

Oestrogenic Regulation of Pro-Opiomelanocortin, Neuropeptide Y and Corticotrophin-Releasing Hormone mRNAs in Mouse Hypothalamus

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Journal of Neuroendocrinology

It is well documented that oestrogen suppresses food intake by an action at the hypothalamic level. Using *in situ* hybridisation, we studied the effect of castration (CX) and short-term administration of oestradiol (E_2) in CX female mice for three neuropeptides involved in feeding behaviour: two anorexigenic peptides, (i) the pro-opiomelanocortin (POMC)-derived peptide α -melanocyte-stimulating hormone and (ii) corticotrophin-releasing hormone (CRH), and the orexigenic peptide, (iii) neuropeptide Y (NPY). POMC-expressing neurones were mostly laterally located in the arcuate nucleus. POMC mRNA expression was decreased following CX and a single injection of E_2 induced an increase in mRNA levels at 12- and 24-h time intervals. In the parvocellular area of the paraventricular nucleus, CRH mRNA levels were similarly decreased after CX and completely restored to normal levels at 12 and 24 h following E_2 injection. On the other hand, the levels of NPY mRNA expressed in neurones located in the inner zone of the arcuate nucleus were increased by CX and decreased to the levels observed in intact animals by E_2 injection (3–24 h). The present data suggest that oestrogen might exert an anorexigenic action by stimulating POMC and CRH mRNA expression and decreasing NPY mRNA expression in the hypothalamus.

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Key words: oestradiol, pro-opiomelanocortin, neuropeptide Y, corticotrophin-releasing hormone, hypothalamus, *in situ* hybridisation.

doi: 10.1111/j.1365-2826.2007.01548.x

Feeding behaviour is regulated by several neuropeptides and monoamine neurotransmitters in a complex manner (1, 2). In many species, oestrogens are involved in food intake regulation. The concentration of plasma oestrogens, especially oestradiol (E_2), inversely correlates with feeding during many physiological states (3). In young mice and rats, ovariectomy induces weight gain mainly related to an increase in feeding (4, 5). Systemic administration of E_2 to ovariectomised mice and rats could prevent these changes in feeding behaviour (6, 7). In the guinea pig, ovariectomy similarly induced an increase in food intake and body weight, an effect which was reversed by oestradiol implants in the hypothalamic ventromedial arcuate region and paraventricular nucleus (PVN) (8). These results suggest that the hypothalamic ventromedial nucleus, arcuate nucleus and PVN might be the anatomical sites of the oestrogenic effects on food intake. There is some evidence for a similar role for oestrogen in the control of body weight in women (9). Postmenopausal oestrogen replacement therapy has a significant effect in reducing the amount of weight gained in women during the decade following menopause (9). The effect of oestrogen on

food intake and body weight appears to be mediated by oestrogen receptor (ER) α , as shown by experiments using ER α null mice (6, 10).

Several neuropeptides are involved in the regulation of feeding behaviour (1, 2, 11, 12). Corticotrophin-releasing hormone (CRH), cholecystokinin, neurotensin, cocaine- and amphetamine-regulated transcript, α -melanocyte-stimulating hormone (α -MSH), thyrotrophin-releasing hormone (TRH), vasopressin and octadecaneuropeptide are anorexigenic, whereas neuropeptide Y (NPY), galanin, agouti-related protein, melanin-concentrating hormone, ghrelin, 26RFa and the orexins stimulate food intake (2, 12–21).

The arcuate nucleus, PVN and ventromedial nuclei are prominent hypothalamic areas involved in the circuitry elaborating and emitting orexigenic and anorexigenic signals (1, 12). The arcuate nucleus contains a high density of neurones that produce two peptides involved in feeding behaviour control: NPY (22) and the pro-opiomelanocortin (POMC)-derived peptide α -MSH (23, 24). In the parvocellular area of the PVN, there is high concentration of CRH neurones (25). Although the effect of oestrogens on feeding behaviour

and body weight is well documented, the exact site of action of oestrogens in the central nervous system is still unknown. To evaluate the possible involvement of NPY, α -MSH and CRH in the anorexigenic effect of oestradiol, we studied the effect of ovariectomy and acute administration of oestradiol on mRNA expression of the three peptides in the mouse hypothalamus.

Materials and methods

Animals and treatments

Eleven- to 12-week-old female C57BL6 mice were received from Charles River (St Constant, Quebec, Canada) and were allowed to acclimate for 3 weeks. The animals were housed individually under constant temperature (22 ± 3 °C) and under a 14 : 10 h light dark cycle lighting (lights on 06.00 h) regimens. The mice had free access to tap water and a certified rodent feed [Laboratory Diet 5002 (pellet); Ralston Purina, St Louis, MO, USA]. The experiment was conducted in an animal facility approved by the Canadian Council on Animal Care (CCAC) and the Association for Assessment and Accreditation of Laboratory Animal Care. The study was performed in accordance with the CCAC Guide for Care and Use of Experimental Animals.

Animals, weighing between 23 and 26 g, were randomised according to their body weights and were assigned to five groups of six animals each: intact control (group 1); ovariectomised control (group 2); and ovariectomised + E_2 (0.05 μ g/mouse) (groups 3–5). On day 1 of the study, animals in groups 2–5 were bilaterally ovariectomised under isoflurane anaesthesia. Mice in group 1 were sham-operated (intact). Before the animals were killed on day 8 of the study, they received a single subcutaneous injection (0.2 ml/mouse) of the vehicle alone (5% ethanol/0.4% methylcellulose; groups 1 and 2) or E_2 (groups 3–5). The injection was performed 3 h (group 3), 12 h (group 4) or 24 h (groups 1, 2 and 5) before the animals were killed. The dose of E_2 was selected on the basis of preliminary experiments conducted the effect of increasing doses (0.01–0.16 μ g/animal) of E_2 on the uterine weight in ovariectomised mice. The selected dose (0.05 μ g/mouse) was the minimal dose that could completely prevent the decrease in uterine weight in 7-day ovariectomised mice. The changes in body weight were evaluated at the beginning and end (day 8) of the experiment. Body weight increase after 8 days was very similar for control and ovariectomised mice (1.0 ± 0.2 g versus 1.1 ± 0.2 g).

Tissue collection

On day 8 of the study, mice were anaesthetised with ketamine/xylazine and perfused transcardially with 90 ml 4% (w/v) paraformaldehyde in 0.1 M phosphate buffer (pH 7.4). Prior to the perfusion, a vaginal smear was collected from intact female mice of group 1 to determine the oestrous cycle using Papanicolaou staining. Mice in oestrous were selected. The brains were removed and postfixed in the same fixative overnight at 4 °C, and then placed in 15% sucrose in the 0.1 M phosphate buffer overnight at 4 °C. Thereafter, the tissues were frozen on dry ice in support medium (OCT, Bayer Corp., Elkhart, IN, USA). Coronal sections throughout the hypothalamus were serially cut at 10 μ m with a cryostat. The sections were then mounted on Superfrost/PLUS Microscope slides (Fisher Scientific, Montreal, Canada) and maintained at -80 °C until use.

Preparation of cRNA probes

The vector used for the production of cRNA probes was constructed by insertion into a pCR-Blunt II-TOPO (Invitrogen, Ontario, Canada) of a cDNA

fragment of 287 bp of mouse POMC1 (Genebank no. NM_008895), 247 bp of mouse NPY (Genebank no. NM_023456) or 468 bp of mouse CRH (Genebank no. NM_0205769). The cDNA fragments located at position 98–384 (POMC), 185–431 (NPY) and 211–678 (CRH) were obtained by amplification using the polymerase chain reaction (PCR). Antisense and sense 35 S-labelled cRNA probes were prepared by *in vitro* transcription with the SP6 and the T7 polymerase promoters, respectively.

In situ hybridisation with the antisense and sense 35 S-labelled cRNA probes was performed as previously described (26). Briefly, the sections were prehybridised at room temperature in a humid chamber for 2 h in 450 μ l/slide of a prehybridisation buffer containing 50% formamide, $5 \times$ SSPE (1 \times SSPE being 0.1 M NaCl, 10 mM NaH_2PO_4 , pH 7.4, mM EDTA), $5 \times$ Denhart's buffer, 200 μ g/ml denatured salmon testis DNA (Sigma, St Louis, MO, USA), 200 μ g/ml yeast tRNA, 2 μ g/ml Poly A (Boehringer-Mannheim, Montreal, Canada) and 4% dextran sulphate. After the prehybridisation procedure, a 100 μ l hybridisation mixture (prehybridisation buffer containing 10 mM dithiothreitol and the 35 S-labelled cRNA probe at a concentration of 10×10^6 c.p.m./ml) was spotted on each slide, sealed under a coverslip and incubated at 37 °C overnight (15–20 h) in a humid chamber.

After hybridisation, coverslips were removed and slides were rinsed in $2 \times$ SSC at room temperature for 30 min. Sections were digested by RNase A (20 μ g/ml in $2 \times$ SSC) at 37 °C for 30 min at room temperature, washed in $0.5 \times$ SSC for 30 min at 37 °C, followed by 90 min at room temperature in $0.5 \times$ SSC, at 60 °C in $0.1 \times$ SSC and finally for 30 min at room temperature in $0.1 \times$ SSC.

Coating

Following hybridisation, the slides were coated with Kodak NTB2 photographic emulsion (Kodak, Rochester, NY, USA). Slides were exposed for 5 days for NPY, 3 days for CRH and 13 days for POMC, developed in Dektol developer for 2 min, and fixed in rapid fixer (Kodak) for 4 min. Thereafter, tissue were rinsed in running water for 30 min, counterstained with Harris modified haematoxylin (Fisher Scientific) and rapidly dehydrated through graded concentrations of ethanol, cleared in toluene and coverslipped with Permount (Fisher Scientific).

Semi-quantitative analysis

Anatomical identification of the arcuate nucleus and PVN was essentially based on the atlas of Paxinos and Franklin (27). Semiquantitative analysis of hybridisation signal was carried out on nuclear emulsion-dipped slides over the confines of reactive cells in the arcuate nucleus and the medial parvocellular area of the PVN using a Zeiss Optical System (Carl Zeiss, Göttingen, Germany) coupled to a Macintosh computer (Power PC 7500/100; Apple Computer Inc., Cupertino, CA, USA) and Image Software (version 1.60 non-FPU, W. Rasband, NIH, Bethesda, MD, USA). The optical density (OD) of the hybridisation signal was measured under dark-field illumination at a $\times 10$ magnification. Sections from the treated and control animals were matched for rostrocaudal level. The arcuate nucleus and PVN of the hypothalamus were digitised and subjected to densitometric analysis, yielding measurements of integrated OD (area of nucleus \times average OD). The OD of each specific region was then corrected for the average background signal, which was determined by sampling cells immediately outside the cell group of interest.

Average OD was obtained from measurements of 18 sections (for POMC and NPY) and 12 sections (for CRH) per mouse (six mice per group). Quantitative data are presented as mean \pm SEM ($n = 6$). Comparison of the mRNA levels between experimental groups was performed using ANOVA (Statview, Abacus Concepts, Berkeley, CA, USA) followed by Dunnett's post-hoc test. $P < 0.05$ was considered statistically significant.

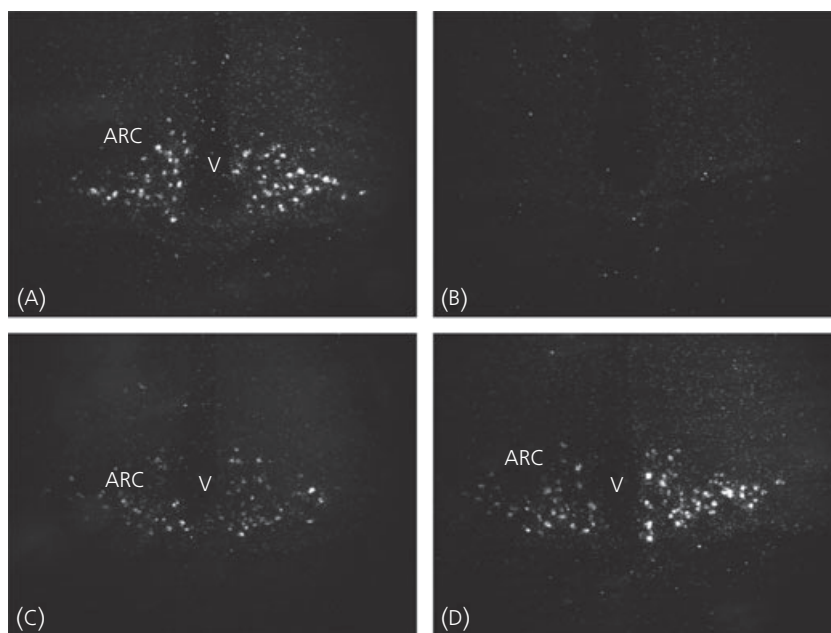


Fig. 1. Representative dark-field micrograph illustrating pro-opiomelanocortin mRNA hybridisation signal in the arcuate nucleus (ARC). (A) Intact mice. (B) Section consecutive to that shown in (A) hybridised with the sense probe (negative control). (C) Castrated mice. (D) Castrated mice which received a single injection of oestradiol 12 h before they were killed. V, Third ventricle ($\times 60$).

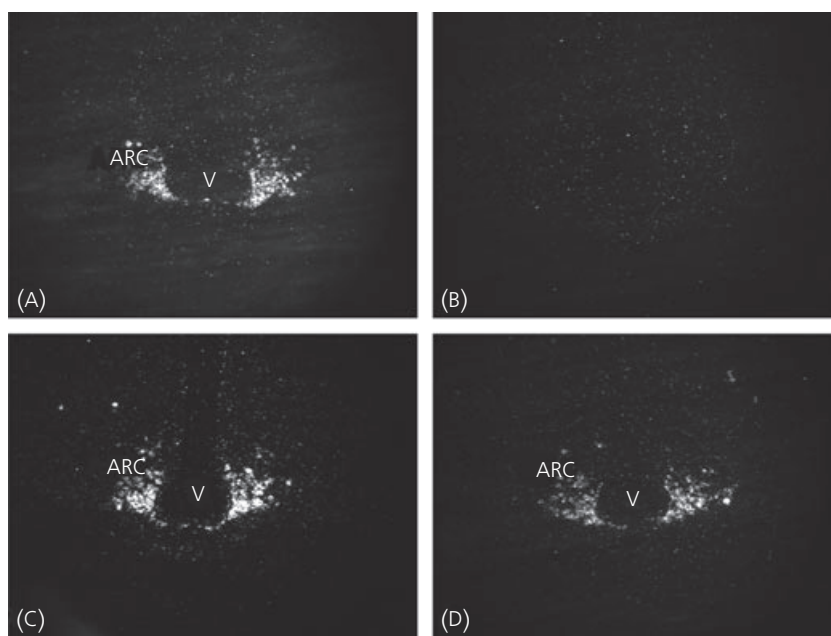


Fig. 2. Representative dark-field micrographs illustrating neuropeptide Y mRNA hybridisation signal in the arcuate nucleus (ARC). (A) Intact mice. (B) Section consecutive to that shown in (A) hybridised with the sense probe (negative control). (C) Castrated mice. (D) Castrated mice which received a single injection of oestradiol 12 h before they were killed. V, Third ventricle ($\times 60$).

Results

After appropriate exposure times, strong hybridisation signal was observed with the three probes (Figs 1–3). Sections hybridised with the sense probes did not exhibit any detectable reaction in the hypothalamic regions that showed a positive hybridisation signal with the antisense probes (Figs 1b, 2b and 3b).

POMC mRNA

As illustrated by light microscopic dark-field micrographs, neurones expressing POMC mRNA were mostly observed in the outer area of the arcuate nucleus (Fig. 1). Seven days after castration, POMC mRNA levels were decreased by 25% ($P < 0.001$). E_2 administration to castrated mice induced a significant increase ($P < 0.001$) of 23%

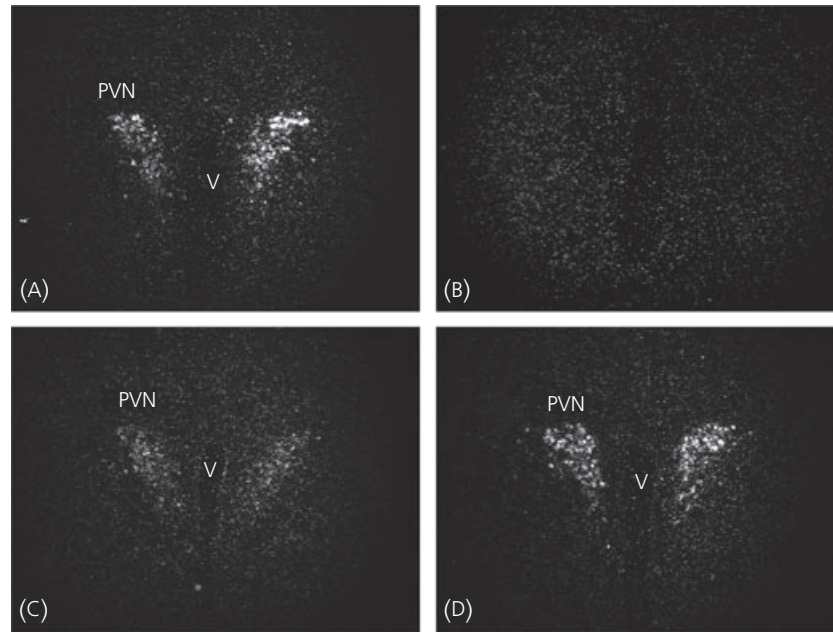


Fig. 3. Representative dark-field micrographs illustrating corticotrophin-releasing hormone mRNA hybridisation signal in the paraventricular nucleus (PVN). (A) Intact mice. (B) Section consecutive to that shown in (A) hybridised with the sense probe (negative control). (C) Castrated mice. (D) Castrated mice which received a single injection of oestradiol 12 h before they were killed. V, Third ventricle ($\times 60$).

and 18%, when compared to the levels detected in vehicle-treated castrated animals, after 12 and 24 h, respectively (Figs 1 and 4). At the 3-h time interval, no significant changes were detected.

NPY mRNA

Following *in situ* hybridisation with the NPY cRNA probe, several radiolabelled neurones were detected in the inner area of the arcuate nucleus. NPY mRNA levels were increased by 35% ($P < 0.001$) in castrated mice compared to the levels measured in intact animals (Figs 2 and 4). At all the time intervals following E_2 administration, mRNA levels were completely restored, the values being not different from those detected in intact animals (Figs 2 and 4).

CRH mRNA

Labelling obtained after *in situ* hybridisation with the [35 S] CRH cRNA probe was essentially distributed throughout the medial parvocellular area of the PVN (Fig. 3). As shown in Figs 3 and 4, following 7-day castration, CRH mRNA expression was markedly decreased (-42% ; $P < 0.001$). E_2 treatment induced 79% and 81% increase in the mRNA levels when compared to levels observed in vehicle-treated castrated animals, at the 12- and 24-h time intervals, respectively, then completely restoring mRNA levels. No significant changes could be detected at the 3-h time interval.

Discussion

The present data clearly demonstrate that in adult female mice 7-day castration as well as short-term administration of E_2 to castrated animals can modify mRNA expression of three neuropep-

tides, POMC, NPY and CRH involved in food intake regulation. The localisation of POMC, NPY and CRH mRNAs in the mouse hypothalamus is very similar to that reported in the rat hypothalamus (26). There is evidence that circulating oestrogens can down-regulate food intake (3–7). This effect of oestradiol on feeding behaviour appears to be mediated by $ER\alpha$, as shown by studies involving use of $ER\alpha$ KO and $ER\beta$ KO female mice (6, 10, 28).

The levels of POMC mRNA which decreased following castration were almost completely restored by a single injection of E_2 , indicating that oestrogens can positively regulate POMC-expressing neurones. Endogenous α -MSH from POMC as well as melanocortin agonists are potent inhibitors of food intake (29, 30). On the basis of the present results, it may be suggested that α -MSH may mediate, at least partly, the anorexigenic influence of oestrogens. The site(s) of action of E_2 still remain(s) to be fully clarified. $ER\alpha$ mRNA and protein are highly expressed in the arcuate nucleus (31, 32). There are reports indicating that, in rat and mouse hypothalamus, a subpopulation of POMC neurones can concentrate oestradiol (33, 34). Interestingly, Qiu *et al.* (35), using whole-cell patch recording in hypothalamic arcuate neurones in slices, reported that all the POMC neurones identified responded to E_2 . These previous studies suggest a direct action of oestrogens on POMC neurones, although the stimulatory effect of E_2 on POMC mRNA expression might be partly mediated by other oestrogen-sensitive neuronal systems, such as the NPYergic system. We have already shown that NPY negatively modulates POMC mRNA expression in the arcuate nucleus (36).

NPY is considered as a potent orexigenic peptide involved in the hypothalamic regulation of food intake and energy balance in several species (2, 12, 15, 37). In the hypothalamus, NPY is synthesised predominantly in the arcuate nucleus from which the axons project to the PVN (22, 38). Food deprivation and attendant weight loss

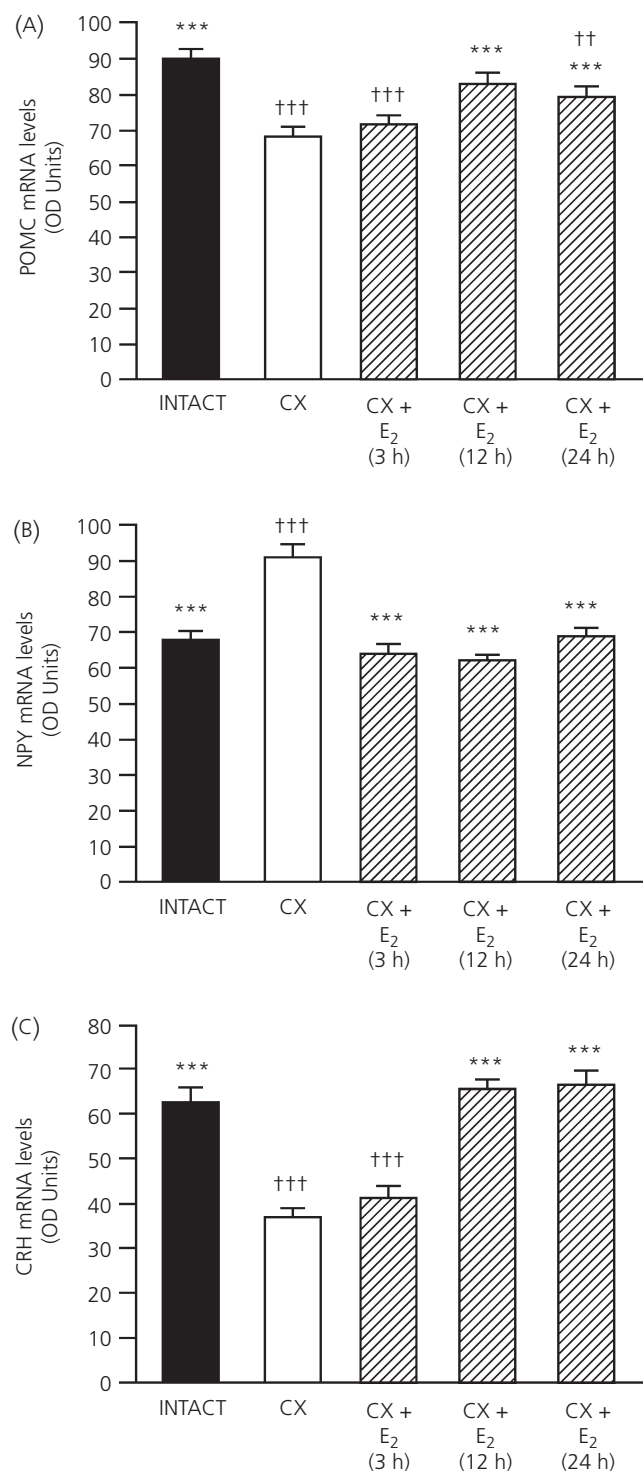


Fig. 4. Effects of castration (CX) and a single injection of oestradiol (E₂) to castrated female mice in the arcuate nucleus (A,B) and paraventricular nucleus (C). ***P < 0.001 versus CX; †††P < 0.001, ††P < 0.01 versus intact mice. For each group, n = 6. POMC, Pro-opiomelanocortin; NPY, neuropeptide Y; CRH, corticotrophin-releasing hormone.

induced increases in NPY gene expression and NPY peptide levels in the rat arcuate nucleus and PVN (12). When the rats consumed food, NPY returned to the normal range (12). In the present experi-

ment, we report that 7-day castration resulted in an increase in NPY mRNA levels in female mouse arcuate neurones, an effect which was completely reversed as soon as 3 h after a single injection of E₂. It has already been demonstrated that administration of E₂ to ovariectomised rats during 18 days decreased NPY peptide concentrations in the PVN region, suggesting that NPY may play a role in weight loss observed during E₂ treatment (39). Since NPY neurones in the arcuate nucleus which project to the PVN possess E₂ receptors (40), it appears likely that E₂ can have a direct influence on NPY expression in the arcuate nucleus.

The expression of CRH mRNA in the PVN was markedly decreased following castration and stimulated 12 and 24 h following E₂ administration to castrated animals. Similar findings were also reported by Roy *et al.* (41) who showed that E₂ could increase CRH mRNA expression in the PVN of the rhesus monkey. CRH is considered as an anorexigenic peptide because central injection of the peptide has been shown to alternate nocturnal and fasting-induced feeding and decrease feeding in different paradigms designed to evaluate ingestive behaviour (42). Microinjection studies revealed that the sites of anorectic action of CRH are within the PVN, possibly mediated by CRHR1 or CRHR2 receptor types (42–44). It then appears likely that CRH might play a role in food intake regulation in the female mouse. The effects of E₂ on CRH neurones might be direct because approximately 40% of CRH containing neurones located in the parvocellular PVN in human hypothalamus express ER α (45). Moreover, the presence of oestrogen responsive elements in the CRH promoter region has been reported (46).

In summary, it appears that the oestrogenic modulation of mRNA expression of three neuropeptides known to be involved in food intake regulation, namely POMC, NPY and CRH, can account for the anorectic effect of oestrogens. The relative role of each of the peptides remains to be established. It is likely that oestrogens exert a direct influence on each of the three neuronal populations, although an indirect influence cannot be totally excluded.

Accepted 11 February 2007

References

- Boberger C, Hokfelt T. Hypothalamic and vagal neuropeptide circuitries regulating food intake. *Physiol Behav* 2001; **74**: 669–682.
- Schwartz MW, Woods SC, Porte PJ, Seeley RJ, Baskin DG. Central nervous system control of food intake. *Nature Med* 2000; **404**: 661–671.
- Wade GN. Gonadal hormones and behavioral regulation of body weight. *Physiol Behav* 1972; **8**: 523–534.
- Mystkowski P, Schwartz MW. Gonadal steroids and energy homeostasis in the leptin era. *Nutrition* 2000; **16**: 937–946.
- Wade GN, Gray JM, Bartness TJ. Gonadal influences on adiposity. *Int J Obes* 1985; **9** (Suppl. 1): 83–92.
- Geary N, Asarian L, Korach KS, Pfaff DW, Ogawa S. Deficits in E₂-dependent control of feeding, weight gain, and cholecystokinin satiation in ER- α null mice. *Endocrinology* 2001; **142**: 4751–4757.
- Geary N. Estradiol, CCK and satiation. *Peptides* 2001; **22**: 1251–1263.
- Butera PC, Czaja JA. Intracranial estradiol in ovariectomized guinea pigs: effects on ingestive behaviors and body weight. *Brain Res* 1984; **322**: 41–48.

- 9 Gambacciani M, Ciaponi M, Cappagli B, Piaggese L, De Simone L, Orlandi R, Genazzani AR. Body weight, body fat distribution, and hormonal replacement therapy in early postmenopausal women. *J Clin Endocrinol Metab* 1997; **82**: 414–417.
- 10 Heine PA, Taylor JA, Iwamoto GA, Lubahn DB, Cooke PS. Increased adipose tissue in male and female estrogen receptor- α knockout mice. *Proc Natl Acad Sci USA* 2000; **97**: 12729–12734.
- 11 Morley JE. Neuropeptide regulation of appetite and weight. *Endocr Rev* 1987; **8**: 256–287.
- 12 Kalra SP, Dube MG, Pu S, Xu B, Horvath TL, Kalra PS. Interacting appetite-regulating pathways in the hypothalamic regulation of body weight. *Endocr Rev* 1999; **20**: 68–100.
- 13 Beck B. [Cholecystokinin, neurotensin and corticotropin-releasing factor, three important anorexic peptides]. *Ann Endocrinol (Paris)* 1992; **53**: 44–56.
- 14 Kristensen P, Judge ME, Thim L, Ribel U, Christjansen KN, Wulff BS, Clausen JT, Jensen PB, Madsen OD, Vrang N, Larsen PJ, Hastrup S. Hypothalamic CART is a new anorectic peptide regulated by leptin. *Nature* 1998; **393**: 72–76.
- 15 Stanley BG. Neuropeptide Y in multiple hypothalamic sites controls eating behavior, endocrine and autonomic systems for body energy balance. In: Colmers WF, Wahlestedt C, eds. *Biology of Neuropeptide Y and Related Peptides*. Totowa, NJ: Humana Press, 1993: 457–472.
- 16 Sakurai T, Amemiya A, Ishii M, Matsuzaki I, Chemelli RM, Tanaka H, Williams SC, Richardson JA, Kozlowski GP, Wilson S, Arch JR, Buckingham RE, Haynes AC, Carr SA, Annan RS, McNulty DE, Liu WS, Terrett JA, Elshourbagy NA, Bergsma DJ, Yanagisawa M. Orexins and orexin receptors: a family of hypothalamic neuropeptides and G protein-coupled receptors that regulate feeding behavior. *Cell* 1998; **92**: 573–585.
- 17 Tritos NA, Maratos-Flier E. Two important systems in energy homeostasis: melanocortins and melanin-concentrating hormone. *Neuropeptides* 1999; **33**: 339–349.
- 18 Owens MJ, Nemeroff CB. Physiology and pharmacology of corticotropin-releasing factor. *Pharmacol Rev* 1991; **43**: 425–473.
- 19 de Mateos-Verchere JG, Leprince J, Tonon MC, Vaudry H, Costentin J. The octadecaneuropeptide [diazepam-binding inhibitor (33–50)] exerts potent anorexigenic effects in rodents. *Eur J Pharmacol* 2001; **414**: 225–231.
- 20 Horvath TL, Diano S, Sotonyi P, Heiman M, Tschop M. Minireview: ghrelin and the regulation of energy balance – a hypothalamic perspective. *Endocrinology* 2001; **142**: 4163–4169.
- 21 Chartrel N, Dujardin C, Anouar Y, Leprince J, Decker A, Clerens S, Do-Rego JC, Vandesande F, Llorens-Cortes C, Costentin J, Beauvillain JC, Vaudry H. Identification of 26RFa, a hypothalamic neuropeptide of the RFamide peptide family with orexigenic activity. *Proc Natl Acad Sci USA* 2003; **100**: 15247–15252.
- 22 Pelletier G, Guy J, Allen YS, Polak JM. Electron microscope immunocytochemical localization of neuropeptide Y (NPY) in the rat brain. *Neuropeptides* 1984; **4**: 319–324.
- 23 Smith AI, Funder JW. Proopiomelanocortin processing in the pituitary, central nervous system, and peripheral tissues. *Endocr Rev* 1988; **9**: 159–179.
- 24 Pelletier G, Leclerc R, Saavedra JM, Brownstein MJ, Vaudry H, Ferland L, Labrie F. Distribution of beta-lipotropin (beta-LPH), adrenocorticotropin (ACTH) and alpha-melanocyte-stimulating hormone (alpha-MSH) in the rat brain. I. Origin of the extrahypothalamic fibers. *Brain Res* 1980; **192**: 433–440.
- 25 Sawchenko PE, Swanson LW. Localization, colocalization and plasticity of corticotropin-releasing factor immunoreactivity in rat brain. *Fed Proc* 1985; **44**: 221–227.
- 26 Compere V, Li S, Leprince J, Tonon MC, Vaudry H, Pelletier G. Effect of intracerebroventricular administration of the octadecaneuropeptide on the expression of pro-opiomelanocortin, neuropeptide Y and corticotropin-releasing hormone mRNAs in rat hypothalamus. *J Neuroendocrinol* 2003; **15**: 197–203.
- 27 Paxinos A, Franklin KBJ. *The Mouse Brain in Stereotaxic Coordinates*. San Diego, CA: Academic Press, 2001.
- 28 Lemieux C, Gelinat Y, Lalonde J, Labrie F, Richard D, Deshaies Y. The selective estrogen receptor modulator acobifene reduces cholesterolemia independently of its anorectic action in control and cholesterol-fed rats. *J Nutr* 2005; **135**: 2225–2229.
- 29 Poggioli R, Vergoni AV, Bertolini A. ACTH-(1–24) and alpha-MSH antagonize feeding behavior stimulated by kappa opiate agonists. *Peptides* 1986; **7**: 843–848.
- 30 Fan W, Boston BA, Kesterson RA, Hruby VJ, Cone RD. Role of melanocortinergic neurons in feeding and the agouti obesity syndrome. *Nature* 1997; **385**: 165–168.
- 31 Laflamme N, Nappi RE, Drolet G, Labrie C, Rivest S. Expression and neuropeptidic characterization of estrogen receptors (ER α and ER β) throughout the rat brain: anatomical evidence of distinct roles of each subtype. *J Neurobiol* 1998; **36**: 357–378.
- 32 Pelletier G. Localization of androgen and estrogen receptors in rat and primate tissues. *Histol Histopathol* 2000; **15**: 1261–1270.
- 33 Morrell J, McGinty F, Pfaff DW. A subset of beta-endorphin or dynorphin-containing neurons in the medial basal hypothalamus accumulate estradiol. *Neuroendocrinology* 1985; **41**: 417–426.
- 34 Jirikowski GF, Merchenthaler I, Rieger GE, Stumpf WE. Estradiol target sites immunoreactive for beta-endorphin in the arcuate nucleus of rat and mouse hypothalamus. *Neurosci Lett* 1986; **65**: 121–126.
- 35 Qiu J, Bosch MA, Tobias SC, Krust A, Graham SM, Murphy SJ, Korach KS, Chambon P, Scanlan TS, Ronnekleiv OK, Kelly MJ. A G-protein-coupled estrogen receptor is involved in hypothalamic control of energy homeostasis. *J Neurosci* 2006; **26**: 5649–5655.
- 36 Garcia de Yebenes E, Li S, Fournier A, St-Pierre S, Pelletier G. Regulation of proopiomelanocortin gene expression by neuropeptide Y in the rat arcuate nucleus. *Brain Res* 1995; **674**: 112–116.
- 37 White JD, Neuropeptide Y. a central regulator of energy homeostasis. *Regul Pept* 1993; **49**: 93–107.
- 38 Chronwall BM. Anatomy and physiology of the neuroendocrine arcuate nucleus. *Peptides* 1985; **6** (Suppl. 2): 1–11.
- 39 Bonavera JJ, Dube MG, Kalra PS, Kalra SP. Anorectic effects of estrogen may be mediated by decreased neuropeptide-Y release in the hypothalamic paraventricular nucleus. *Endocrinology* 1994; **134**: 2367–2370.
- 40 Sar M, Sahu A, Crowley WR, Kalra SP. Localization of neuropeptide-Y immunoreactivity in estradiol-concentrating cells in the hypothalamus. *Endocrinology* 1990; **127**: 2752–2756.
- 41 Roy BN, Reid RL, Van Vugt DA. The effects of estrogen and progesterone on corticotropin-releasing hormone and arginine vasopressin messenger ribonucleic acid levels in the paraventricular nucleus and supraoptic nucleus of the rhesus monkey. *Endocrinology* 1999; **140**: 2191–2198.
- 42 Morley JE, Levine AS. Corticotrophin releasing factor, grooming and ingestive behavior. *Life Sci* 1982; **31**: 1459–1464.
- 43 Heinrichs SC, Menzaghi F, Pich EM, Hauger RL, Koob GF. Corticotropin-releasing factor in the paraventricular nucleus modulates feeding induced by neuropeptide Y. *Brain Res* 1993; **611**: 18–24.
- 44 Monnikes H, Schmidt BG, Raybould HE, Tache Y. CRF in the paraventricular nucleus mediates gastric and colonic motor response to restraint stress. *Am J Physiol* 1992; **262**: G137–G143.
- 45 Bao AM, Hestiantoro A, Van Someren EJ, Swaab DF, Zhou JN. Colocalization of corticotropin-releasing hormone and oestrogen receptor- α in the paraventricular nucleus of the hypothalamus in mood disorders. *Brain* 2005; **128**: 1301–1313.
- 46 Torpy DJ, Papanicolaou DA, Chrousos GP. Sexual dimorphism of the human stress response may be due to estradiol-mediated stimulation of hypothalamic corticotropin-releasing hormone synthesis. *J Clin Endocrinol Metab* 1997; **82**: 982.