hours and then anesthetized with sodium pentobarbital (35 mg/kg, intravenously). The intraventricular injection site was prepared by surgical techniques described by Jacob *et al.* (*7*). The abdominal area was shaved, topical anesthetic was applied, and a midline incision was made. The edges of the wound were sutured around a ring clamp suspended above the animal, creating a "well." Mineral oil was applied to the exposed viscera, and the ascending colon was located. A small incision was then made about 30 mm distal to the caecum, and an open-tipped cannula was introduced into the lumen and directed distally about 15 mm. The "well" was then covered with clear plastic, and a heating lamp was positioned above the animal to maintain a "well" temperature between 38° and 40°C. Recordings were begun approximately 30 minutes after the pentobarbital injection. Two different cannula-transducer systems were employed. One system consisted of a polyethylene cannula (PE 350) attached by a Y-tube to a Statham pressure transducer (0 to 5 cm-H_2O) and a Harvard infusion pump set to deliver at a rate of 194 μl/minute. The other system consisted of a glass cannula (2 mm inner diameter) attached directly to a Narco BioSystems pressure transducer (0 to 300 mm-Hg).

A typical response elicited by an intraventricular injection of TRH consisted primarily of an increase in magnitude of the intraluminal pressure changes associated with activity of the large intestine (Fig. 1). This response was observed in 80 percent of the animals (N = 20). Of the four animals not responding, three had received a dose of 10 μg of TRH, which was the lowest dose tested. We were unable to demonstrate a dose-response relationship using doses of 10, 20, 30, 50, and 100 μg. Pressure changes elicited by 10 μg and 20 μg of TRH often exceeded responses achieved at higher doses. This observation might be explained by the fact that the responsiveness of the large intestine is influenced by a large number of parameters, and that these parameters are impossible to control completely in a short-term preparation in situ such as ours.

Although there was a marked variation in the pressure patterns (*8*) recorded from individual rabbits, the patterns were unaltered by TRH administration in all but one animal. The frequency of the pressure changes also appeared to be unaffected in all but two animals. However, in the two apparent exceptions, changes that prior to TRH administration had been below the sensitivity limits of our instruments may have been unmasked. Base-line pressure (tone) responded variably to TRH, with most animals showing no change or a slight increase (0.8 cm-H_2O).

Because we had administered TRH in-

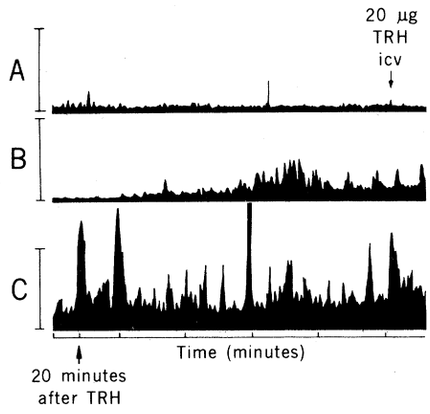

Fig. 1. Pressure changes in rabbit colon induced by intracerebroventricularly (icv) administered TRH (*15*). (A) Pressure recordings during the 5-minute interval immediately preceding the TRH injection. (B) An increase in colonic pressure changes became evident approximately 2 minutes after the TRH injection. (C) Twenty minutes after TRH administration colonic activity increased considerably. Bar height represents half the maximum pressure change recorded during the course of the experiment.

traventricularly, we suspected that the effects were evoked centrally. To strengthen this assumption, we administered TRH intravenously (500 μg) to three animals. After an immediate spike of activity, no further deviation from control activity was noted (Fig. 2). The initial sharp rise in intraluminal pressure we observed may be explained by the findings of Ormston (*9*) that TRH in high concentrations could cause a distinct contraction of guinea pig ileum in vitro and an ensuing increase in tone, and that TRH could potentiate the contractions of guinea pig ileum induced by acetylcholine and electrical stimulation. However, the response to centrally administered TRH described above differed from the response to the intravenously administered TRH in that the onset was more gradual and the increase in activity was of longer duration, often persisting well over 30 minutes. It appears then that the response elicited by intraventricularly administered TRH is centrally mediated. The possibility that slow TRH leakage from the cerebrospinal fluid, coupled with the direct stimulatory capability of TRH, caused the slow onset and long duration of action following intraventricular administration seems unlikely because of the high dose of TRH required to stimulate the guinea pig ileum in vitro and the short half-life of TRH in plasma (*10*).

An investigation with certain pharmacological agents indicated that the peripheral mediation of the TRH response of the colon in rabbits relies primarily on cholinergic receptors. A large dose of atropine methyl bromide (2 mg/kg, intravenous) was able to reduce TRH-induced activity to control levels although tone changes were not always reversed (Fig. 3). Two animals, however, showed resistance to inhibition by muscarinic blockade, which suggests that under certain circumstances other noncholinergic factors may be involved. Ganglionic blockade produced by tetraethylammonium (7 mg/kg, intravenous) and chlorisondamine (1 mg/kg, intravenous) appeared to be as equally effective as atropine at inhibiting TRH-induced colonic activity. The effectiveness of atropine and chlorisondamine at inhibiting the effects of TRH on the colon supports the concept that the parasympathetic nervous system mediates the gastrointestinal response to intraventricularly administered TRH. This view conforms with Gellhorn's theory (*11*) that the parasympathetic anxiety effects, such as abdominal cramp and diarrhea, result from a delayed excitation following a strong stimulation of the hypothalamus.

It has been a common observation that colonic disturbances frequently accompany situations of mental stress, as evidenced by the irritable-colon syndrome (*12*), yet little effort has been made to elucidate cerebral events associated with the modulation of large-intestine motility. Although the role played by the autonomic nervous system in the peripheral modification of intestinal motility has been investigated extensively, the biochemical processes leading to the central initiation

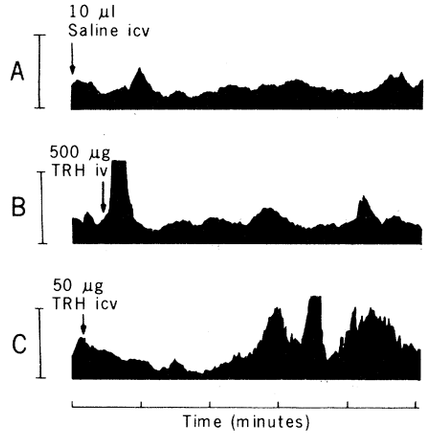

Fig. 2. A comparison between the pressure responses of rabbit colon after an intravenous (iv) injection of TRH and after an intracerebroventricular (icv) injection of TRH. (A) Intraluminal pressure recordings during the 5-minute interval immediately preceding an intravenous injection of TRH. (B) Colonic pressure changes after an intravenous injection of TRH. (C) Colonic response to TRH injected intraventricularly 22 minutes after the intravenous injection. Bar height represents half the maximum pressure change recorded during the course of the experiment.

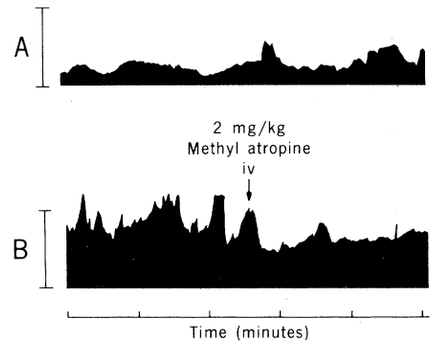

Fig. 3. Effects of methyl atropine (injected intravenously) on TRH-induced pressure changes in the rabbit colon. (A) Colonic pressure changes just prior to an intraventricular injection of TRH. (B) The changes in TRH-induced colonic activity after atropine administration. Methyl atropine was given 16 minutes after the TRH. Bar height represents half the maximum pressure change recorded during the course of the experiment.

of autonomic input are poorly understood. The available information implicates the hypothalamus and limbic systems as possible activation centers (*13*). Since TRH is found in high concentrations in the hypothalamus, it is not unreasonable to suggest that the colonic effects of TRH observed by us may arise from the stimulation of these activation centers. It is also interesting that thyroid disorders are sometimes accompanied by alterations in bowel function (*12, 14*).

The evidence presented here suggests that the neuroactive tripeptide, TRH, may play a role in the biochemical processes involved in the central activation of extrinsic nervous input to the large intestine.

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15. A typical polygraph recording apparatus was used to accumulate all tracings. The area under the curve was shaded to enhance contrast, which facilitated photographic duplication. The base line for each figure was arbitrarily chosen and remained constant within each figure.
16. This work was supported by DHEW Training Grant GM-00109 and HL-15426. The TRH was generously supplied by Abbott Laboratories, North Chicago, Ill.

20 September 1976; revised 19 November 1976

Adequate Response of Plasma 1,25-Dihydroxyvitamin D to Parturition in Paretic (Milk Fever) Dairy Cows

Abstract. *The concentration of 1,25-dihydroxyvitamin D was measured by means of a radioactive receptor assay in the plasma of cows during the period immediately prior to, during, and following parturition. Nonparetic cows showed initially a slight decrease in plasma 1,25-dihydroxyvitamin D which was followed by a significant increase during parturition and 2 days postpartum. The highest concentration achieved in the control or nonparetic cows was 100 picograms per milliliter. In the paretic animals the plasma 1,25-dihydroxyvitamin D concentration increased sharply during the day preceding calving and reached a maximum of 200 picograms per milliliter at parturition. This level was maintained during the ensuing 2.5 days. These results demonstrate that parturient paresis cannot be the result of insufficient synthesis or secretion of 1,25-dihydroxyvitamin D.*

Parturient paresis (milk fever) is a metabolic disease occurring at or near parturition, especially in high-producing dairy cows. The onset is associated with the initiation of lactation and is characterized by a rapid decline in serum calcium and phosphorous concentrations. Ultimately, the animal suffers from a low-calcium tetanic state which is usually rapidly corrected by the infusion of calcium gluconate. If the disease is left untreated, approximately 60 to 70 percent of the animals will succumb to this condition (*1*).

The underlying cause of parturient paresis remains unknown despite many years of investigation. Several hypotheses have appeared; one of the more popular is the idea that bone and intestine are resistant to the parathyroid hormone which is secreted in response to the hypocalcemia brought about by milk formation (*2*). Because parathyroid hormone also stimulates production of 1,25-dihydroxyvitamin D_3 [1,25-$(OH)_2D_3$], a major calcium-mobilizing hormone (*3*), it is possible that parturient paresis results from a failure to produce 1,25-$(OH)_2D_2$ in response to hypocalcemia and parathyroid hormone. The absence of adequate 1,25-$(OH)_2D_3$ would then cause skeletal resistance to parathyroid hormone and inadequate calcium absorption, giving rise to parturient paresis.

Recently we developed an assay for 1,25-$(OH)_2D_3$ which is highly specific and which can be carried out on reasonably small amounts of plasma (*4*). By using this assay we have measured the plasma concentrations of 1,25-$(OH)_2D_3$ in paretic and nonparetic animals prior to, during, and immediately following parturition. The results clearly demonstrate that paretic animals are fully capable of increasing their plasma 1,25-$(OH)_2D_3$ levels in response to the hypocalcemia brought about by parturition and milk formation.

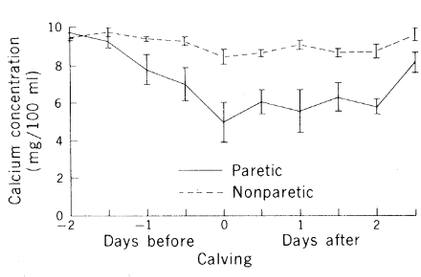
Eight Holstein cows (5 years or older) were fed a basal diet of low-moisture silage made from alfalfa, plus 1.8 kg of grain. This diet was adequate in energy and protein according to National Research Council requirements for maintenance and pregnancy (*5*). The diet supplied 80 to 85 g of calcium and 30 to 35 g of phosphorus daily from 35 days prepartum to 2.5 days postpartum.

Blood (50 ml) was collected from the jugular vein every 12 hours from 2 days before to 2.5 days after calving in heparinized tubes (5 unit/ml) packed in ice. In the case of parturient paretic animals, blood was always taken just prior to calcium infusions (500 ml of 25 percent calcium borogluconate). The plasma was separated immediately in a refrigerated centrifuge at 4°C and divided into 5-ml portions which were stored at −10°C until assayed.

The assay for 1,25-$(OH)_2D_3$ was carried out according to the method of Eisman *et al.* (*4*). Calcium was measured in the presence of 0.1 percent $LaCl_3$ by atomic absorption spectrophotometry with a Perkin-Elmer Model 403 instrument.

Four of the eight cows showed clinical signs of parturient paresis, that is, cold extremities, lateral recumbency, and hypocalcemia. These animals responded to infusions of calcium borogluconate and were, therefore, diagnosed as having parturient paresis.

Plasma calcium levels in the paretic cows decreased from 9.5 mg/100 ml at 2 days before calving to 5 mg/100 ml at calving (Fig. 1). On the other hand, the nonparetic animals showed only a slight decrease in serum calcium concentration during this period. Although the calcium infusions relieved the paretic symptoms, an elevated level of calcium in the serum was not sustained since the values were not appreciably higher at 0.5 days after the infusion. These results are consistent with previous reports for parturient pa-



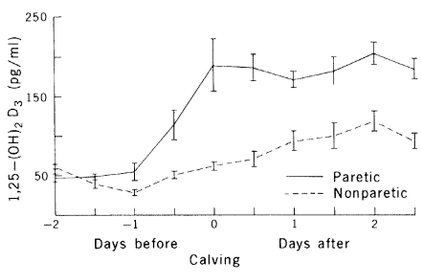


Fig. 1 (left). The concentration of calcium in the plasma of paretic and nonparetic cows at parturition. Day 0 represents parturition (calving), while negative figures represent days prior to calving. The data are presented as the mean ± standard error (vertical bars) for four animals in each group. Fig. 2 (right). The concentration of 1,25-$(OH)_2D_3$ in the plasma of paretic and nonparetic cows at parturition. Designations are as described in Fig. 1. Vertical bars represent standard errors, and there were four values for each point on the curve.