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Thyrotropin-releasing hormone increased heat production without the involvement of corticotropin-releasing factor in neonatal chicks

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Abstract

Thyrotropin-releasing hormone (TRH) is a hypothalamic signal in the hypothalamic–pituitary–thyroid (HPT) axis, and is well known as a hyperthermic hormone in the brain of chicks. The thermogenetic effect leads to the hypothesis that central TRH increases heat production (HP) in chicks. The purpose of the present study was to clarify whether central TRH affects HP of neonatal chicks, and if such an effect is mediated by corticotropin-releasing factor (CRF) since the thermogenetic effect of TRH is mediated by CRF. Intracerebroventricular (ICV) injection of TRH (14 and 55 nmol) dose-dependently increased oxygen consumption, carbon dioxide production and HP, and a similar effect was also observed with CRF (2.1 and 21 pmol). The TRH-induced increase in HP could not be attenuated by astressin, a CRF receptor antagonist, while the effect of CRF was completely diminished by astressin. The present study demonstrates that central TRH increases HP in chicks but the effect was not related to CRF.

Keywords: Chicks; Corticotropin-releasing factor; Heat production; Intracerebroventricular injection; Thyrotropin-releasing hormone

1. Introduction

Thyrotropin-releasing hormone (TRH) is well known as a hypothalamic signal of the hypothalamic-pituitary-thyroid (HPT) axis. It causes not only a stimulation of thyrotrophic hormone secretion, but also appears to act in thermoregulation (Metcalf, 1974), locomotor activity (Lin et al., 1983) and prolactin secretion (Collu et al., 1976; Ikegami et al., 1992) in mammals. In addition, intracerebroventricular (ICV) injection of TRH altered heat production (HP) of rats (Lin et al., 1980) demonstrating that central TRH is involved in the regulation of energy expenditure in mammals.

ICV injection of TRH induces hyperthermia in chicks (Takahashi et al., 2005) as well as mammals (Metcalf, 1974). In addition to the thermogenetic effect, TRH increases respiratory rate and locomotion activity in chicks (Nisticò et al., 1978). These facts lead to the hypothesis that central TRH is involved in the regulation of energy expenditure in chicks as in mammals. However, there are no reports that investigated the effect of TRH on HP in neonatal chicks.

Although ICV injection of TRH showed the thermogenetic effect in neonatal chicks, the plasma thyroid hormone levels were not stimulated by the injection of TRH (Takahashi et al., 2005). Furthermore, peripherally administered thyroid hormones did not enhance body temperature (Takahashi et al., 2005). These results suggest that the effects of ICV-injected TRH are not related to thyroid hormones. If central TRH increases energy expenditure in neonatal chicks, this effect would be mediated by thyroid hormones-independent pathways.

We recently reported that ICV injection of corticotropin-releasing factor (CRF), a hypothalamic signal of the hypothalamic–pituitary–adrenal (HPA) axis, also induces thermogenesis in neonatal chicks (Tachibana et al., 2004) and that

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some of the actions of TRH in the brain are mediated by CRF (Takahashi et al., 2005). Furthermore, TRH-induced thermogenesis was completely abolished by the CRF receptor antagonist astressin (Takahashi et al., 2005), suggesting that CRF contributes to the TRH-induced thermogenesis. These results imply that CRF is involved in the regulation of energy expenditure in neonatal chicks and mediates the effect of TRH.

The purpose of the present study was to investigate the effect of ICV injection of TRH on HP in chicks using an open-circuit calorimeter system. Whether the effect of TRH on energy expenditure is mediated by CRF was also determined.

2. Materials and methods

2.1. Animals

Day-old male layer chicks were purchased from a local hatchery (Murata Hatchery, Fukuoka Japan) and kept in a room at 30 °C under continuous lighting. The birds were allowed free access to a commercial diet (Toyohashi Feeds and Mill, Aichi, Japan), and water except as noted elsewhere. All experimental procedures were performed according to the National Research Council publication, Guide for Care and Use of Laboratory Animals and the guidance for Experiments in the Faculty of Agriculture and in the Graduate Course of Kyushu University and the Law (No. 105) and Notification (No. 6) of the Japanese Government.

2.2. ICV injection

TRH, ovine CRF (both purchased from Peptide Institute, Osaka, Japan) and astressin (Sigma Chemical CO., USA) were dissolved in a 0.1% Evans Blue solution prepared in saline. The control group was injected with the same volume of this Evans Blue solution. The ICV injection was conducted according to the method of Davis et al. (1979). This method is not stressful for chicks since the ICV injection of saline solution, which was used as the control, did not affect feeding behavior (Furuse et al., 1999) or corticosterone release (Saito et al., 2005) when compared with intact chicks without injections. The injection volume was 10 μl in all experiments. At the end of each experiment, chicks were sacrificed with an intraperitoneal overdose of sodium pentobarbital. Confirmation of drug injection was made by observation of the presence of Evans Blue dye in the lateral ventricle. The results obtained from chicks which did not have Evans Blue dye in the lateral ventricle were not used.

2.3. Respiratory measurement

Oxygen (O_2) consumption, carbon dioxide (CO_2) production and respiratory quotient (RQ) were measured using an open-circuit calorimeter system to determine HP (MK-5000RQ, Muromachi Kikai Co. Ltd., Tokyo, Japan). For these measurements, an acrylic chamber (150 × 150 × 150 mm) with a stainless steel grid floor was used. Fresh atmospheric air was drawn at a rate of 500 ml/min and then passed through O_2 and CO_2 detectors (MM202R, Muromachi Kikai Co., Ltd., Tokyo, Japan). The concentrations of these gases were recorded every 3 min. The analyzer was calibrated using primary gas standards of high purity (Sumitomo Seika Chemicals Co. Ltd., Osaka, Japan) every 1 h. HP was calculated by the method of Romijn and Lokhorst (1961) as follows: HP (kcal/min) = the volume of O_2 consumed (ml/min) × 3.871 + the volume of CO_2 produced (ml/min) × 1.194. The units for HP were converted to joules from calories by multiplying by 4.184, and the obtained values were normalized with the body weight.

2.4. Experiment 1: effect of ICV injection of TRH on HP and RQ

Each chick (2 days old) was transferred to the test chamber for 1 h to allow acclimation to the chamber. Then the chick was injected with either 0 (control), 14 or 55 nmol of TRH. O_2 consumption and CO_2 production were measured for 1 h after injection. During the acclimation and experimental periods, the chicks were not given feed or water. The number of chicks in each group was as follows: 0 nmol (control), 6; 14 nmol, 5; 55 nmol, 6.

2.5. Experiment 2: effect of ICV injection of CRF on HP and RQ

The experimental procedures were the same as Experiment 1 but the chicks (2 and 3 days old) were injected with 0 (control), 2.1 or 21 pmol of CRF. The number of chicks in each group was as follows: 0 pmol (control), 6; 2.1 pmol, 7; 21 pmol, 6. In the 0 and 21 pmol groups, 4 (n = 3 at 21, 33 and 55 min) and 1 (n = 1 at 21 min) of the 3 min O_2 consumption and CO_2 production samples, respectively, could not be measured due to mechanical reasons.

2.6. Experiment 3: effect of ICV co-injection of astressin on CRF-induced change in HP

The experimental procedures were the same as Experiment 1 except that the chicks (3 and 4 days old) were injected with saline (control), 21 pmol of CRF alone or 21 pmol of CRF plus 6 nmol astressin. In our preliminary experiment, the dose of astressin did not affect O_2 consumption [$F(1, 9) = 0.1$, $P = 0.8218$] and CO_2 production [$F(1, 9) = 0.0$, $P = 0.8727$]. The number of chicks in each group was as follows: saline (control), 7; CRF alone, 7; CRF plus astressin, 6.

2.7. Experiment 4: effect of ICV co-injection of astressin on TRH induced change in HP

The experimental procedures were the same as Experiment 1 but the chicks (3 and 4 days old) were injected with saline (control), 55 nmol TRH alone or 55 nmol TRH plus 6 nmol astressin. The number of chicks in each group was 6. In the saline and TRH alone groups, 2 (n = 1 at 12 and 15 min) and 1 (n = 1 at 12 min) of the 3 min O_2 consumption and CO_2 production samples, respectively, could not be measured due to mechanical reasons.

2.8. Statistical analysis

Statistical analysis was performed using the StatView program (version 5; SAS Institute, NC). Data were statistically analyzed with repeated two-way analysis of variance (ANOVA) with respect to drug treatment and time. Significant differences were set at $P<0.05$. Results are expressed as means±S.E.M.

3. Results

3.1. Experiment 1: effect of ICV injection of TRH on HP and RQ

The changes in O_2 consumption, CO_2 production and HP after the ICV injection of TRH are shown in Fig. 1. The TRH treatment significantly increased O_2 consumption [$F(2, 14)=9.8$, $P<0.01$] and CO_2 production [$F(2, 14)=6.2$, $P<0.05$] in a dose-dependent manner. HP was significantly [$F(2, 14)=9.3$, $P<0.01$] increased by TRH in a dose-dependent manner over time [$F(18, 252)=5.4$, $P<0.05$]. The TRH-induced increase in HP was time-dependent because a significant [$F(36, 252)=1.6$, $P<0.05$] interaction was observed. RQ was not significantly [$F(2, 14)=1.4$, $P>0.05$] affected by the TRH treatment (data not shown).

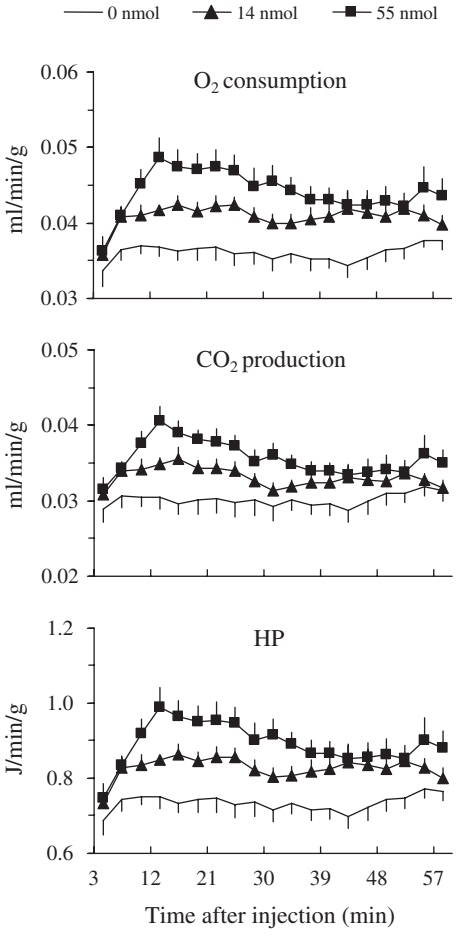

Fig. 1. Changes in O_2 consumption, CO_2 production and heat production after ICV injection of TRH. The number of chicks in each group was as follows: 0 nmol (control), 6; 14 nmol, 5; 55 nmol, 6. Data are expressed as means±S.E.M.

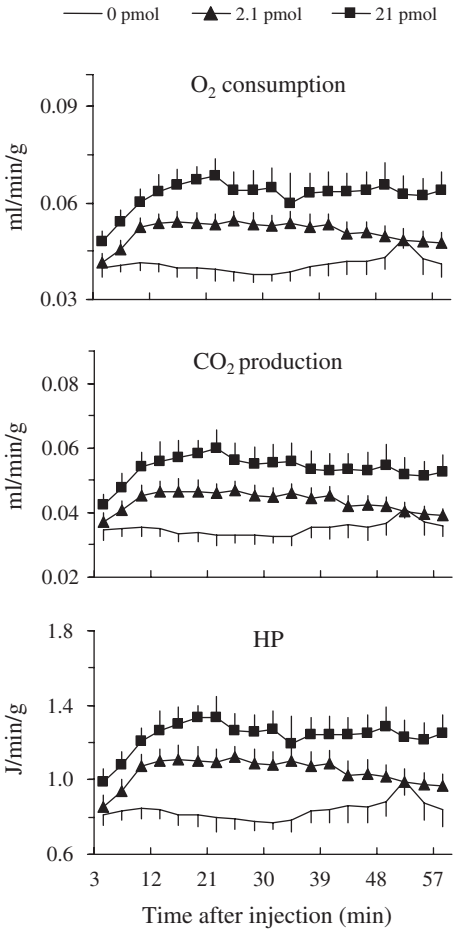

Fig. 2. Changes in O_2 consumption, CO_2 production and heat production after ICV injection of CRF. The number of chicks in each group was as follows: 0 pmol (control), 6; 2.1 pmol, 7; 21 pmol, 6. Data are expressed as means±S.E.M.

3.2. Experiment 2: effect of ICV injection of CRF on HP and RQ

Fig. 2 shows the effect of CRF on the time-course changes in O_2 consumption, CO_2 production and HP. The ICV injection of CRF significantly and dose-dependently increased O_2 consumption [$F(2, 12)=4.9$, $P<0.05$] and CO_2 production [$F(2, 12)=4.2$, $P<0.05$]. HP was significantly [$F(2, 12)=3.9$, $P<0.05$] increased by the CRF treatment in a dose-dependent manner, and changed over time [$F(18, 216)=12.6$, $P<0.01$]. The effect of CRF was time-dependent because a significant [$F(36, 216)=2.7$, $P<0.01$] interaction was found. RQ was not significantly [$F(2, 12)=0.0$, $P>0.05$] affected by the CRF treatment (data not shown).

3.3. Experiment 3: effect of ICV co-injection of astressin on CRF-induced change in HP

The effect of ICV co-injection of astressin on CRF-induced changes in O_2 consumption, CO_2 production and HP is shown in Fig. 3. These treatments significantly affected O_2 consumption [$F(2, 17)=20.3$, $P<0.01$] and CO_2 production [$F(2, 17)=25.0$, $P<0.01$]. HP was also significantly affected by these

treatments [$F(2, 17)=21.3$, $P<0.01$] and changed with time [$F(18, 306)=12.6$, $P<0.01$]. The CRF treatment alone increased O_2 consumption, CO_2 production and HP as shown in Experiment 2, but the values for CRF plus astressin treatments were comparable with the control. The change in each group over time was different since an interaction was significant in O_2 consumption [$F(36, 306)=2.1$, $P<0.01$], CO_2 production [$F(36, 306)=1.8$, $P<0.01$] and HP [$F(36, 306)=2.1$, $P<0.01$]. RQ was not significantly [$F(2, 17)=1.0$, $P>0.05$] affected by these treatments (data not shown).

3.4. Experiment 4: effect of ICV co-injection of astressin on TRH-induced change in HP

Fig. 4 shows the effect of astressin on TRH-induced changes in O_2 consumption, CO_2 production and HP. These treatments significantly affected O_2 consumption [$F(2, 13)=10.9$, $P<0.01$] and CO_2 production [$F(2, 13)=7.6$, $P<0.01$]. HP was also significantly affected by these treatments [$F(2, 13)=10.7$, $P<0.01$], and changed over time [$F(18, 234)=13.3$, $P<0.01$]. The TRH treatment alone increased HP as shown in Experiment 1, and astressin did not change this response. The time-course change in O_2 consumption, CO_2 production and HP was different between groups since the interactions were significant [O_2 consumption, $F(36, 234)=4.2$, $P<0.01$; CO_2 production $F(36, 234)=2.5$, $P<0.01$; HP, $F(36, 234)=3.8$, $P<0.01$]. RQ was not significantly [$F(2, 13)=0.9$, $P>0.05$] affected by these treatments (data not shown).

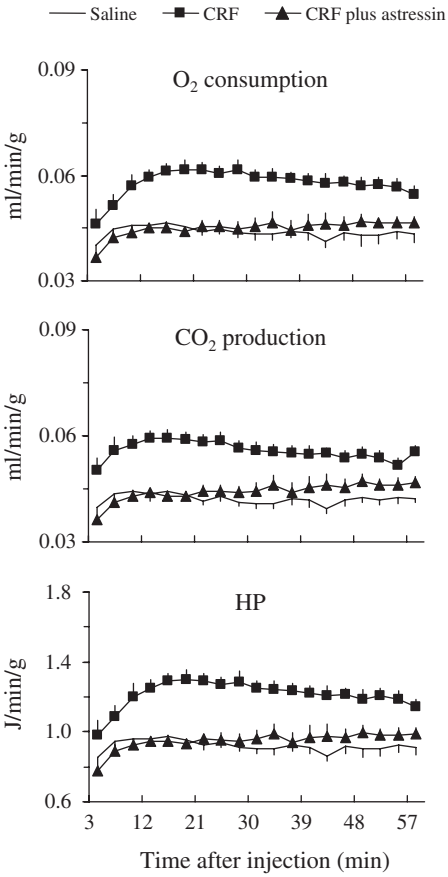

Fig. 3. Effect of ICV co-injection of astressin on CRF-induced changes in O_2 consumption, CO_2 production and heat production. The doses of CRF and astressin were 21 pmol and 6 nmol, respectively. The number of chicks in each group was as follows: saline (control), 7; CRF alone, 7; CRF plus astressin, 6. Data are expressed as means±S.E.M.

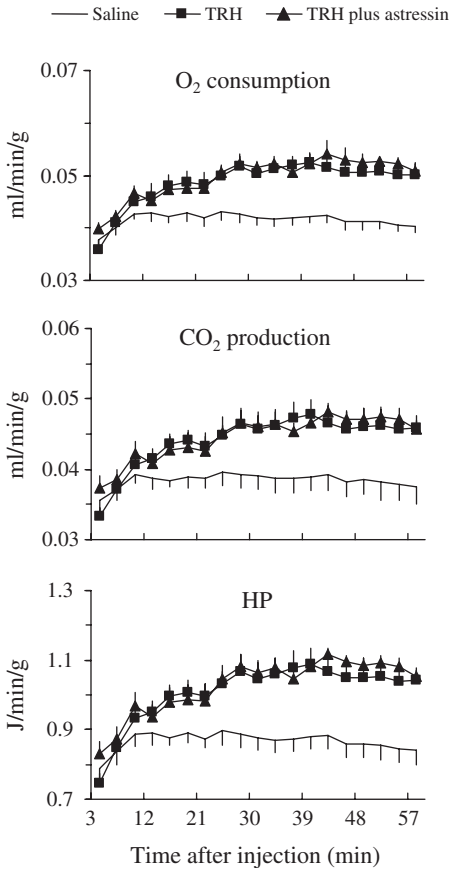

Fig. 4. Effect of ICV co-injection of astressin on TRH-induced changes in O_2 consumption, CO_2 production and heat production. The doses of CRF and astressin were 55 nmol and 6 nmol, respectively. The number of chicks in each group was 6. Data are expressed as means±S.E.M.

4. Discussion

The TRH-induced hyperthermia and increase in locomotor activity suggested that TRH enhanced energy expenditure in chicks (Nisticò et al., 1978; Takahashi et al., 2005). This idea was strongly supported by the present results that ICV injection of TRH dose-dependently elevated HP (Fig. 1).

TRH is the hypothalamic signal of HPT axis. The HPT axis seems to be well developed immediately after the hatching since intravenous administration of TRH increases circulating 3,5,3′-triiodothyronine (T3) concentration (Decuypere and Scanes, 1983). The circulating thyroid hormones are changed by cold exposure in chicks, suggesting that these hormones are involved in thermogenesis (Decuypere and Kuhn, 1988). In the previous report, however, elevated rectal temperature induced by ICV-injected TRH was not mediated through thyroid hormones in newborn chicks because ICV injection of TRH did not elevate plasma thyroid hormones concentrations (Takahashi et al.,

2005). Furthermore, thyroid hormones did not have thermogenetic effects in neonatal chicks (Takahashi et al., 2005). A previous study also reported that HP was increased by an intraperitoneal injection of T3, but the effect was larger at 2 week-old than at 1 week-old chicks (Hwang-Bo et al., 1990). These phenomena indicate that the effect of T3 on HP would be age-dependent and the TRH-induced increase in HP is not related to thyroid hormones in neonatal chicks. There would be T3-independent pathways concerning TRH-induced energy expenditure in chicks.

In rats, injection of CRF into the brain increased O_2 consumption and body temperature (Brown et al., 1982; Diamant and de Wied, 1991) and is involved in prostaglandin- and serotonin-induced O_2 consumption and thermogenesis (Le Feuvre et al., 1991; Rothwell, 1990). The similar effect was also observed in neonatal chicks by the previous study that ICV injection of CRF induced hyperthermia (Tachibana et al., 2004). In addition, ICV injection of CRF dose-dependently increased O_2 consumption, CO_2 production and HP in the present study (Fig. 2). This effect was similar to those of TRH in neonatal chicks (Fig. 1). Since TRH-induced corticosterone release and thermogenesis were completely abolished by co-injection of astressin, a CRF receptor antagonist (Takahashi et al., 2005), the TRH-induced increase in HP was expected to be mediated through the action of CRF in neonatal chicks. However, the TRH-induced increase in HP was not affected by astressin (Fig. 4) although the astressin treatment completely blocked the effect of CRF (Fig. 3). The dose of astressin also had no effect on the TRH-induced increase in HP when the lower dose of TRH (28 nmol) was used in our additional experiment (data not shown). Therefore, the mechanism underlying the effect of TRH on HP was different from that of CRF.

In conclusion, the present results demonstrated that, within the brain, TRH increased HP via CRF-independent mechanisms. Which mechanisms are involved in the TRH-induced increase in HP would be clarified in the future.

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References

Brown MR, Fisher CA, Durer J, Spiess J, Rivier C, Vale W. Corticotrophin-releasing factor: effects on the sympathetic nervous system and oxygen consumption. Life Sci 1982;30:207–10.

Collu R, Clermont MJ, Ducharme JR. Effects of thyrotropin-releasing hormone on prolactin, growth hormone and corticosterone secretions in adult male rats treated with pentobarbital or morphine. Eur J Pharmacol 1976;37:133–40.

Davis JL, Masuoka DT, Gerbrandt LK, Cherkin A. Autoradiographic distribution of L-proline in chicks after intracerebral injection. Physiol Behav 1979;22:693–5.

Decuypere E, Kuhn ER. Alterations in thyroid hormone physiology induced by temperature and feeding in newly hatched chickens. Acta Physiol Pol 1988;39:380–94.

Decuypere E, Scanes CG. Variation in the release of thyroxine, triiodothyronine and growth hormone in response to thyrotrophin releasing hormone during development of the domestic fowl. Acta Endocrinol 1983;102:220–3.

Diamant M, de Wied D. Autonomic and behavioral effects of centrally administered corticotropin-releasing factor in rats. Endocrinology 1991;129:446–54.

Furuse M, Ando R, Bungo T, Ao R, Shimojo M, Masuda Y. Intracerebroventricular injection of orexins does not stimulate food intake in neonatal chicks. Br Poult Sci 1999;40:698–700.

Hwang-Bo J, Muramatsu T, Okumura J. Age dependency of triiodothyronine-induced thermogenesis in young chicks: inhibition by propylthiouracil. Poult Sci 1990;69:1599–601.

Ikegami H, Jikihara H, Koike K, Morishige K, Kurachi H, Yamamoto N, et al. Intraventricular administration of thyrotrophin-releasing hormone (TRH) suppresses prolactin secretion and synthesis: a possible involvement of dopamine release by TRH from rat hypothalamus. J Endocrinol 1992;133:59–66.

Le Feuvre RA, Aisenthal L, Rothwell NJ. Involvement of corticotrophin releasing factor (CRF) in the thermogenic and anorexic actions of serotonin (5-HT) and related compounds. Brain Res 1991;555:245–50.

Lin MT, Chandra A, Chern YF, Tsay BL. Effects of thyrotrophic-releasing hormone (TRH) on thermoregulation in the rat. Experientia 1980;36:1077–8.

Lin MT, Chan HK, Chen CF, Teh GW. Involvement of both opiate and catecholaminergic receptors in the behavioural excitation provoked by thyrotropin-releasing hormone: comparisons with amphetamine. Neuropharmacology 1983;22:463–9.

Metcalf G. TRH: a possible mediator of thermoregulation. Nature 1974;252:310–1.

Nisticò G, Rotiroti D, De Sarro A, Stephenson JD. Behavioural, electrocortical and body temperature effects after intracerebral infusion of TRH in fowls. Eur J Pharmacol 1978;50:253–60.

Romijn C, Lokhorst W. Some aspects of energy metabolism in birds. Proc 2nd Symp Energy Metabolism. Wageningen; 1961. p. 49–58.

Rothwell NJ. Central activation of thermogenesis by prostaglandins: dependence on CRF. Horm Metab Res 1990;22:616–8.

Saito ES, Kaiya H, Tachibana T, Tomonaga S, Denbow DM, Kangawa K, et al. Inhibitory effect of ghrelin on food intake is mediated by the corticotropin-releasing factor system in neonatal chicks. Regul Pept 2005;125:201–8.

Tachibana T, Saito ES, Saito S, Tomonaga S, Denbow DM, Furuse M. Comparison of brain arginine-vasotocin and corticotrophin-releasing factor for physiological responses in chicks. Neurosci Lett 2004;360:165–9.

Takahashi H, Iigo M, Ando K, Tachibana T, Denbow DM, Furuse M. Regulation of body temperature by thyrotropin releasing hormone in neonatal chicks. Dev Brain Res 2005;157:58–64.