Pharmacology Biochemistry & Behavior, Vol. 14, pp. 901–905, 1981. Printed in the U.S.A.

Cardiovascular and Behavioral Changes after ICV Infusion of TRH in the Conscious Goat

LEA ERIKSSON[1] AND ARIEL GORDIN

Department of Physiology, College of Veterinary Medicine, Helsinki
and
Endocrine Research Laboratory, University of Helsinki and Minerva Foundation, Helsinki, Finland

Received 9 February 1981

ERIKSSON, L. AND A. GORDIN. *Cardiovascular and behavioral changes after ICV infusion of TRH in the conscious goat.* PHARMAC. BIOCHEM. BEHAV. 14(6) 901–905, 1981.—Thyrotropin releasing hormone was infused during 5 min into the lateral brain ventricle (ICV) of conscious goats in doses ranging from 125 to 4000 ng. Changes in blood pressure, heart rate and behavior were studied. TRH in doses of 500–4000 ng raised the mean blood pressure and both the magnitude and the duration of the response was related to the dose. The onset of the rise occurred in average 8 min after the start of the infusion. The heart rate fell somewhat with all doses. No consistent changes were observed in the respiration rate. TRH caused several behavioral changes beginning about 6 min after the start of infusion. The goats often decreased their locomotor activity. They were frequently bleating except after the lowest dose. These results support the view that TRH affects several vital functions in the central nervous system.

Behavior Blood pressure TRH Intraventricular administration Goats

IN ADDITION to the well known endocrine effects, the thyrotropin releasing hormone (TRH) also appears to have direct actions in the central nervous system. TRH exerts a great variety of behavioral effects and it interacts with several drugs [17]. Many of these effects can also be elicited in hypophysectomized and thyreoidectomized animals. Both systemic and central administration of TRH has been shown to cause behavioral changes, such as: arousal, excitation, increase in locomotion, suppression of food and water intake, decrease in aggression, changes in the sleep-wakefulness cycle and changes in the EEG.

TRH can influence also many vegetative functions. Changes in body temperature, rise of blood pressure, tachypnea, changes in intestinal motility, defecation, urination, mydriasis etc. have been reported [3, 6, 9, 10, 13, 14, 16, 20]. The cardiovascular effects of TRH are of special interest, because this neuropeptide is one of the most potent, centrally acting pressor agents.

Since a great deal of the above listed experiments have been performed in anesthetized animals, it was of interest to study the behavioral and cardiovascular effects of centrally administered TRH in unanesthetized animals. A conscious goat with a permanent ICV cannula provides an excellent model for such studies. In these calm and co-operative animals behavioral patterns can easily be recorded.

METHOD

Animals and Animal Care

Five adult female goats (weighing 31–38 kg) were used for repeated experiments. The animals were housed in metabolism cages by means of collars. All experiments were conducted in these cages. The minimum interval between experiments on each animal was 3 days. The goats were given hay and water ad lib.

Procedure

A permanent cannula of stainless steel or platinum-iridium was implanted into the lateral brain ventricle under general anesthesia. The anesthesia was performed with pentobarbital and petidin. The implantation and infusion techniques have been described earlier [1,2]. The goats were allowed to recover for two weeks after the operation before beginning the experiments.

TRH (Ferring International, Malmö, Sweden) was infused into the lateral ventricle in doses of 125, 500, 1000 or 4000 ng/0.1 ml of physiologic saline at a constant rate of 20 μl/min for 5 min. Infusions of physiologic saline (0.1 ml) were used for the controls.

[1]Send reprint requests to Lea Eriksson, Department of Physiology, College of Veterinary Medicine, PO Box 6, SF-00551 Helsinki 55, Finland.

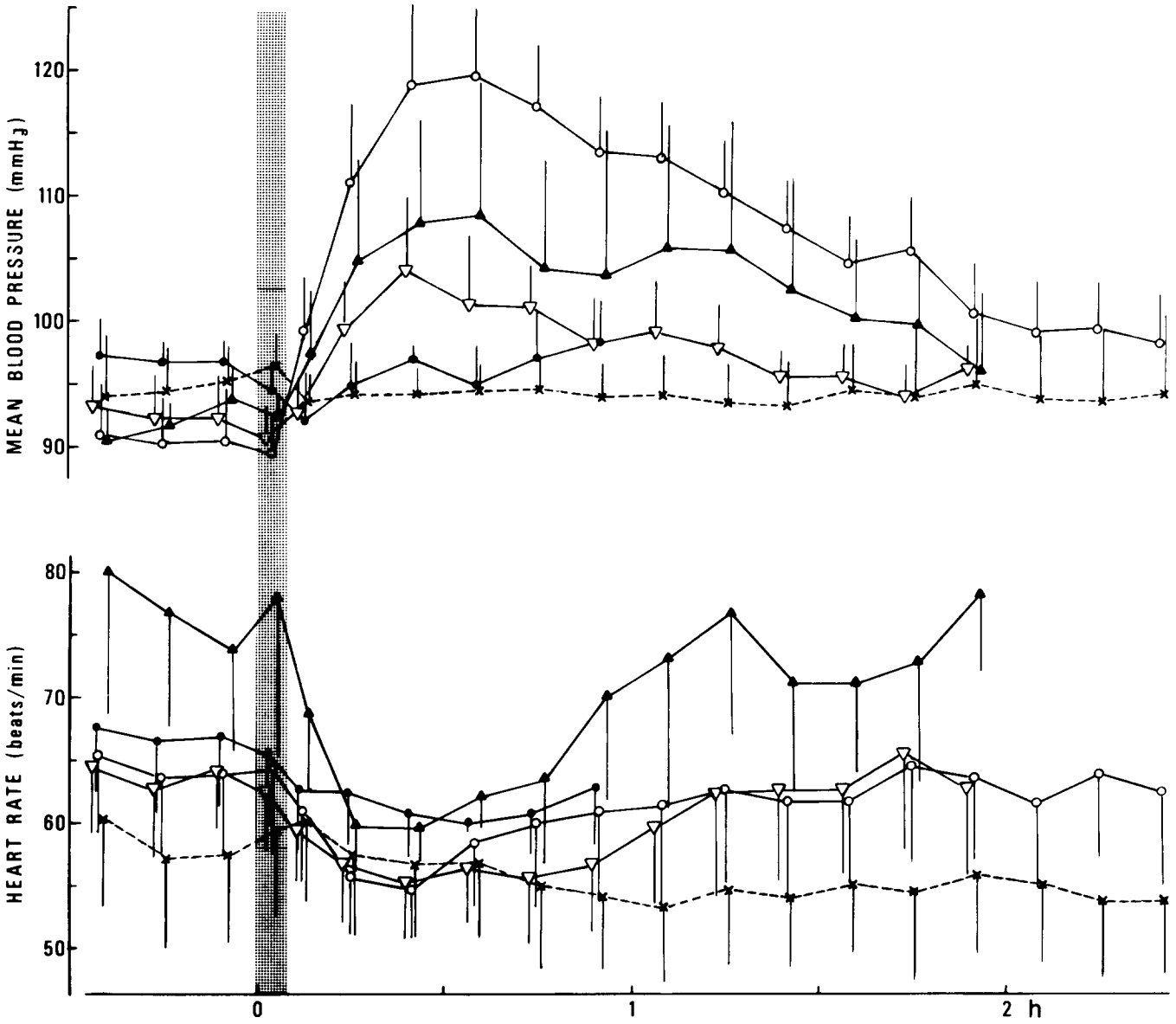

FIG. 1. Mean blood pressure (upper panel) and heart rate (lower panel) after infusion of different doses of TRH into the lateral ventricle of conscious goats. The infusion is indicated by the shaded area. The number of experiments (n) and the number of animals (n_a) are shown within parentheses. TRH 4000 ng ○—○ (n=6, n_a=5), 1000 ng ▲—▲ (n=4, n_a=3), 500 ng ▽—▽ (n=5, n_a=4), 125 ng ●—● (n=4, n_a=3), 0.15 M NaCl X----X (n=3, n_a=3). Means±SE are indicated.

Blood Pressure Recording

To record blood pressure, a permanent polyvinyl tube (i.d. 0.8 mm) was inserted into the carotid artery as described previously [7] at least two days before the first experiment. The mean and systolic/diastolic blood pressures and heart rate were recorded with a Beckman Dynograph (type S-II) or a Grass Polygraph (model 79D) using a Statham (P23AA) pressure transducer. Due to the narrowness of the catheter the values of systolic and diastolic pressures were only approximate values. Thus the changes in pulse pressure are given in percentage.

Behavioral Observations

The goats were well adapted to laboratory conditions and handling. They could move relatively freely in their cages and alertly followed their environment without any signs of stress or fear. The infusion could be performed without the animals noticing it. The behavioral parameters followed

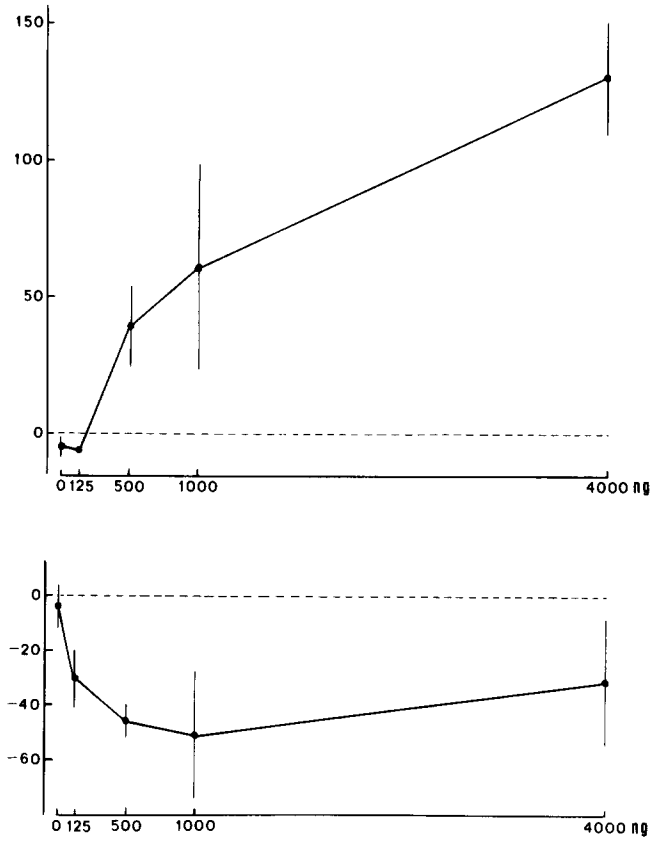

FIG. 2. Changes in mean blood pressure (upper panel) and heart rate (lower panel) during the first hour after the start of infusion (from the single experiments) plotted against the dose of TRH. The results of isotonic saline infusions are used as control. The number of experiments is same as in Fig. 1. Means ± SE are indicated. Arbitrary units, area under the curve, are used.

were: alertness, spontaneous motility, drinking of water, eating, ruminating, defecation, urination, vocalization and pattern of respiration.

RESULTS

Blood Pressure and Heart Rate

The changes in blood pressure and heart rate are shown in Figs. 1 and 2. TRH raised the blood pressure except after the lowest dose. Both the magnitude and the duration of the response was related to the dose. The onset of the rise occurred 4–15 min after the start of infusion and the pressure fell to the preinfusion level within 2 hr after 500 and 1000 ng, but lasted longer than 3 hr after the highest dose. The pulse pressure increased after 1000 ng and 4000 ng of TRH with in average 50 and 60 per cent, respectively. The heart rate fell somewhat with all doses of TRH after a latency time of 2–10 min. The magnitude of responses was relatively variable and the number of experiments rather small. Thus, the rise in blood pressure was statistically significant only after 4000 ng of TRH ($p<0.005$) and the fall in heart rate after 500 ng ($p<0.01$), when compared with the control infusions by the Student's t-test.

With the highest doses of TRH, oscillation of blood pressure (Mayer-like waves, see Table 1) occurred in two of the goats. The waves appeared about 30 min after the infusion, disappearing and reappearing irregularly. The frequency varied between 4–17 per min.

Behavior

TRH caused some clear behavioral changes, beginning about 6 min (3–10 min) after the start of the infusion (Table 1). In most experiments, the goats did not move their legs as usually but stood almost still for 10–30 min. After TRH they were often bleating for 5–10 min, except after the lowest dose. Grinding of the teeth and licking of lips was frequently seen. After the highest dose of TRH two of the four goats appeared somewhat uncomfortable. In spite of some passivity all the goats followed their environments and ate

TABLE 1

CARDIOVASCULAR AND BEHAVIORAL CHANGES DURING THE FIRST HOUR FOLLOWING ICV INFUSION OF TRH IN CONSCIOUS GOATS

	TRH				
	4000 ng	1000 ng	500 ng	125 ng	0.15 M NaCl
Rise in mean blood pressure (≧ 5 mm Hg)	5/5	4/4	4/5	0/4	0/3
Fall in heart rate (≧5 beats/min)	4/5	4/4	5/5	3/4	1/3
Oscillation of blood pressure (Mayer-like waves)	3/5	1/4	0/5	0/4	0/3
Bleating	4/5	2/4	3/5	0/4	0/3
Standing still	3/5	4/4	4/5	2/4	0/3
Grinding of teeth	4/5	0/4	1/5	0/4	0/3
Licking of lips	2/5	2/4	2/5	0/4	0/3

The changes are indicated as number of positives/number of experiments.

readily the hay they were offered. On the other hand, they did not drink water. No consistent change in the respiration rate was observed.

No deviations from normal behavior were noted after infusion of saline.

DISCUSSION

Cardiovascular Effects of TRH

Centrally given TRH has been found to raise blood pressure in several species, e.g. in curarized or pentobarbital-anesthetized rabbits [3,10], in conscious or chloralose-anesthetized cats [6], in pentobarbital-anesthetized dogs [6], in urethane-anesthetized rats [13] and in conscious goats (present study). Calculation of the smallest effective dose per kg body weight for the different species gives the following values: 0.5–1 ng for rabbit, administered intracisternally [3], 10 ng for rat, given into the lateral ventricle [13], 15 ng for goat, given into the lateral ventricle.

The site of action of TRH in elevating blood pressure has not yet been located. Quite a rapid and wide distribution of the solution occurs following intraventricular or intracisternal drug administration. The most rapid onset of action has been reported in rabbits after intracisternal injection (within 20 sec) [3], whereas a slower onset was observed after injection into the lateral ventricle [10]. On the other hand, intracisternal injection of TRH in the cat did not raise the blood pressure at all, whereas injection into the lateral ventricle was effective [6]. In rats the blood pressure rose within 2 min following injection into the lateral ventricle [13]. The delay of response in goats after the start of ICV infusion was somewhat longer (in average 8 min), which appears quite natural when taking into consideration the larger volume of the ventricular system. In several studies concerning behavioral effects of TRH, microinjections into different brain loci have been used. Corresponding investigations for evaluation of pressor action of TRH are still lacking, but should be performed before further conclusions as to the site of action of TRH in elevating blood pressure can be drawn. In any case, the extreme potency and the fast action of TRH support the view, that this neuropeptide might be one of the natural contributors in the blood pressure control system.

Likewise, the mechanism of action of TRH appears complex and there is a great deal of discrepancy in opinions [6, 10, 17]. This aspect was not studied in the present work.

In conscious goats, TRH infusions decreased the heart rate. Activation of the baroreflex by the pressor response appears to be a sufficient explanation. However, bradycardia was observed even after the smallest dose, which did not raise the blood pressure. Furthermore, with higher TRH doses the heart rate often started to slow down even before the rise in blood pressure. Slowdown of heart rate seen in unanesthetized goats can thus in part be a direct effect of centrally given TRH. In curarized rabbits, bradycardia has also been reported during the peak pressor response [3]. On the other hand, TRH has a distinct chronotropic effect in anesthetized rats, cats and dogs [6,13]. Anesthesia and differences of species can at least partly explain these varying results. Further studies are needed to settle the role of TRH in the control of heart rate.

Behavioral Effects of TRH

A characteristic behavioral change observed in goats after centrally administered TRH (3.5–130 ng/kg) was unwillingness of the animal to move. The difference was obvious when comparing with the saline treated control animals or the intact goats. The behavioral pattern differs distinctly also from that reported in laboratory rodents. Both rats [8,15] and rabbits [9,23] became excited and increased their locomotor activity after ICV or intracerebrally administered TRH. Those experiments involved, however, relatively high doses. Furthermore, some studies on rats fail to support the view that TRH might be a locomotor stimulant [5,18]. In cats, small doses of TRH (10 ng into mesencephalon or 5 μg ICV) have been observed to have a sedative and debiliting effect in addition to several other actions [14,16], while a high dose (200 μg ICV) increased the total time when animals stayed awake [12]. In the present study two of the four goats appeared somewhat restless and uncomfortable following the highest dose of TRH (4000 ng). It seems possible, therefore, that unphysiologically high doses of TRH can induce excitation and increase locomotion even in goats.

Another characteristic change in the goats was their intensified vocalization. Similar response has been reported in cats following TRH injection (10–20 ng) into the mesencephalic reticular formation [16]. It is not possible to answer without an EEG-registration to what extent this may reflect increased alertness. In a previous study we have observed that a histamine H_1-agonist, 2-pyridylethylamine, induced vigorous bleating in goats after ICV infusion [21]. In that study, the animals appeared on the whole very alert and active, a response clearly different from that seen after TRH. Beale and co-workers [3] have reported a long-lasting EEG-activation in both curarized and uncurarized rabbits after intracisternal injection of 20 ng to 200 μg of TRH.

The analeptic activity of TRH is also well documented [17]. In electrophysiological studies TRH has been reported to have predominantly inhibitory and, to a smaller extent facilitatory effects on neurons in several brain loci [11,19].

TRH has been reported to suppress both eating and drinking in food-deprived rats already with doses of 0.2 μg ICV [22]. In our experiment, the goats showed no signs of acute loss of appetite, but ate readily the hay they were offered. Quantitative measurements of food intake within a fixed time interval are, however, necessary to achieve comparable results.

TRH can apparently cause many different and partly controversial effects depending on which of the multiple receptor sites [4] have been stimulated. Different routes of application, various doses, differences in animal size and species, as well as the basal arousal state of the animal are factors that affect the final response. Anyway, TRH must be regarded as one of the several peptides of the brain that modify behavior.

ACKNOWLEDGEMENTS

Our thanks are due to Mrs. Irja Lundelin and Miss Annukka Enervi for skillful technical aid. The gift of TRH from Ferring International, Malmö, Sweden is gratefully acknowledged. This work has been supported by grants from the Academy of Finland and the Signe and Ane Gyllenberg Foundation.

REFERENCES

1. Åkerlund, L.-E., I. Andersson and K. Olsson. A cannula system for frequent infusions into the CSF of the cerebral ventricles of the goat. *Physiol. Behav.* **10:** 161–162, 1973.
2. Andersson, B., K. Olsson and R. G. Warner. Dissimilarities between the central control of thirst and the release of antidiuretic hormone (ADH). *Acta physiol. scand.* **71:** 57–64, 1967.
3. Beale, J. S., R. P. White and S.-P. Huang. EEG and blood pressure effects of TRH in rabbits. *Neuropharmacology* **16:** 499–506, 1977.
4. Burt, D. R. and S. H. Snyder. Thyrotropin releasing hormone (TRH): apparent receptor binding in rat brain membranes. *Brain Res.* **93:** 309–328, 1975.
5. Costall, B., S.-C. G. Hui, G. Metcalf and R. J. Naylor. A study of the changes in motor behaviour caused by TRH on intracerebral injection. *Eur. J. Pharmac.* **53:** 143–150, 1979.
6. Delbarre, B., D. Senon and H. Schmitt. Further attempts to characterize the action of cAMP and TRH in control blood pressure regulation. In: *Molecular Biology and Pharmacology of Cyclic Nucleotides*, edited by G. Folco and R. Paoletti. Amsterdam: Elsevier/North Holland, 1978, pp. 251–254.
7. Eriksson, L., O. Fernández and K. Olsson. Differences in the antidiuretic response to intracarotid infusions of various hypertonic solutions in the conscious goats. *Acta physiol. scand.* **83:** 554–562, 1971.
8. Heal, D. J. and A. R. Green. Administration of thyrotropin releasing hormone (TRH) to rats releases dopamine in n. accumbens but not n. caudatus. *Neuropharmacology* **18:** 23–31, 1979.
9. Horita, A. and M. A. Carino. Thyrotropin-releasing hormone (TRH)-induced hyperthermia and behavioral excitation in rabbits. *Psychopharmac. Communs.* **1:** 403–414, 1975.
10. Horita, A., M. A. Carino, H. Lai and T. R. LaHann. Behavioral and autonomic effects of TRH in animals. In: *Central Nervous System Effects of Hypothalamic Hormones and Other Peptides*, edited by R. Collu, A. Barbeau, J. R. Ducharme and J.-G. Rochefort. New York: Raven Press, 1979, pp. 65–74.
11. Ishibashi, S., Y. Oomura and T. Okajima. Facilitatory and inhibitory effects of TRH on lateral hypothalamic and ventromedial neurons. *Physiol. Behav.* **22:** 785–787, 1979.
12. King, C. D. Inhibition of slow wave sleep and rapid eye movement sleep by thyrotropin releasing hormone in cats. *Pharmacologist* **17:** 211, 1975.
13. Koivusalo, F., I. Paakkari, J. Leppäluoto and H. Karppanen. The effect of centrally administered TRH on blood pressure, heart rate and ventilation in rat. *Acta physiol. scand.* **106:** 83–86, 1979.
14. Metcalf, G. TRH: a possible mediator of thermoregulation. *Nature* **252:** 310–311, 1974.
15. Miyamoto, M. and Y. Nagawa. Mesolimbic involvement in the locomotor stimulant action of thyrotropin-releasing hormone (TRH) in rats. *Eur. J. Pharmac.* **44:** 143–152, 1977.
16. Myers, R. D., G. Metcalf and J. C. Rice. Identification by microinjection of TRH-sensitive sites in the cat's brain stem that mediate respiratory, temperature and other autonomic changes. *Brain Res.* **126:** 105–115, 1977.
17. Nemeroff, C. B., P. T. Loosen, G. Bissette, P. J. Manberg, I. C. Wilson, M. A. Lipton and A. J. Prange, Jr. Pharmaco-behavioral effects of hypothalamic peptides in animals and man: focus on thyrotropin-releasing hormone and neurotensin. *Psychoneuroendocrinology* **3:** 279–310, 1979.
18. Nomoto, T. and T. Tonoue. Actions of thyrotropin-releasing hormone in the central nervous system. *J. Pharmacobiodynamics* **3:** s9 and s17, 1980.
19. Renaud, L. P. and J. B. Martin. Thyrotropin releasing hormone (TRH): depressant action on central neuronal activity. *Brain Res.* **86:** 150–154, 1975.
20. Smith, J. R., T. R. LaHann, R. M. Chesnut, M. A. Carino and A. Horita. Thyrotropin-releasing hormone: stimulation of colonic activity following intracerebroventricular administration. *Science* **196:** 660–662, 1977.
21. Tuomisto, L. and L. Eriksson. Cardiovascular and behavioural changes after i.c.v. infusions of histamine and agonists in conscious goat. *Agents Actions* **10:** 165–166, 1980.
22. Vijayan, E. and S. M. McCann. Suppression of feeding and drinking activity in rats following intraventricular injection of thyrotropin releasing hormone (TRH). *Endocrinology* **100:** 1727–1730, 1977.
23. Weitzman, R. E., H. M. Firemark, T. H. Glatz and D. A. Fisher. Thyrotropin-releasing hormone stimulates release of arginine vasopressin and oxytocin in vivo. *Endocrinology* **104:** 904–907, 1979.