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14. Effects on CNS and Anterior Pituitary Function

EFFECTS OF ANTERIOR PITUITARY HORMONES AND THEIR RELEASING HORMONES ON PHYSIOLOGICAL AND BEHAVIORAL FUNCTIONS IN RATS

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SUMMARY

The effects of direct administration of TRH, TSH, LHRH, LH, ACTH, GH, FSH and prolactin into cerebral ventricle system on metabolic, respiratory, cardiovascular and behavioral responses were assessed in unanesthetized rats. Intraventricular administration of TRH, TSH, LHRH or LH caused hypothermia, decreased metabolism and/or cutaneous vasodilation at room temperature (22°C). Intraventricular administration of FSH, ACTH or prolactin caused hyperthermia, increased metabolism and/or cutaneous vasoconstriction. Intraventricular administration of GH caused an insignificant change in thermoregulatory responses. There was no change in respiratory evaporative heat loss in response to either of the drugs tested. In addition, intraventricular administration of TRH, LHRH or LH caused tachycardia, hypertension and a reduction in the epinephrine-induced reflex bradycardia. In contrast, intraventricular administration of prolactin caused bradycardia, hypotension and an enhancement in the epinephrine-induced reflex bradycardia in conscious rats. There was no change in cardiovascular function in response to intraventricular administration of TSH, FSH, ACTH or GH. Furthermore, following intraventricular administration of TRH, but not TSH, LHRH, LH, FSH, GH, ACTH or prolactin three main categories of behavior were provoked: activity of normal type—forward locomotion stimulation, head and body rearing; stereotype activity—increased grooming and head swaying; and abnormal type behavior—tail elevation and piloerection in rats. The data indicate that most of the anterior pituitary hormones and their releasing hormones act through a central mechanism to influence physiological and/or behavioral functions.

INTRODUCTION

The possibility that peptides or anterior pituitary hormones play neurotransmitter roles in the central nervous system has long been suggested but only recently begun to be supported by various lines of evidence. For example, direct administration of luteinizing hormone-releasing hormone (LHRH) into the anterior hypothalamus in ovariectomized, estrogen-treated female rats facilitated the induction of lordosis reflex [8, 9]. In addition to its thyrotrophic action on the release of thyroid-stimulating hormone (TSH), thyrotropin-releasing hormone (TRH) was also shown to be active in altering neural discharge rates of different central neurons and in affecting behavior [3, 10].

In the present study, we have attempted to further assess the effects of administration of six established anterior pituitary hormones (including TSH, LH, FSH, GH, ACTH and prolactin) and two hypothalamic releasing hormones (including TRH and LHRH) into the cerebral ventricle system on metabolic, respiratory, cardiovascular and behavioral responses in unanesthetized rats, in order to explore the probable neurotransmitter roles of these hormones or peptides in the central nervous system.

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MATERIALS AND METHODS

Adult male Sprague–Dawley rats weighing between 250–300 g were used in all experiments. Both the thermoregulatory and the cardiovascular experiments were performed on the conscious animals minimally restrained in rat stocks, while the behavioral experiments were performed on the conscious animals in a freely moving state. Between experiments the animals were housed individually in wire mesh cages in a room of 22 ± 1.0°C with natural light–dark cycles. The animals were given free access to tap water and granular chicken feed supplied by Taiwan Sugar Corporation (Taipei, Taiwan, Republic of China).

Cannula implantation

For direct administration of the drugs into the lateral cerebral ventricle of animal's brain, stainless-steel cannulas consisting of a guide tube with a snug fitting trocar and a cannula insert introduced into the guide tube at the time of injection, were used [6, 7]. The cannula guide tubes with trocars were implanted using the stereotaxic atlas and co-ordinates of König and Klippel[4]. The animals were anesthetized with pentobarbital sodium (6 mg/100 g, i.p.) and placed in a Kopf stereotaxic apparatus. The following co-ordinates were used: A, 6.0 mm; L, 1.5 mm; and H, 2.0 mm.

After appropriately located craniotomy holes had been trephined, two self-tapping screws were attached to the calvarium of the parietal bones, and the cannula guide tubes were inserted to the desired depth through the craniotomy holes. They were anchored with fast-drying dental cement to the cranial surface that had been scraped clean of periosteum.

A period of 2 weeks was allowed to permit the animals to recover from the operation. At the time of injection, the cannula insert was connected to a 10-μl Hamilton microsyringe by PE-20 polyethylene tube.

Measurements of thermoregulatory responses

The effects of these drugs on metabolic, respiratory, vasomotor and body temperature responses in conscious rats were assessed in a small animal partitional calorimeter [5–7]. Metabolic rate (M) was calculated from the animal's oxygen consumption. Respiratory evaporative heat loss (E_{res}) was calculated by measuring the increase in water vapor content in the helmet effluent air over that of the ambient air. Rectal (T_r), foot skin (T_f) and tail skin (T_t) temperatures were measured using copper-constantan thermocouples. All measurements were taken once per one minute throughout the experiments, each variable being measured as a d.c. potential on a Hewlett–Packard digital voltmeter (DVM 3455) interfaced to an on-line computer Hewlett–Packard 9825. Each minute all temperatures, M, and E_{res} were calculated instantaneously by the computer and relayed immediately back to the laboratory where they were displayed by an on-line printer Hewlett–Packard 9871.

Measurements of cardiovascular function

The femoral arterial pressure was monitored with a Stathman P23AC tranducer, and heart rate was monitored with a Grass 7C tachometer triggered by arterial pulses. The right femoral vein was cannulated for intravenous injection. All recordings were made on a four-channel Grass 7C polygraph. Both arterial blood pressure and heart rate were measured in unanesthetized animals according to the method of Thuránsky [11].

Measurements of behavioral responses

All experiments were performed between 10.00 h–12.00 h or between 15.00 h–17.00 h as pilot results showed that responses to TRH during these two periods were comparable. Conscious animals were acclimated to an electronic activity monitor (Stoelting Company, Chicago, IL) for 1 h per day for 1 week before the experiments. Selectable sensing rate and sensitivity enable the research to simit attention to the type of movements desired. In the present study, both gross movements (such as forward walking, running or rearing) and fine movements (such as grooming, scratching, tremor, head swaying, gnawing, or licking) were monitored, In addition, other types of behavioral responses such as backward walking, circling, tail elevation or piloerection were also observed grossly. Animals were injected intraventricularly with drugs or control vehicles and then placed in the center of the sensor for motor activity counts.

Drug solutions

Rats were injected with an intraventricular dose of thyrotropin-releasing hormone (TRH; Sigma Chemical Co, St. Louis, MO), thyroid-stimulating hormone (TSH; Sigma Chemical Co), follicle-stimulating hormone (FSH; NIAMDD-rat-FSH-RP-1), luteinizing hormone (LH; NIAMDD-rat-LH-RP-1), luteinizing hormone-releasing hormone (LHRH; NIAMDD-rat-LHRH-RP-1), adrenocorticotropic hormone (ACTH; Sigma Chemical Co), growth hormone (GH) or prolactin (NIAMDD-rat-RP-1).

Data analysis

The maximal changes in T_r, T_f, T_t, M and E_{res} produced within 90 min after drug injections were collected at ambient temperatures (T_a) of 8°, 22° and 30°C. On the test day the animals were placed on sensor of an electrotonic activity monitor right after injection of drugs. The activity recorded every 10 min for 60 min. Motor activity counts in 10-min intervals were plotted against time after injection and differences between arithmetic means of activity counts were evaluated by Student's t-test.

Histological verification

After the completion of experiments, the animals were killed with an overdose of sodium pentobarbital. Later, sections of the fixed brain were cut and stained with hematoxylin-eosin so that the stereotaxic coordinates of cerebroventricular cannulas were verified.

RESULTS

Effects of anterior pituitary hormones and hypothalamic releasing hormones on thermoregulatory responses. Animals were exposed to each T_a for a period of at least 90 min to attain thermal balance before the drugs were administered. Direct administration of TRH, TSH, LHRH or LH caused hypothermia at $T_a = 22$°C. The hypothermia induced by TRH and TSH was due to both decreased metabolism and cutaneous vasodilatation, while the hypothermia induced by LHRH or LH was due to decreased metabolism only (Table 1). Intraventricular administration of FSH, ACTH or prolactin caused hyperthermia in rats (Table 1). The hyperthermia induced by FSH was due solely to increased metabolism, while the hyperthermia induced by ACTH or prolactin was due to both increased metabolism and cutaneous vasoconstriction. Intraventricular administration of GH produced an insignificant change in thermoregulatory responses (Table 1). Again, there was no change in respiratory evaporative heat loss in response to either of these hormones.

Table 1. Maximal changes in rectal temperature (T_r), foot skin temperature (T_f), tail skin temperature (T_t), metabolic rate (M) and respiratory evaporative heat loss (E_{res}) produced within a 90-min period after an intraventricular dose of 0.9% saline or drugs in conscious rats at room temperature (22°C)

Treatments	T_r (Δ°C)	T_f (Δ°C)	T_t (Δ°C)	ΔM (W/kg)	ΔE_{res} (W/kg)
1. 0.9% Saline (n = 8)	0.1 ± 0.06	0.5 ± 0.19	0.4 ± 0.17	0.4 ± 0.11	0.03 ± 0.01
2. *TRH* 20 μg (n = 8)	−1.0 ± 0.08*	2.5 ± 0.33*	3.2 ± 0.35*	−1.0 ± 0.12*	0.04 ± 0.02
3. TSH 6 μg (n = 8)	−1.7 ± 0.14*	5.0 ± 0.67*	4.7 ± 0.61*	−1.2 ± 0.13*	0.03 ± 0.01
4. LHRH 40 μg (n = 8)	−0.9 ± 0.07*	−0.4 ± 0.18	−0.5 ± 0.15	−1.3 ± 0.10*	0.03 ± 0.01
5. LH 20 μg (n = 8)	−1.0 ± 0.9*	−0.5 ± 0.21	0.6 ± 0.22	−1.1 ± 0.09*	0.03 ± 0.01
6. FSH 5 μg (n = 8)	1.5 ± 0.12*	−0.4 ± 0.21	−0.4 ± 0.22	2.4 ± 0.12*	0.04 ± 0.02
7. GH 6 μg (n = 8)	0.3 ± 0.13	0.4 ± 0.19	−0.5 ± 0.22	0.3 ± 0.10	0.03 ± 0.01
8. ACTH 4 μg (n = 8)	1.1 ± 0.09*	−2.5 ± 0.35*	−2.2 ± 0.37*	1.0 ± 0.11*	0.03 ± 0.01
9. Prolactin 9 μg (n = 8)	1Ö ± 0.08*	−3.7 ± 0.29*	−1.7 ± 0.26*	1.0 ± 0.12*	0.04 ± 0.02

* Significantly different from corresponding control values (saline group), $P < 0.05$ (Student's t-test). The values are expressed as the mean ± SE; n = numbers of rats tested.

Effects of anterior pituitory hormones and hypothalamic peptides on cardiovascular responses. Table 2 summarizes the effects of intraventricular injection of hormones on the spontaneous levels of both the mean arterial blood pressure and heart rate. It was found that intraventricular injection of TRH, LHRH or LH caused tachycardia and hypertension. In contrast, intraventricular administration of prolactin caused bradycardia and hypotension in rats. There was no change in cardiovascular responses in response to intraventricular injection of TSH, FSH, ACTH or GH.

Table 3 summarizes the effects of hormones on the reflex bradycardia induced by epinephrine. Over the dose range (1–5 μg/kg) of epinephrine, a dose-dependent vasopressor and bradycardia response was obtained. In animals pretreated with an intraventricular dose of TRH, LHRH or LH the bradycardic responses were significantly attenuated, although the vasopressor responses were not significantly different from control. In contrast, in animals pretreated with an intraventricular-dose of prolactin, the bradycardic responses were significantly enhanced, although the vasopressor responses were not significantly different from control. The cardiovascular responses induced by i.v. epinephrine were not significantly affected by pretreatment of animals with an intraventricular dose of TSH, FSH, ACTH or GH in rats.

Effects of anterior pituitary hormones and hypothalamic peptides on behavioral responses. Following

Table 2. Effects of intraventricular administration of TRH, TSH, LHRH, LH, FSH, ACTH, GH and prolactin on cardiovascular responses in rats

Treatments	Control values (before drug)	Maximal values (after drug)	Difference
Mean arterial pressure (mmHg)			
1. 0.9% Saline	105 ± 16.4 (8)	107 ± 18.6 (8)	2 ± 0.55 (8)
2. TRH 20 μg	104 ± 14.4 (8)	135 ± 15.7 (8)	31 ± 2.35 (8)*
3. TSH 8 μg	102 ± 13.7 (8)	106 ± 16.2 (8)	4 ± 1.27 (8)
4. LHRH 40 μg	103 ± 14.5 (8)	114 ± 12.8 (8)	12 ± 1.78 (8)*
5. LH 20 μg	101 ± 15.6 (8)	121 ± 13.9 (8)	20 ± 4.12 (8)*
6. FSH 5 μg	102 ± 17.5 (8)	97 ± 14.3 (8)	−5 ± 2.11 (8)
7. ACTH 4 μg	95 ± 13.8 (8)	100 ± 11.3 (8)	5 ± 1.13 (8)
8. GH 6 μg	104 ± 15.2 (8)	107 ± 13.2 (8)	3 ± 1.20 (8)
9. Prolactin 4 μg	101 ± 12.8 (8)	81 ± 12.1 (8)	−20 ± 2.47 (8)*
Heart rate (beats/min)			
1. 0.9% saline	402 ± 20.4 (8)	405 ± 19.8 (8)	3 ± 1.02 (8)
2. TRH 20 μg	404 ± 19.8 (8)	456 ± 20.2 (8)	52 ± 4.33 (8)*
3. TSH 8 μg	410 ± 21.6 (8)	408 ± 21.5 (8)	−2 ± 1.12 (8)
4. LHRH 40 μg	401 ± 18.3 (8)	421 ± 19.7 (8)	20 ± 2.63 (8)*
5. LH 20 μg	405 ± 21.2 (8)	439 ± 20.7 (8)	34 ± 4.17 (8)*
6. FSH 5 μg	403 ± 20.7 (8)	412 ± 21.6 (8)	9 ± 3.24 (8)
7. ACTH 4 μg	400 ± 19.3 (8)	404 ± 20.9 (8)	4 ± 1.14 (8)
8. GH 6 μg	408 ± 18.5 (8)	405 ± 21.1 (8)	−3 ± 1.23 (8)
9. Prolactin 4 μg	402 ± 19.8 (8)	370 ± 18.6 (8)	−32 ± 2.53 (8)*

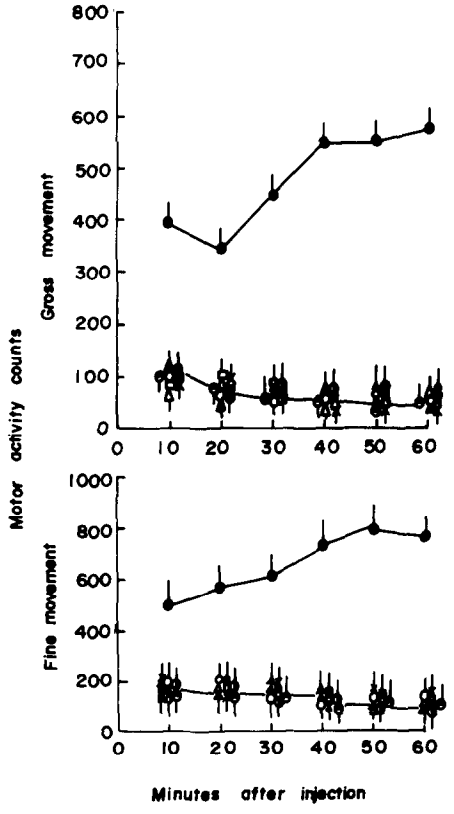
* Difference is statistically significant from the corresponding control values (saline group), $P < 0.05$ (Student's t-test). The values are expressed as means ± SE. The numbers in parentheses denote the numbers of rats tested.

Table 3. Effects of TRH, TSH, LHRH, LH, FSH, ACTH, GH and prolactin on the cardiovascular responses induced by epinephrine (EPI) in conscious rats

Treatments	Mean arterial pressure (mmHg)			Heart rate (beats/min)		
	Control	After EPI	Difference	Control	After EPI	Difference
1. 0.9% Saline, i.c.v. + EPI, i.v., 2.5 μg/kg, $n = 8$)	104 ± 17.8	176 ± 20.4	72 ± 11.6	407 ± 21.7	324 ± 16.8	−83 ± 7.97
2. *TRH* 40 × g, i.c.v. + EPI 2.5 μg, i.v., $n = 8$	103 ± 15.4	172 ± 18.9	69 ± 12.3	404 ± 18.5	364 ± 16.7	−40 ± 5.71*
3. *TSH* 8 μg, i.c.v. + EPI 2.5 μg/kg, i.v., $n = 8$	105 ± 16.2	175 ± 21.2	70 ± 13.5	402 ± 19.0	322 ± 15.9	−80 ± 8.01
4. LHRH 40 μg, i.c.v. + EPI 2.5 μg/kg, i.v., $n = 8$	106 ± 14.7	181 ± 19.2	75 ± 13.4	401 ± 17.6	353 ± 15.9	−48 ± 6.42*
5. LH 20 μg, i.c.v. + EPI 2.5 μg/kg, i.v., $n = 8$	105 ± 18.6	179 ± 19.9	74 ± 13.1	403 ± 16.8	360 ± 15.5	−43 ± 5.26*
6. FSH 6 μg, i.c.v. + EPI 2.5 μg/kg, i.v., $n = 8$	102 ± 16.6	173 ± 21.4	71 ± 10.8	400 ± 17.9	322 ± 15.3	−78 ± 8.83
7. ACTH 4 μg, i.c.v. + EPI 2.5 μg/kg, i.v., $n = 8$	101 ± 15.8	174 ± 20.3	73 ± 12.2	405 ± 19.1	320 ± 17.4	−85 ± 9.54
8. GH 6 μg, i.c.v. + EPI 2.5 μg/kg, i.v., $n = 8$	100 ± 16.8	168 ± 22.6	68 ± 13.5	399 ± 18.7	321 ± 18.8	−78 ± 8.66
9. Prolactin 9 μg, i.c.v. + EPI 2.5 μg/kg, i.v., $n = 8$	105 ± 14.7	175 ± 19.7	70 ± 12.2	409 ± 21.0	289 ± 14.3	−120 ± 9.11*

* Significantly different from the corresponding control values (saline group), $P < 0.05$ (Student's t-test). The values are expressed as means ± SE; n = numbers of rats tested; i.c.v. = intracerebroventricularly; i.v. = intravenously.

intraventricular administration of TRH,, but not TSH, FSH, ACTH, LHRH, LH, GH or prolactin, three main categories of behavioral were provoked: activity of normal type—forward locomotion stimulation, head and body rearing; stereotype activity—increased grooming and head swaying; and abnormal type behavior—tail elevation and piloerection in rats (Fig. 1 and Fig. 2).



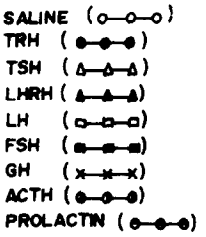


Fig. 1. Motor activity counts including both gross and fine movements in acclimated rats after intraventricular administration of TRH, TSH, LHRH, LH, FSH, ACTH, GH and prolactin. Each point depicts the mean ± SE. The drug was injected at $t = 0$ ($n = 8$ each).

DISCUSSION

Thyrotropin-releasing hormone was the first hypothalamic peptide to be isolated. TRH was found in and was active in neural pathways outside the hypothalamic regions [1, 2, 12]. In animals with longterm hypophysectomy, TRH can reverse the CNS (behavioral, cardiovascular and thermoregulation) depression induced by barbiturates and alcohol [3, 10]. The present results also showed that intraventricular administration of TRH caused behavioral responses such as forward locomotion stimulation, head and

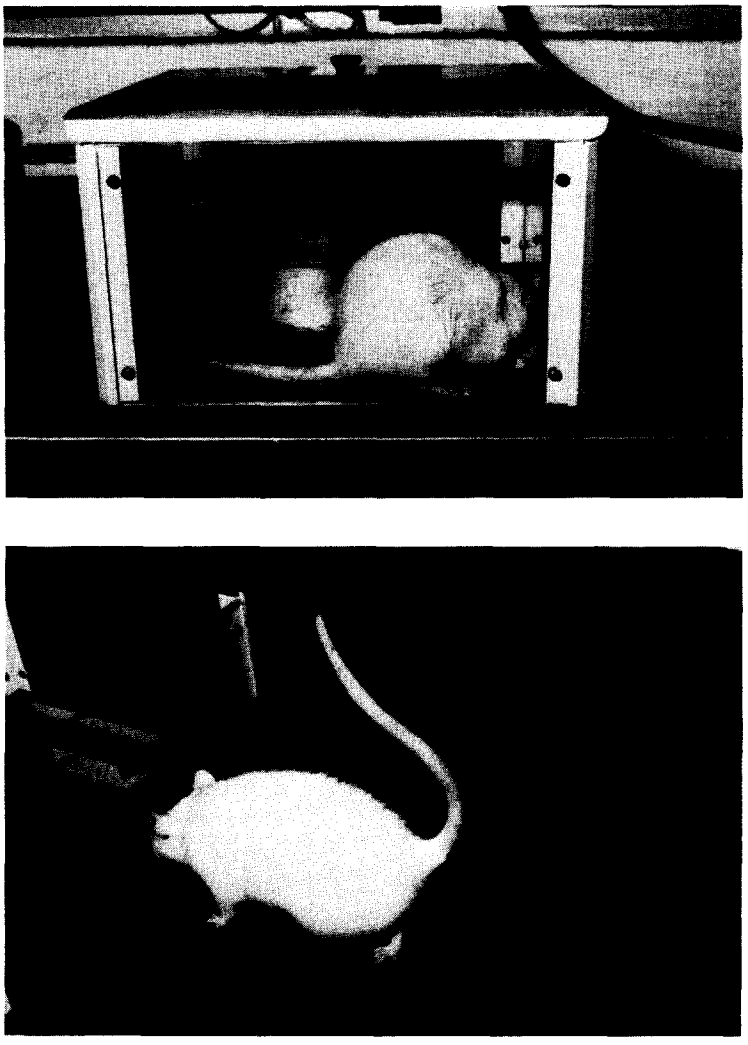


Fig. 2. (A) Intraventricular administration of 0.9% saline produced no behaviors distinguishable from that elicited by a normal rat. (B) Intraventricular administration of 20 μg of TRH caused tail elevation, piloerection and other excitement in a rat.

body rearing, increased grooming, head swaying, tail elevation and piloerection in rats. Our recent results showed that the behavioral excitation induced by TRH was antagonized by pretreatment of rats with either a narcotic receptor antagonist naloxone, an α-adrenergic antagonist yohimbine, or a dopaminergic antagonist haloperidol, but not with a β-adrenergic receptor antagonist propranolol. The data indicate that both opiate and catecholaminergic receptors are involved in the TRH-induced behavioral excitation (Lin, Chan, Chen and Teh, unpublished data) in rats. The results also showed that TRH acted through a central transmitter mechanism to induce hypothermia, cutaneous vasodilatation, decreased metabolism, and a reduction in the reflex bradycardia induced by epinephrine.

The principal hormone action of adrenocorticotropic hormone is to stimulate adrenocortical secretion and growth. In addition to its hormone action, the results demonstrated that central administration of ACTH caused hyperthermia, increased metabolism and cutaneous vasoconstriction in rats.

Thyroid-stimulating hormone stimulates thyroid secretion and growth. The present results showed that central injection of TSH caused hypothermia, cutaneous vasodilatation and decreased metabolism in rats. This is consistent with that produced by central injection of its releasing hormone TRH. Thus, it appears that TRH acts through the release of TSH from the anterior pituitary gland to induce hypothermic action in rats.

Luteinizing hormone stimulates ovulation and luteinization of ovarian follicles in female and testosterone secretion in male. The results also demonstrated that central administration of LH caused hypothermia, decreased metabolism, tachycardia, hypertension and a reduction in the reflex bradycardia induced by epinephrine. Central injection of its

releasing hormone LHRH was also shown to cause the same effects. This indicates that LHRH acts through the release of LH from the anterior pituitary gland to influence both the thermoregulatory and the baroreflex mechanisms in rats.

Follicle-stimulating hormone stimulates follicle growth in female and spermatogenesis in male. The present results also showed that central administration of FSH caused hyperthermia and increased metabolism in rats.

Prolactin stimulates secretion of milk and maternal behavior and maintains corpus luteum in female rodents. The present results showed that central administration of prolactin caused hyperthermia, cutaneous vasoconstriction, increased metabolism, bradycardia, hypotension and an enhancement in the reflex bradycardia induced epinephrine in rats.

In summary, the present results demonstrate that TRH, TSH, LH, LHRH, ACTH, FSH and prolactin, but not GH, act through a central transmitter mechanism to influence physiological and/or behavioral functions in rats.

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REFERENCES

1. Burgus R., Dunn T. F., Desiderio D., Ward D. N., Vale W., and Guilienmin R.: Characterization of ovine hypothalamic TRH-releasing factor. *Nature, Lond.* **226** (1970) 331–332.
2. Hökfelt T., Fuxe K., Johansson O., Jeffcoate S. and White M.: Distribution of TRH in the central nervous system as revealed by immunocytochemistry. *Eur. J. Pharmacol.* **34** (1975) 389–392.
3. Horst W. D. and Spirt N.: A possible mechanism for the anti-depressant action of thyrotrophic-releasing hormone. *Life Sci.* **15** (1974) 1073–1082.
4. König J. F. and Klippel R. A.: In *The Rat Brain—A Stereotaxic Atlas of the Forebrain and Lower Parts of the Brain Stem.* William and Wilkins, Baltimore (1963).
5. Lin M. T.: Effects of specific inhibitors of 5-hydroxytryptamine uptake on thermoregulation in rats. *J. Physiol. Lond.* **284** (1978) 147–154.
6. Lin M. T., Chandra A., Tsay B. L. and Chern Y. F.: Hypothalamic and striatal dopamine receptor activation inhibits heat production in the rat. *Am. J. Physiol.* **242** (1982) R471–R481.
7. Lin M. T., Wu J. J., Chandra A. and Tsay B. L.: A noradrenergic-cyclic AMP link in the hypothalamic pathways which mediate febrile reaction induced by endotoxin and prostaglandin E_2 in rats. *J. Pharmac. exp. Ther.* **222** (1982) 251–257.
8. Moss R. L. and McCann S. M.: Induction of mating behavior in rats by luteinizing releasing factor. *Science* **181** (1973) 177–179.
9. Moss R. L. and McCann S. M.: Activation of luteinizing hormone releasing factor (LRF) on initiation of lordosis behavior in the estrone-primed ovariectomized female rat. *Neuroendocrinology* **17** (1975) 309–318.
10. Prange A. J., Wilson I. C., Lara P. P., Alltop L. B. and Breese G. R.: Effects of thyrotropin-releasing hormone in depression. *Lancet* **ii** (1972) 999–1002.
11. Thuránsky K.: Continuous blood pressure measurement in unanesthetized animals. *Acta physiol. Hung.* **29** (1966) 33–39.
12. Winokur A. and Utiger R. D.: Thyrotropin-releasing hormone—regional distribution in rat brain. *Science* **185** (1974) 265–267.