ELSEVIER

Pharmacology, Biochemistry and Behavior 70 (2001) 251–255

PHARMACOLOGY BIOCHEMISTRY AND BEHAVIOR

www.elsevier.com/locate/pharmbiochembeh

Intracerebroventricular injection of fusaric acid attenuates the anorexia by glucagon-like peptide-1 in the neonatal chick

T. Bungo[a,*], S.-I. Kawakami[b], A. Ohgushi[c], K. Sashihara[c], N. Saito[d], K. Sugahara[e], S. Hasegawa[f], D.M. Denbow[g], M. Furuse[c]

[a]*Laboratory of Animal Science, Department of Agrobiological Science, Faculty of Agriculture, Ehime University, Matsuyama 790-8566, Japan*

[b]*Department of Animal Production, Kyushu National Agricultural Experiment Station, Kumamoto 861-1192, Japan*

[c]*Department of Animal and Marine Bioresources Science, Graduate School of Bioresource and Bioenvironmental Sciences, Kyushu University, Fukuoka 812-8581, Japan*

[d]*Laboratory of Animal Physiology, Graduate School of Bioagricultural Science, Nagoya University, Nagoya 464-8601, Japan*

[e]*Department of Animal Science, Faculty of Agriculture, Utsunomiya University, Utsunomiya 321-8505, Japan*

[f]*Laboratory of Biological Function and Metabolism, Division of Bioscience, Graduate School of Science and Technology, Kobe University, Kobe 657-8501, Japan*

[g]*Department of Animal and Poultry Science, Virginia Polytechnic Institute and State University, Blacksburg, VA 24061-0332 USA*

Received 13 November 2000; received in revised form 30 May 2001; accepted 29 June 2001

Abstract

It is known that central injection of glucagon-like peptide-1 (GLP-1) suppresses feeding in rats and chicks, but the systems for GLP-1 are still open with special reference to the chick. The present study was done to determine whether a noradrenergic mechanism contributes to the anorexigenic effect of GLP-1 on the neonatal chick. Central administration of norepinephrine (NE) suppressed food intake with narcolepsy as GLP-1 in chicks. However, in spite of that dopamine (DA) did not affect food intake, coadministration of inhibitor of dopamine-β-hydroxylase (DBH), fusaric acid (FA), attenuated the suppressive effect of GLP-1 on feeding behavior. It is suggested that there may be the interactive relationships between GLP-1 and noradrenergic system in the neonatal chick.

Keywords: Glucagon-like peptide-1 (GLP-1); Noradrenergic system; Fusaric (5-butylpicolinic) acid; Central nervous system; Food intake; Chick

1. Introduction

Glucagon-like peptide-1 (GLP-1) is known as an inhibitor of food intake in rats (Navarro et al., 1996; Tang-Christensen et al., 1996; Turton et al., 1996) and chickens (Furuse et al., 1997a,b), but the systems for this anorexia of GLP-1 are poorly defined. One of the systems suggested by Larsen et al. (1997) is that GLP-1 activates the hypothalamo–pituitary–adrenocortical (HPA) axis in the rat. This is primarily stimulation of corticotropin releasing factor (CRF) neurons, and this activation may be responsible for the inhibition of feeding behavior. In fact, plasma corticosterone concentration was rapidly increased after central administration of GLP-1 in the rat. On the contrary, central GLP-1 did not alter plasma corticosterone concentration in the neonatal chick (Furuse et al., 1997c). Thus, the action of GLP-1 in the central nervous system of chickens might at least have no relation to CRF.

As the side effect of GLP-1, we observed that intracerebroventricular (ICV) administration of GLP-1 induced sedation or narcolepsy (sleep-like behavior) in chicks (Bungo et al., 1999b). On the other hand, the reduction in food intake after central injection of norepinephrine (NE) was superimposed upon a sleep-inducing effect has

* Corresponding author. Tel.: +81-89-946-9820; fax: +81-89-946-9820.

E-mail address: bungo@agr.ehime-u.ac.jp (T. Bungo).

0091-3057/01/$ – see front matter
PII: S0091-3057(01)00613-X

been reported previously the domestic fowl (Denbow et al., 1981) and the Leghorn chick (Steinman et al., 1987). On the relationship between sleep and NE, it has reported that central injection of fusaric acid (FA) (an inhibitor of dopamine-β-hydroxylase (DBH), a late-limiting enzyme in NE synthesis) suppressed REM sleep in cats (Satoh and Tanaka, 1973). Young animals sleep more than adults, and they also spend a large percentage of their time in REM sleep (Fraser and Broom, 1990). We recently reported that central injection of FA increased food intake in chicks since FA may inhibit narcolepsy and/or REM sleep by endogenous NE (Bungo et al., 1999a). These findings hypothesized that the functional link between GLP-1 and NE may exist and/or that suppression of food intake by GLP-1 may be mediated by increased NE release in the central nervous system.

The present study was to elucidate whether suppressed food intake by the central injection of GLP-1 has relation to NEergic system by using FA. In advance of experiments on FA, the effects of catecholamines (NE and dopamine (DA)) that have relation to DBH on regulating food intake in the neonatal chick were also examined.

2. Materials and methods

2.1. Animals

Day-old male broiler chicks (Cobb) were purchased from a local hatchery (Mori Hatchery, Fukuoka, Japan). Birds were maintained in a room with 24 h light and at a temperature of 28 °C. They were given free access to a commercial starter diet (Toyohashi Feeds and Mill, Aichi, Japan) and water. Chicks were maintained in accordance with the recommendations of the National Research Council (National Research Council, 1985). They were distributed into experimental groups based on their body weight, so that the average body weight was as uniform as possible for each treatment.

2.2. Intracerebroventricular (ICV) injections

The birds were ICV injected with the solutions (10 μl) using a microsyringe according to the methods by Davis et al. (1979). NE (bitartrate salt) and DA (hydrochloride) were purchased from Sigma (St. Louis, MO, USA), and chicken GLP-1 and FA were purchased from Peptide Institute (Osaka, Japan) and Funakoshi (Tokyo, Japan), respectively. Drugs were dissolved in a 0.1% Evans Blue solution, which was prepared in a 0.85% saline. At the end of the experiments, birds were sacrificed by decapitation after which the location of the injection site was confirmed. Data from the individuals that were not verified by the presence of Evans Blue dye in the lateral ventricle were deleted.

2.3. Experimental procedure

2.3.1. Effect of ICV injection of NE on food consumption

Although ICV administration of NE failed to stimulated food intake in young chickens (Denbow et al., 1981) because of narcolepsy, it is unclear as to whether central injection of NE influences feeding behavior in neonatal chicks or not. This experiment was conducted to determine the effect of NE on food intake in the neonatal chick. Birds (2-day-old) were in free access to food. Birds were injected by the ICV route with saline or NE (25, 50 and 100 μg) and food intake was determined at 30, 60 and 120 min. The numbers of birds used were: control, 10; NE, 25 μg, 9; 50 μg, 8 and 100 μg, 8, respectively.

2.3.2. Effect of ICV administration of DA on food consumption

Since FA, which is used on the following experiments, inhibits NE synthesis from DA, this experiment was conducted to determine whether DA influenced food intake of chicks. Birds (2-day-old) were in free access to food. Birds had ICV administration of saline or DA (25, 50 and 100 μg). Food intake was determined at 30, 60 and 120 min. The numbers of birds used were: control, 9; DA, 25 μg, 9; 50 μg, 9 and 100 μg, 9, respectively.

2.3.3. Effect of ICV injection of FA on GLP-1-suppressed food consumption

This experiment was conducted to determine whether the anorexic effect of GLP-1 was affected by altering NE synthesis through the action of FA. After being deprived of food for 3 h, birds (2-day-old) were injected by ICV route with saline, GLP-1 (0.03 μg) or GLP-1 coinjected with FA. Food intake was measured at 1 and 2 h. The numbers of birds used were: control, 5; GLP-1 alone, 8 and GLP-1 with FA, 6, respectively.

2.3.4. Dose response of FA on GLP-1-suppressed food consumption

This experiment was conducted to determine whether FA attenuated the anorexigenic effect of GLP-1 in a dose-dependent-manner. Birds (2-day-old) with 3 h fasting were divided into four groups, i.e., saline, GLP-1 (0.03 μg) alone and GLP-1 coinjected with two levels of FA (50 and 100 μg). Food intake was determined for 2 h immediately after treatment. The numbers of birds used were: control, 8; GLP-1 alone, 9; GLP-1 with FA 50 μg, 6 and GLP-1 with FA 100 μg, 8.

2.4. Data analysis

Data were analyzed by one-way ANOVA by the General Linear Model procedure using a commercially available package (SAS Institute and SAS User's Guide, 1985), and comparisons between means were made using Duncan's multiple range test in the effects of ICV injection of NE and

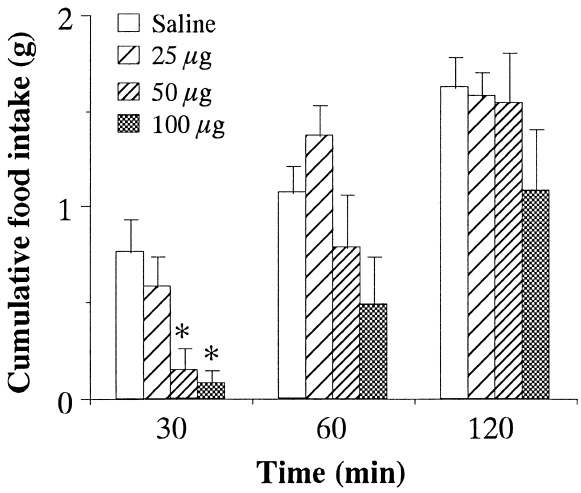

Fig. 1. Cumulative food intake over a 2-h period after ICV injection of norepinephrine or saline in 2-day-old chicks. Values are means ± S.E.M. * $P < .05$ compared to control saline. Numbers of birds used were: 10 (saline), 9 (25 μg), 8 (50 μg) and 8 (100 μg).

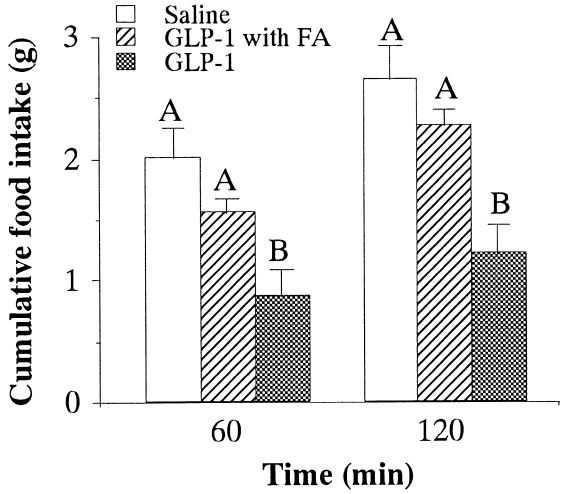

Fig. 3. Cumulative food intake (after 3-h fasting) of chicks injected ICV with saline, GLP-1 (0.03 μg) or GLP-1 coinjected with fusaric acid (FA; 100 μg). Values are means ± S.E.M. Means with a different letter are significantly different at $P < .05$. Numbers of birds used were: 5 (saline), 6 (GLP-1 with FA) and 8 (GLP-1 alone).

DA, and GLP-1 coinjected with FA. In the dose response experiment, regression analysis was applied for the values obtained by three doses of FA (0, 50 and 100 μg) with GLP-1. The results are presented as mean ± SEM.

3. Results

3.1. Effect of ICV injection of NE on food consumption

The result on the effect of central injection of NE is shown in Fig. 1. Food intake was significantly decreased by 50 and 100 μg NE when compared with control and the level of 10 μg at 30 min postinjection, $F(3,31) = 5.31$, $P < .05$. On the other hand, each level of NE failed to suppress food intake of chicks at 60 and 120 min when compared with control ($P > .05$).

3.2. Effect of ICV administration of DA on food consumption

Fig. 2 depicts the effect of central injection of DA in chicks. Throughout the 2 h experimental period, food intake was not affected by each level of DA (25, 50 and 100 μg) when compared with the saline control ($P > .05$).

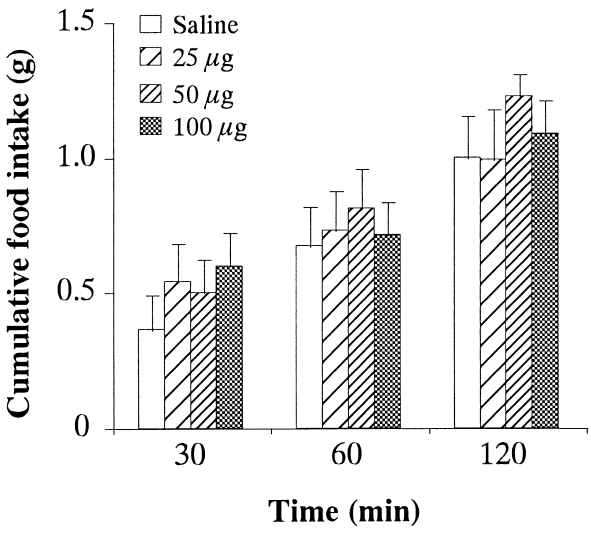

Fig. 2. Cumulative food intake over a 2-h period after ICV injection of dopamine or saline in 2-day-old chicks. Values are means ± S.E.M. Numbers of birds used were: 9 (saline), 9 (25 μg), 9 (50 μg) and 9 (100 μg).

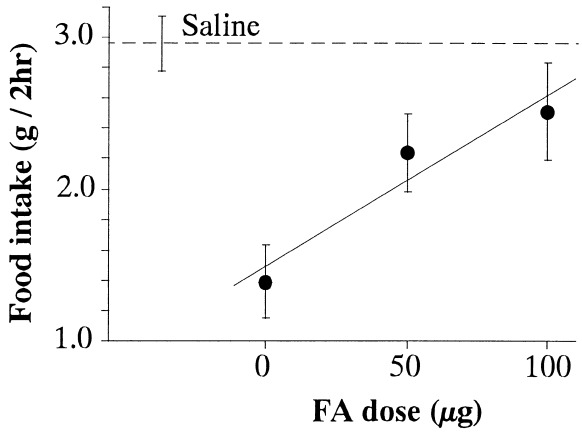

Fig. 4. Food intake (after 3-h fasting) during 2 h of chicks injected ICV with saline, GLP-1 (0.03 μg) or GLP-1 coinjected with two levels of FA (50 and 100 μg). Values are means ± S.E.M. The following regression equation was obtained between food intake and the doses of FA: food intake (g) = 1.4607 + 0.0113X (R^2=.3074, P=.006). Numbers of birds used were: 8 (saline), 9 (GLP-1 alone), 6 (GLP-1 with FA 50 μg) and 8 (GLP-1 with FA 100 μg).

3.3. Effect of ICV injection of FA on GLP-1-suppressed food consumption

The result on the effect of central injection of GLP-1 alone and GLP-1 with FA is given in Fig. 3. Although central administration of GLP-1 significantly decreased food intake, an anorexigenic effect of GLP-1 was attenuated by FA (100 μg) used in this experiment at 60 min, $F(2,16) = 7.96$, $P < .05$ and 120 min, $F(2,16) = 11.27$, $P < .05$.

3.4. Dose response of FA on GLP-1-suppressed food consumption

Fig. 4 shows the effect of each level of FA upon hypophagia by GLP-1. Two levels of FA (50 and 100 μg) attenuated the suppression of food intake by in a dose-related fashion. Dose level of FA (100 μg) produced a recovery from anorexic effect of GLP-1 to approximately 80% of baseline (control) level (see Fig. 4). The following regression equation was obtained between food intake and the doses of FA: food intake (g) = 1.4607(S.E. 0.2385) + 0.0113(S.E. 0.0037) X ($R^2 = 0.3074$: $P = .006$).

4. Discussion

As regards the NEergic system, the paraventricular nucleus (PVN) of the hypothalamus is the sensitive site to NE-stimulated feeding in the rat (Leibowitz, 1978). This response is mediated by α_2-receptors (Schlemmer et al., 1981), since central injection of clonidine (α_2-adrenoceptor agonist) stimulates feeding behavior in rats (Goldman et al., 19852) and chickens (Bungo et al., 199a; Choi et al., 1995). On the other hand, NEergic mechanisms are important in the sleep-waking cycle, for example it is believed that the locus coeruleus prevents motor activity during rapid eye movement (REM) sleep (Kruk and Pycock, 1991). Denbow et al. (1981) observed that ICV injection of NE failed to stimulated food intake in the domestic fowl, presumably because it induced a narcoleptic response. Similar to Denbow et al. (1981), we found that higher level of NE reduced food intake of satiated chicks (see Fig. 1). Harsing et al. (1989) also suggested that stimulation of α_2-adrenoceptors with pre- and postsynaptic locations or inhibitor of α_1-adrenoceptors in the central nervous system might shift the depression/vigilance balance to the direction of depression, which might be accompanied by decreased activity of cortical NEergic neural transmission. On the other hand, we observed that ICV administration of GLP-1 induced sleep-like behavior in the neonatal chick (Bungo et al., 1999b). Thus, there is the possibility that the anorexigenic effect of GLP-1 was due to hypersecretion of NE. Actually, it was demonstrated that fasted chicks were also suppressed feeding behavior by central injection of NE (unpublished data; food intake at 30 min after treatment was: control, 1.08 ± 0.15 g; NE 25 μg, 0.39 ± 0.10 g; NE 50 μg, 0.56 ± 0.13 g; NE 100 μg, 0.51 ± 0.10 g). In the purpose of inhibition for this hypersecretion of NE, we used FA (an inhibitor of DBH), because FA suppressed REM sleep that has relation to NEergic mechanism (Satoh and Tanaka, 1973). In fact, it was confirmed that NE concentration was decreased to the level of 80% by central injection of FA compared with control saline (unpublished data).

Recently, we reported that food intake of satiated chicks was enhanced by the central injection of FA in a dose-dependent manner (Bungo et al., 1999a), but not fasted chicks (unpublished data). The dose of FA used in the effect of ICV injection of FA on GLP-1-suppressed food consumption, was highest level in the previous report (Bungo et al., 1999a). Because FA attenuate the synthesis of NE, we predicted that coinjection of FA does not have strong influence on the anorexia by GLP-1 in the beginning of the experiment. Thus, food intake was measured at 60 and 120 min. As the result of this experiment, central administration of GLP-1 significantly decreased food intake as previously reported (Furuse et al., 1997a; Furuse et al., 1997b). However, an anorexigenic effect of GLP-1 was attenuated by FA used in this experiment at both time determined (see Fig. 3). Although there was no systematic attempt to quantify other behavioral measurement, FA seemed to inhibit sleep-like behavior induced by GLP-1 in chicks. Moreover, two levels of FA attenuated the suppression of food intake by GLP-1 in a dose-related fashion (Fig. 4). Therefore, it seemed that anorexic effect by central injection of GLP-1 might have relation to NEergic system. Anyway, this result was very interesting, because the suppressed food intake by central administration of GLP-1 could not be attenuated by a strong orexigenic factor, neuropeptide Y (NPY) in the neonatal chick (Furuse et al., 1997a). We observed in the neonatal chick that central coinjection with NPY and clonidine, a specific α_2-agonist, decreased food intake enhanced by NPY alone (Bungo et al., 2000).

On the other hand, there is the possibility for the attenuation of the anorexia of GLP-1 induced by FA that DA in the ventromedial hypothalamic nucleus (VMH) might mediate an increase in food intake. The amount of DA may be increased in VMH as a result of DBH inhibition with FA. Meguid et al. (1997) reported that DA concentration in VMH decreased during eating. However, evidence that this possibility may be wrong can be seen in the effect of ICV administration of DA on food consumption (Fig. 2). This result coincided with other report that central injection of DA had no effect on food intake in broiler and White Leghorn chickens (Denbow et al., 1981; Denbow et al., 1983). Recently, we found that central injection of FA decreased the concentration of DA, but not modified that of serotonin (5-HT) in the hypothalamus of chicks (unpublished data). However, the depression of DA induced anorexia in chicks (Bungo et al., 2001). Collectively, it is unlikely that both DA and 5-HT might not have direct relationship with the effect of FA on food intake.

From the standpoint of the relationship between GLP-1 and NE, coworker found that after 2 h central injection GLP-1 produced a decrease of catecholamines in VMH, but not PVN and suggested that ICV injection of GLP-1 may induced a drastic release in catecholamines (Tachibana et al., unpublished data). This result implies that the regulation of GLP-1 on the suppressive effect of food intake may be through the action of NE.

In conclusion, NEergic system affects directly or indirectly the anorexic effect of GLP-1 on food intake of chicks. However, these relationships remain to be studied in future.

Acknowledgments

This study was supported by grant-in-aid for scientific research from the Ministry of Education, Science and Culture, Japan, by Kyowa Hakko Kogyo, Tokyo, Japan and Uehara Memorial Foundation, Japan.

References

Bungo T, Shimojo M, Masuda Y, Choi Y-H, Denbow MD, Furuse M. Induction of food intake by a noradrenergic system using clonidine and fusaric acid in the neonatal chick. Brain Res 1999a;826:313–6.

Bungo T, Kawakami S-I, Ohgushi A, Shimojo M, Masuda Y, Saito N, Sugahara K, Hasegawa S, Furuse M. Intracerebroventricularly administered glucagon-like peptide-1 (7–36) amide induced sleep-like behavior in the neonatal chick. Jpn Poult Sci 1999b;36:377–81.

Bungo T, Choi Y-H, Denbow DM, Shimojo M, Masuda Y, Furuse M. Intracerebroventricularly administered neuropeptide Y and clonidine: effects on feeding behavior in the neonatal chick. Jpn Poult Sci 2000;37:27–32.

Bungo T, Ando R, Kawakami S-I, Ohgushi A, Furuse M. The role of central catecholaminergic systems in regulation of food intake of chicks. J Poult Sci 2001;38:35–40.

Choi Y-H, Furuse M, Okumura J, Denbow DM. The interaction of clonidine and nitric oxide on feeding behavior in the chicken. Brain Res 1995;699:161–4.

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

Denbow DM, Cherry JA, Siegel PB, Van Krey HP. Eating, drinking and temperature response of chicks to brain catecholamine injection. Physiol Behav 1981;27:265–9.

Denbow DM, Van Krey HP, Lacy MP, Dietrick TJ. Feeding, drinking and body temperature of Leghorn chicks: effects of ICV injections of biogenic. Physiol Behav 1983;31:85–90.

Fraser AF, Broom DM. Rest and sleep. Farm animal behaviour and welfare. 3rd ed. Oxon: Cab International, 1990. pp. 135–46.

Furuse M, Matsumoto M, Okumura J, Sugahara K, Hasegawa S. Intracerebroventricular injection of mammalian and chicken glucagon-like peptide-1 inhibits food intake of the neonatal chick. Brain Res 1997a;755:167–9.

Furuse M, Matsumoto M, Mori R, Sugahara K, Kano K, Hasegawa S. Influence of fasting and neuropeptide Y on the suppressive food intake induced by intracerebroventricular injection of glucagon-like peptide-1 in the neonatal chick. Brain Res 1997b;764:289–92.

Furuse M, Matsumoto M, Saito N, Sugahara K, Hasegawa S. The central corticotropin-releasing factor and glucagon-like peptide-1 in food intake of the neonatal chick. Eur J Pharmacol 1997c;339:211–3.

Goldman CK, Marino L, Leibowitz SF. Postsynaptic α_2-noradrenergic receptors mediate feeding induced by paraventricular nucleus injection of norepinephrine and clonidine. Eur J Pharmacol 1985;115:11–9.

Harsing LG Jr, Kapocsi J, Vizi ES. Possible role of alpha-2 and alpha-1 adrenoceptors in the experimentally-induced depression of the central nervous system. Pharmacol, Biochem Behav 1989;32:927–35.

Kruk ZL, Pycock CJ. Noradrenaline. Neurotransmitters and drugs. 3rd ed. New York: Chapman and Hall, 1991. pp. 50–86.

Larsen PJ, Tang-Christensen M, Jessop DS. Central administration of glucagon-like peptide-1 activates hypothalamic neuroendocrine neurons in the rat. Endocrinology 1997;138:4445–55.

Leibowitz SF. Paraventricular nucleus: a primary site mediating adrenergic stimulation of feeding and drinking. Pharmacol, Biochem Behav 1978;8:163–75.

Meguid MM, Yang Z-J, Laviano A. Meal size and number: relationship to dopamine levels in the ventromedial hypothalamic nucleus. Am J Physiol 1997;272:R1925–30.

National Research Council. Guide for the care and use of Laboratory animals. NIH Publ. No. 85-23. Washington, DC: Department of Health and Human Services, 1985.

Navarro M, de Fonseca FR, Alvarez E, Chowen JA, Zueco JA, Gomez R, Eng J, Blázquez E. Colocalization of glucagon-like peptide-1 (GLP-1) receptors, glucose transporter GLUT-2, and glucokinase mRNAs in rat hypothalamic cells: evidence for a role of GLP-1 receptor agonists as an inhibitory signal for food and water. J Neurochem 1996;67:1982–91.

SAS Institute, SAS User's Guide. Statistics. 5th ed. Cary, NC: SAS Institute, 1985.

Satoh T, Tanaka R. Selective suppression of rapid eye movement sleep (REM) by fusaric acid, an inhibitor of dopamine-β-oxidase. Experientia 1973;29:177–8.

Schlemmer RF, Elder JK, Casper RC, Davis JM. Clonidine-induced hyperphagia in monkeys: evidence for α_2-noradrenergic receptor mediation. Psychopharmacology 1981;73:99–100.

Steinman JL, Fujikawa DG, Wasterlain CG, Cherkin A, Morley JE. The effects of adrenergic, opioid and pancreatic polypeptidergic compounds on feeding and other behaviors in neonatal Leghorn chicks. Peptides 1987;8:585–92.

Tang-Christensen M, Larsen PJ, Göke R, Fink-Jensen A, Jessop DS, Møller M, Sheikh SP. Central administration of GLP-1-(7–36) amide inhibits food and water intake in rats. Am J Physiol 1996;271:R848–56.

Turton MD, O'Shea D, Gunn I, Beak SA, Edwards CMB, Meeran K, Choi SJ, Tayler GM, Heath MM, Lambert PD, Wilding JPH, Smith DM, Ghatei MA, Herbert J, Bloom SR. A role for glucagon-like peptide-1 in the central regulation of feeding. Nature 1996;379:69–72.