[177]. Nesfatin-1 has been shown to colocalize with several well-described peptides, including CART, CRH, OXT, and AVP [178]. Double-labeling immunohistochemistry in these areas has revealed that nesfatin-1 is colocalized with feeding-related factors such as CRH, OXT, POMC and CART [177–179]. Central administration of α-MSH increases NUCB2 mRNA in the hypothalamus [180]. Anorectic effect by icv administration of nesfatin-1 was mediated by OXT in the PVN [181,182]. Icv administered nesfatin-1 decreased food intake and inhibited gastroduodenal motility in mice [183]. Nesfatin-1 and OXT both suppresses food intake in *fa/fa* Zucker rats, and leptin-induced satiety is unaltered by immunoneutralizing nesfatin-1 IgG [176,182]. These results suggest that nesfatin-1 induces anorexia in a leptin-independent and melanocortin-dependent manner [176,182].

4.6. Prolactin-releasing peptide (PrRP)

Prolactin-releasing peptide (PrRP) was isolated as an endogenous ligand of an orphan G-protein-coupled receptor (GPR10/hGR3) and belongs to the RFamide peptide [183]. Initial studies showed that PrRP could stimulate prolactin release both in vitro [184] and in vivo [185,186], giving rise to the name of this peptide. However, recent morphological and physiological studies have shown that PrRP is not a hypophysiotropic prolactin-releasing factor [187–189], but have suggested rather that PrRP was involved in a wider range of neuroendocrine and autonomic functions [190,191].

PrRP-synthesizing cells have been identified in the dorsomedial hypothalamic nucleus (DMH), the A1 region of the ventrolateral medulla (VLM) and the A2 region of the NTS in the medulla oblongata [192–196]. Icv administration of PrRP significantly increased plasma OXT and AVP levels [197] and to stimulate ACTH secretion via CRH from the parvocellular cells in the PVN [198]. As stress activates medullary and hypothalamic PrRP neurons, PrRP and NA may both function cooperatively in neuroendocrine responses to stress [180,199]. Icv administration of anti-PrRP antibodies to rats attenuates OXT secretion in response to conditioned fear [199]. Our previous study showed that central administration of PrRP induced the expression of c-*fos* gene in the PVN and increased plasma corticosterone levels in conscious rats [200]. Moreover, we showed that the restraint stress and acute inflammatory stress upregulated the expression of PrRP gene in the NTS and the VLM. The nociceptive stimulus upregulated the expression of PrRP gene in the ventrolateral medulla. We also showed that pretreatment with an anti-PrRP antibody significantly attenuated nociceptive stimulus induced the expression of the c-*fos* gene in the PVN. These results indicate that PrRP may be potent and important mediator of stress responses.

PrRP neurons in the brainstem were activated by CCK [77] and PrRP mediates CCK-induced satiety [201]. Icv or microinjection of PrRP inhibits feeding [180,202] but does not induce nausea [203]. Icv co-administration of PrRP and leptin resulted in additive reduction in food intake and body weight gain, and that PrRP mRNA levels were reduced in Zucker (*fa/fa*) rats with mutated leptin receptor and in fasted rats [78]. Thus, PrRP is regulated by leptin. PrRP promotes release of the feeding inhibition factors, α-MSH and neurotensin [203]. It is possible that α-MSH and neurotensin contribute to the inhibitory effect of PrRP. Icv administration of PrRP also increased the core temperature and oxygen consumption in male rats [204]. These results indicate that PrRP may affect energy homeostasis by the reduction of food intake and the increase in energy expenditure. Icv administration of PrRP activated OXT neurons at the PVN in mice, which was significantly reduced in GPR10 knockout mice, which is the phenotype of PrRP knockout mice [199]. The roles of PrRP on energy homeostasis were supported by studies on GPR10 knockout mice, which became hyperphagic and obese [205]. More recent study showed icv administration of RFamide-related peptides (RFRP-1 and RFRP-3), which are belong to RFamide peptide such as PrRP, increased the plasma OXT level and activated the OXT neurons [206]. RFamide peptide, including PrRP and RFRP, may play a role in the control of energy metabolism.

4.7. Secretin

Secretin is best known for its role as a duodenal hormone released in response to acidification of the intestinal lumen [207]. Secretin, however, can also activate vagal sensory nerves [208,209]. Secretin is synthesized within the brain and can activate hypothalamic neurons [210–213]. Peripheral administration of secretin induced Fos expression in the SON [209,214]. Icv administration of secretin also increases Fos expression in SON neurons and increases secretion of OXT and AVP, and secretin receptors are found in the SON and the magnocellular area of the PVN [215]. Secretin also activates vagal primary afferent neurons [210]. Furthermore, lacking secretin receptors mice exhibit defects in social and cognitive behaviors [216]. Although the treatment of secretin was beneficial in autism and associated gastrointestinal abnormalities [217], its efficacy was not confirmed in subsequent clinical trials [218]. Moreover, these studies have suggested on the existence of a specific relationship between autism and inflammatory bowel disease [218]. Recently, the combined administration of secretin and OXT inhibited chronic colitis in rats [219]. These results suggested that the administration of both secretin and OXT would develop a novel treatment of inflammation-associated intestinal disorder.

5. Perspective

Although OXT was discovered over 60 years ago, the primary role of OXT has not been known yet. In this review, we know that OXT has relationship with various physiological and pathophysiological functions. OXT works as a hormone in the periphery and as a neurotransmitter in the CNS. The importance of OXT in milk ejection and uterine

contraction is well known. Recently, we showed the central effects of some neuropeptides, such as adrenomedullin family and other peptides in OXT release in rats. OXT is also involved in lots of physiological and pathological functions such as appetite, anxiety, antinociception, social recognition and stress, with many neuropeptides. In each function, the relationship between OXT and neuropeptides is not fully understood. OXT may be an important key in some disease and develop a novel treatment for them. We anticipate that further studies can clarify the relationship with between OXT and neuropeptides.

Acknowledgments

We thank Dr. John Menzies (University of Edinburgh, UK) for critical reviews of this paper. This study was supported by Grant-in-Aid for Scientific Research on Innovative Areas No. 23113518, Grant-in-Aid for Scientific Research (B) No. 22390044, and Grant-in-Aid for challenging Exploratory Research No. 23659127 from the Ministry of Education, Culture, Sports, Science and Technology, and Grant-in-Aid for the Third-term Comprehensive 10-year Strategy for Cancer Control from the Ministry of Health, Labour and Welfare, Japan.

References

[1] H. Dale, On some physiological actions of ergot, The Journal of Physiology 34 (1906) 163–206.
[2] V. du Vigneaud, C. Ressler, S. Trippett, The sequence of amino acids in oxytocin, with a proposal for the structure of oxytocin, The Journal of Biological Chemistry 205 (1953) 949–957.
[3] V. du Vigneaud, C. Ressler, J. Swan, C. Roberts, P. Katsoyannis, S. Gordon, The synthesis of an octapeptide amide with the hormonal activity of oxytocin, Journal of the American Chemical Society 75 (1953) 4879–4880.
[4] V. du Vigneaud, C. Ressler, J. Swan, C. Roberts, The synthesis of oxytocin, Journal of the American Chemical Society 76 (1954) 3115–3121.
[5] L.W. Swanson, H.G.H.G. Kuypers, The paraventricular nucleus of the hypothalamus: cytoarchitectonic subdivisions and organization of projections to the pituitary, dorsal vagal complex, and spinal cord as demonstrated by retrograde fluorescence double-labeling methods, The Journal of Comparative Neurology 194 (1980) 555–570.
[6] M.V. Sofroniew, Morphology of vasopressin and oxytocin neurones and their central and vascular projections, Progress in Brain Research 60 (1983) 101–114.
[7] M. Carmichael, R. Humbert, J. Dixen, G. Palmisano, W. Greenleaf, J. Davidson, Plasma oxytocin increases in the human sexual response, The Journal of Clinical Endocrinology and Metabolism 64 (1987) 27–31.
[8] M. Carmichael, V. Warburton, J. Dixen, J. Davidson, Relationships among cardiovascular, muscular, and oxytocin responses during human sexual activity, Archives of Sexual Behavior 23 (1994) 59–79.
[9] J. Russell, G. Leng, Sex, parturition and motherhood without oxytocin? The Journal of Endocrinology 157 (1998) 342–359.
[10] J. Leckman, W. Goodman, W. North, P. Chappell, L. Price, D. Pauls, G. Anderson, M. Riddle, C. McDougle, L. Barr, et al., The role of central oxytocin in obsessive compulsive disorder and related normal behavior, Psychoneuroendocrinology 19 (1994) 723–749.
[11] S. Stock, K. Uvnas-Moberg, Increased plasma levels of oxytocin in response to afferent electrical stimulation of the sciatic and vagal nerves and in response to touch and pinch in anaesthetized rats, Acta Physiologica Scandinavica 132 (1988) 29–34.
[12] K. Uvnäs-Moberg, G. Bruzelius, P. Alster, T. Lundeberg, The antinociceptive effect of non-noxious sensory stimulation is mediated partly through oxytocinergic mechanisms, Acta Physiologica Scandinavica 149 (1993) 199–204.
[13] P. Burbach, L.J. Young, J. Russell, Oxytocin: synthesis, secretion and reproductive functions, in: J.D. Neill (Ed.), Knobil and Neill's Physiology of Reproduction, Elsevier, Amsterdam, 2006, pp. 3055–3127.
[14] M. Sofroniew, Projections from vasopressin, oxytocin, and neurophysin neurons to neural targets in the rat and human, The Journal of Histochemistry and Cytochemistry 28 (1980) 475–478.
[15] R. Ivell, K. Furuya, B. Brackmann, Y. Dawood, F. Khan-Dawood, Expression of the oxytocin and vasopressin genes in human and baboon gonadal tissues, Endocrinology 127 (1990) 2990–2996.
[16] R. Ivell, M. Balvers, W. Rust, R. Bathgate, A. Einspanier, Oxytocin and male reproductive function, Advances in Experimental Medicine and Biology 424 (1997) 253–264.
[17] J. Frayne, H.D. Nicholson, Effect of oxytocin on testosterone production by isolated rat Leydig cells is mediated via a specific oxytocin receptor, Biology of Reproduction 52 (1995) 1268–1273.
[18] J. Frayne, H.D. Nicholson, Localization of oxytocin receptors in the human and macaque monkey male reproductive tracts: evidence for a physiological role of oxytocin in the male, Molecular Human Reproduction 4 (1998) 527–532.
[19] N.C. Foo, D. Carter, D. Murphy, R. Ivell, Vasopressin and oxytocin gene expression in rat testis, Endocrinology 128 (1991) 2118–2128.
[20] H.D. Nicholson, M.P. Hardy, Luteinising hormone differentially regulates the secretion of testicular oxytocin and testosterone by purified rat Leydig cells in vitro, Endocrinology 130 (1992) 671–677.
[21] S. Filippi, G.B. Vannelli, S. Granchi, M. Luconi, C. Crescioli, R. Mancina, A. Natali, S. Brocchi, L. Vignozzi, E. Bencini, I. Noci, F. Ledda, G. Forti, M. Maggi, Identification, localisation and functional activity of oxytocin receptors in epididymis, Molecular and Cellular Endocrinology 193 (2002) 89–100.
[22] G.C. Harris, J. Frayne, H.D. Nicholson, Epididymal oxytocin in the rat; its origin and regulation, International Journal of Andrology 19 (1996) 278–286.
[23] K. Whittington, S. Assinder, M. Gould, H. Nicholson, Oxytocin, oxytocin associated neurophysin and the oxytocin receptor in human prostate, Cell and Tissue Research 318 (2004) 375–382.
[24] S. Ogawa, S. Kudo, Y. Kitsunai, S. Fukuchi, Increase in oxytocin secretion at ejaculation in male, Clinical Endocrinology 13 (1980) 95–97.
[25] M.S. Carmichael, R. Humbert, J. Dixen, G. Palmisano, W. Greenleaf, J.M. Davidson, Plasma oxytocin increases in the human sexual response, The Journal of Clinical Endocrinology and Metabolism 64 (1987) 27–31.
[26] M.R. Murphy, J.R. Seckl, S. Burton, S.A. Checkley, S.L. Lightman, Changes in oxytocin and vasopressin secretion during sexual activity in men, The Journal of Clinical Endocrinology and Metabolism 65 (1987) 738–741.
[27] M. Ludwig, G. Leng, Dendritic peptide release and peptide-dependent behaviours, Nature Review Neuroscience 7 (2006) 126–136.
[28] M. Castel, J. Morris, The neurophysin-containing innervation of the forebrain of the mouse, Neuroscience 24 (1988) 937–966.
[29] G. de Vries, R. Buijs, The origin of vasopressinergic and oxytocinergic innervation of the rat brain with special reference to the lateral septum, Brain Research 273 (1983) 307–317.
[30] H. van Tol, E. Bolwerk, B. Liu, J. Burbach, Oxytocin and vasopressin gene expression in the hypothalamo–neurohypophyseal system of the rat during the estrous cycle, pregnancy, and lactation, Endocrinology 122 (1988) 945–951.

[31] H. Zingg, D. Lefebvre, Oxytocin and vasopressin gene expression during gestation and lactation, Brain Research 464 (1988) 1–6.
[32] A. Thomas, J. Amico, Sequential estrogen and progesterone (P) followed by P withdrawal increases the level of oxytocin messenger ribonucleic acid in the hypothalamic paraventricular nucleus of the male rat, Life Sciences 58 (1996) 1615–1620.
[33] D. Lefebvre, R. Farookhi, A. Giaid, J. Neculcea, H. Zingg, Uterine oxytocin gene expression. II. Induction by exogenous steroid administration, Endocrinology 134 (1994) 2562–2566.
[34] N. Ostrowski, Oxytocin receptor mRNA expression in rat brain: implications for behavioral integration and reproductive success, Psychoneuroendocrinology 23 (1998) 989–1004.
[35] A. Fuchs, S. Periyasamy, M. Alexandrova, M. Soloff, Correlation between oxytocin receptor concentration and responsiveness to oxytocin in pregnant rat myometrium: effects of ovarian steroids, Endocrinology 113 (1983) 742–749.
[36] M. Soloff, M.M. Fernstrom, S. Periyasamy, S. Soloff, S. Baldwin, M. Wieder, Regulation of oxytocin receptor concentration in rat uterine explants by estrogen and progesterone, Canadian Journal of Biochemical Cellular Biology 61 (1983) 625–630.
[37] M. Maggi, A. Magini, A. Fiscella, S. Giannini, G. Fantoni, F. Toffoletti, G. Massi, M. Serio, Sex steroid modulation of neurohypophysial hormone receptors in human nonpregnant myometrium, The Journal of Clinical Endocrinology and Metabolism 74 (1992) 385–392.
[38] C. Breton, C. Pechoux, G. Morel, H. Zingg, Oxytocin receptor messenger ribonucleic acid: characterization, regulation, and cellular localization in the rat pituitary gland, Endocrinology 136 (1995) 2928–2936.
[39] A. Larcher, J. Neculcea, C. Breton, A. Arslan, F. Rozen, C. Russo, H. Zingg, Oxytocin receptor gene expression in the rat uterus during pregnancy and the estrous cycle and in response to gonadal steroid treatment, Endocrinology 136 (1995) 5350–5356.
[40] C. Pedersen, J. Ascher, Y. Monroe, A. Prange, Oxytocin induces maternal behavior in virgin female rats, Science 216 (1982) 648–650.
[41] S. Fahrbach, J. Morrell, D. Pfaff, Possible role for endogenous oxytocin in estrogen-facilitated maternal behavior in rats, Neuroendocrinology 40 (1985) 526–532.
[42] L. Young, J. Winslow, Z. Wang, B. Gingrich, Q. Guo, M. Matzuk, T. Insel, Gene targeting approaches to neuroendocrinology: oxytocin, maternal behavior, and affiliation, Hormones and Behavior 31 (1997) 221–231.
[43] S. Meddle, V. Bishop, E. Gkoumassi, F. van Leeuwen, A. Douglas, Dynamic changes in oxytocin receptor expression and activation at parturition in the rat brain, Endocrinology 148 (2007) 5095–5104.
[44] T. Insel, Regional changes in brain oxytocin receptors post-partum: time-course and relationship to maternal behaviour, Journal of Neuroendocrinology 2 (1990) 539–545.
[45] C. Pedersen, J. Caldwell, C. Walker, G. Ayers, G. Mason, Oxytocin activates the postpartum onset of rat maternal behavior in the ventral tegmental and medial preoptic areas, Behavioral Neuroscience 108 (1994) 1163–1171.
[46] M.J. Millan, M.H. Millan, A. Członkowski, A. Herz, Vasopressin and oxytocin in the rat spinal cord: distribution and origins in comparison to [Met] enkephalin, dynorphin and related opioids and their irresponsiveness to stimuli modulating neurohypophyseal secretion, Neuroscience 13 (1984) 179–187.
[47] T. Lundeberg, B. Meister, E. Bjorkstrand, K. Uvnas-Moberg, Oxytocin modulates the effects of galanin in carrageenan-induced hyperalgesia in rats, Brain Research 608 (1993) 181–185.
[48] B. Puder, R. Papka, Hypothalamic paraventricular axons projecting to the female rat lumbosacral spinal cord contain oxytocin immunoreactivity, Journal of Neuroscience Research 64 (2001) 53–60.
[49] X. Xu, Z. Wiesenfeld-Hallin, Intrathecal oxytocin facilitates the spinal nociceptive flexor reflex in the rat, Neuroreport 5 (1994) 750–752.
[50] J. Yang, Y. Yang, J.M. Chen, W.Y. Liu, C.H. Wang, B.C. Lin, Central oxytocin enhances antinociception in the rat, Peptides 28 (2007) 1113–1119.
[51] J. Yang, Intrathecal administration of oxytocin induces analgesia in low back pain involving the endogenous opiate peptide system, Spine 19 (1994) 867–871 (Phila Pa 1976).
[52] M. Conde's-Lara, I. Maie, A. Dickenson, Oxytocin actions on afferent evoked spinal cord neuronal activities in neuropathic but not in normal rats, Brain Research 1045 (2005) 124–133.
[53] T. Lundeberg, K. Uvnas-Moberg, G. Agren, G. Bruzelius, Antinociceptive effects of oxytocin in rats and mice, Neuroscience Letters 170 (1994) 153–157.
[54] X.L. Gu, L.C. Yu, Involvement of opioid receptors in oxytocin-induced antinociception in the nucleus accumbens of rats, Journal of Pain 8 (2007) 85–90.
[55] A. Schorscher-Petcu, S. Sotocinal, S. Ciura, A. Dupré, J. Ritchie, R.E. Sorge, J.N. Crawley, S.B. Hu, K. Nishimori, L.J. Young, E. Tribollet, R. Quirion, J.S. Mogil, Oxytocin-induced analgesia and scratching are mediated by the vasopressin-1A receptor in the mouse, The Journal of Neuroscience 30 (2010) 8274–8284.
[56] G. Gimpl, F. Fahrenholz, The oxytocin receptor system: structure, function, and regulation, Physiological Reviews 81 (2001) 629–683.
[57] S. Arnaudeau, N. Leprêtre, J. Mironneau, Oxytocin mobilizes calcium from a unique heparin-sensitive and thapsigargin-sensitive store in single myometrial cells from pregnant rats, Pflugers Archiv: European Journal of Physiology 428 (1994) 51–59.
[58] C.Y. Ku, A. Qian, Y. Wen, K. Anwer, B.M. Sanborn, Oxytocin stimulates myometrial guanosine triphosphatase and phospholipase-C activities via coupling to G alpha q/11, Endocrinology 136 (1995) 1509–1515.
[59] S. Phaneuf, G. Asboth, G.N. Europe-Finner, S.P. Watson, A. Lopez Bernal, Second messenger pathways for oxytocin and prostaglandins in human myometrium, Biochemical Society Transactions 23 (1995) 21S.
[60] T. Schöneberg, E. Kostenis, J. Liu, T. Gudermann, J. Wess, Molecular aspects of vasopressin receptor function, Advances in Experimental Medicine and Biology 449 (1998) 347–358.
[61] M. Thibonnier, L.N. Berti-Mattera, N. Dulin, D.M. Conarty, R. Mattera, Signal transduction pathways of the human V1-vascular, V2-renal, V3-pituitary vasopressin and oxytocin receptors, Progress in Brain Research 119 (1998) 147–161.
[62] M. Birnbaumer, Vasopressin receptors, Trends in Endocrinology and Metabolism 11 (2000) 406–410.
[63] B. Chini, M. Manning, Agonist selectivity in the oxytocin/vasopressin receptor family: new insights and challenges, Biochemical Society Transactions 35 (2007) 737–741.
[64] R.J. Windle, N. Shanks, S.L. Lightman, C.D. Ingram, Central oxytocin administration reduces stress-induced corticosterone release and anxiety behavior in rats, Endocrinology 138 (1997) 2829–2834.
[65] R.J. Windle, S. Wood, N. Shanks, P. Perks, G.L. Conde, A.P. da Costa, C.D. Ingram, S.L. Lightman, Endocrine and behavioural responses to noise stress: comparison of virgin and lactating female rats during non-disrupted maternal activity, Journal of Neuroendocrinology 9 (1997) 407–414.
[66] J.A. Amico, R.C. Mantella, R.R. Vollmer, X. Li, Anxiety and stress responses in female oxytocin deficient mice, Journal of Neuroendocrinology 16 (2004) 319–324.
[67] R.J. Windle, L.E. Gamble, Y.M. Kershaw, S.A. Wood, S.L. Lightman, C.D. Ingram, Gonadal steroid modulation of stress-induced hypothalamo-pituitary-adrenal activity and anxiety behavior: role of central oxytocin, Endocrinology 147 (2006) 2423–2431.
[68] M.M. McCarthy, C.H. McDonald, P.J. Brooks, D. Goldman, An anxiolytic action of oxytocin is enhanced by estrogen in the mouse, Physiology and Behavior 60 (1996) 1209–1215.
[69] T.L. Bale, A.M. Davis, A.P. Auger, D.M. Dorsa, M.M. McCarthy, CNS region-specific oxytocin receptor expression: importance in regulation of anxiety and sex behavior, Journal of Neuroscience 21 (2001) 2546–2552.

[70] M. Heinrichs, T. Baumgartner, C. Kirschbaum, U. Ehlert, Social support and oxytocin interact to suppress cortisol and subjective responses to psychosocial stress, Biological Psychiatry 54 (2003) 1389–1398.
[71] P. Kirsch, C. Esslinger, Q. Chen, D. Mier, S. Lis, S. Siddhanti, H. Gruppe, V.S. Mattay, B. Gallhofer, A. Meyer-Lindenberg, Oxytocin modulates neural circuitry for social cognition and fear in humans, Journal of Neuroscience 25 (2005) 11489–11493.
[72] B. Olson, M. Drutarosky, M. Chow, V. Hruby, E. Stricker, J. Verbalis, Oxytocin and an oxytocin agonist administered centrally decrease food intake in rats, Peptides 12 (1991) 113–118.
[73] J.G. Verbalis, R.E. Blackburn, B.R. Olson, E.M. Stricker, Central oxytocin inhibition of food and salt ingestion: a mechanism for intake regulation of solute homeostasis, Regulatory Peptides 45 (1993) 149–154.
[74] A. Sclafani, L. Rinaman, R.R. Vollmer, J.A. Amico, Oxytocin knockout mice demonstrate enhanced intake of sweet and nonsweet carbohydrate solutions, American Journal of Physiology. Regulatory, Integrative and Comparative Physiology 292 (2007) R1828–R1833.
[75] D.F. Swaab, J.S. Purba, M.A. Hofman, Alterations in the hypothalamic paraventricular nucleus and its oxytocin neurons (putative satiety cells) in Prader-Willi syndrome: a study of five cases, The Journal of Clinical Endocrinology and Metabolism 80 (1995) 573–579.
[76] N. Vrang, P.J. Larsen, P. Kristensen, M. Tang-Christensen, Central administration of cocaine-amphetamine-regulated transcript activates hypothalamic neuroendocrine neurons in the rat, Endocrinology 141 (2000) 794–801.
[77] M. Maruyama, H. Matsumoto, K. Fujisawa, C. Kitada, S. Hinuma, H. Onda, M. Fujino, K. Inoue, Imunocytochemical localization of prolactinreleasing peptide in the rat brain, Endocrinology 140 (1999) 2326–2333.
[78] M. Maruyama, H. Matsumoto, K. Fujisawa, J. Noguchi, C. Kitada, S. Hinuma, H. Onda, O. Nishimura, M. Fujino, T. Higuchi, K. Inoue, Central administration of prolactin-releasing peptide stimulates oxytocin release in rats, Neuroscience Letters 276 (1999) 193–196.
[79] P.K. Olszewski, M.M. Wirth, T.J. Shaw, M.K. Grace, C.J. Billington, S.Q. Giraudo, A.S. Levine, Role of a-MSH in the regulation of consummatory behavior: immunohistochemical evidence, American Journal of Physiology 281 (2001) R673–R680.
[80] E. Bjkstrand, K. Uvn-Moberg, Central oxytocin increases food intake and daily weight gain in rats, Physiology & Behavior 59 (1996) 947–952.
[81] L.R. McMahon, P.J. Wellman, Assessment of the role of oxytocin receptors in phenylpropanolamine-induced anorexia in rats, Pharmacology, Biochemistry, and Behavior 57 (1997) 767–770.
[82] J.G. Verbalis, R.E. Blackburn, G.E. Hoffman, E.M. Stricker, Establishing behavioral and physiological functions of central oxytocin: insights from studies of oxytocin and ingestive behaviors, Advances in Experimental Medicine and Biology 395 (1995) 209–225.
[83] Z. Diaz-Cabiale, J.A. Narvaez, M. Petersson, K. Uvnas-Moberg, K. Fuxe, Oxytocin/alpha2-adrenoceptor interactions in feeding responses, Neuroendocrinology 71 (2000) 209–218.
[84] Y. Maejima, Y. Iwasaki, Y. Yamahara, M. Kodaira, U. Sedbazar, T. Yada, Peripheral oxytocin treatment ameliorates obesity by reducing food intake and visceral fat mass, Aging 3 (2011) 1169–1177 (Albany NY).
[85] E.A. Lawson, D.A. Donoho, J.I. Blum, E.M. Meenaghan, M. Misra, D.B. Herzog, P.M. Sluss, K.K. Miller, A. Klibanski, Decreased nocturnal oxytocin levels in anorexia nervosa are associated with low bone mineral density and fat mass, The Journal of Clinical Psychiatry 72 (2011) 1546–1551.
[86] P. Chiodera, R. Volpi, L. Capretti, C. Marchesi, L. d'Amato, A. De Ferri, L. Bianconi, V. Coiro, Effect of estrogen or insulin-induced hypoglycemia on plasma oxytocin levels in bulimia and anorexia nervosa, Metabolism 40 (1991) 1226–1230.
[87] G.K. Frank, W.H. Kaye, M. Altemus, C.G. Greeno, CSF oxytocin and vasopressin levels after recovery from bulimia nervosa and anorexia nervosa, bulimic subtype, Biological Psychiatry 48 (2000) 315–318.
[88] R.E. Blackburn, E.M. Stricker, J.G. Verbalis, Central oxytocin mediates inhibition of sodium appetite by naloxone in hypovolemic rats, Neuroendocrinology 56 (1992) 255–263.
[89] R.E. Blackburn, A.D. Demko, G.E. Hoffman, E.M. Stricker, J.G. Verbalis, Central oxytocin inhibition of angiotensin-induced salt appetite in rats, American Journal of Physiology 263 (1992) R1347–R1353.
[90] R. Puryear, K.V. Rigatto, J.A. Amico, M. Morris, Enhanced salt intake in oxytocin deficient mice, Experimental Neurology 171 (2001) 323–328.
[91] R.E. Blackburn, W.K. Samson, R.J. Fulton, E.M. Stricker, J.G. Verbalis, Central oxytocin and ANP receptors mediate osmotic inhibition of salt appetite in rats, American Journal of Physiology 269 (1995) R245–R251.
[92] K. Rigatto, R. Puryear, I. Bernatova, M. Morris, Salt appetite and the renin-angiotensin system: effect of oxytocin deficiency, Hypertension 42 (2003) 793–797.
[93] J.A. Amico, M. Morris, R.R. Vollmer, Mice deficient in oxytocin manifest increased saline consumption following overnight fluid deprivation, American Journal of Physiology. Regulatory, Integrative and Comparative Physiology 281 (2001) R1368–R1373.
[94] R.R. Vollmer, X. Li, J.R. Karam, J.A. Amico, Sodium ingestion in oxytocin knockout mice, Experimental Neurology 202 (2006) 441–448.
[95] P. Popik, P.E. Vos, J.M. van Ree, Neurohypophyseal hormone receptors in the septum are implicated in social recognition in the rat, Behavioural Pharmacology 3 (1992) 351–358.
[96] J.N. Ferguson, L.J. Young, E.F. Hearn, M.M. Matzuk, T.R. Insel, J.T. Winslow, Social amnesia in mice lacking the oxytocin gene, Nature Genetics 25 (2000) 284–288.
[97] E. Choleris, J.A. Gustafsson, K.S. Korach, L.J. Muglia, D.W. Pfaff, S. Ogawa, An oestrogen dependent 4-gene micronet regulating social recognition: a study with oxytocin and estrogen receptor-a and -b knockout mice, Proceedings of the National Academy of Sciences of the United States of America 100 (2003) 6192–6197.
[98] J.N. Ferguson, J.M. Aldag, T.R. Insel, L.J. Young, Oxytocin in the medial amygdala is essential for social recognition in the mouse, Journal of Neuroscience 2 (2001) 8278–8285.
[99] E. Choleris, S.R. Little, J.A. Mong, S.V. Puram, R. Langer, D.W. Pfaff, Microparticle-based delivery of oxytocin receptor antisense DNA in the medial amygdala blocks social recognition in female mice, Proceedings of the National Academy of Sciences of the United States of America 104 (2007) 4670–4675.
[100] K. Kitamura, K. Kangawa, M. Kawamoto, Y. Ichiki, S. Nakamura, H. Matsuo, T. Eto, Adrenomedullin: a novel hypotensive peptide isolated from human pheochromocytoma, Biochemical and Biophysical Research Communications 192 (1993) 553–560.
[101] M. Ogoshi, K. Inoue, Y. Takei, Identification of a novel adrenomedullin gene family in teleost fish, Biochemical and Biophysical Research Communications 311 (2003) 1072–1077.
[102] J. Roh, C.L. Chang, A. Bhalla, C. Klein, S.Y. Hsu, Intermedin is a calcitonin/calcitonin gene-related peptide family peptide acting through the calcitonin receptor-like receptor/receptor activity-modifying protein receptor complexes, The Journal of Biological Chemistry 279 (2004) 7264–7274.
[103] Y. Takei, K. Inoue, M. Ogoshi, T. Kawahara, H. Bannai, S. Miyano, Identification of novel adrenomedullin in mammals: a potent cardiovascular and renal regulator, FEBS Letters 556 (2004) 53–58.
[104] S. Eguchi, Y. Hirata, H. Iwasaki, K. Sato, T.X. Watanabe, T. Inui, K. Nakajima, S. Sakakibara, F. Marumo, Structure-activity relationship of adrenomedullin, a novel vasodilatory peptide, in cultured rat vascular smooth muscle cells, Endocrinology 135 (1994) 2454–2458.
[105] A.C. Conner, D.L. Hay, S.G. Howitt, K. Kilk, U. Langel, M. Wheatley, D.M. Smith, D.R. Poyner, Interaction of calcitonin-gene-related peptide with its receptors, Biochemical Society Transactions 30 (2002) 451–455.

[106] Y. Fujisawa, Y. Nagai, A. Miyatake, Y. Takei, K. Miura, T. Shoukouji, A. Nishiyama, S. Kimura, Y. Abe, Renal effects of a new member of adrenomedullin family, adrenomedullin2, in rats, European Journal of Pharmacology 497 (2004) 75–80.
[107] Y. Fujisawa, Y. Nagai, A. Miyatake, K. Miura, T. Shokoji, A. Nishiyama, S. Kimura, Y. Abe, Roles of adrenomedullin 2 in regulating the cardiovascular and sympathetic nervous systems in conscious rats, American Journal of Physiology. Heart and Circulatory Physiology 290 (2006) H1120–H1127.
[108] M. Saita, A. Shimokawa, T. Kunitake, K. Kato, T. Hanamori, K. Kitamura, T. Eto, H. Kannan, Central actions of adrenomedullin on cardiovascular parameters and sympathetic outflow in conscious rats, American Journal of Physiology 274 (1998) R979–R984.
[109] H. Takahashi, T.X. Watanabe, M. Nishimura, T. Nakanishi, M. Sakamoto, M. Yoshimura, Y. Komiyama, M. Masuda, T. Murakami, Centrally induced vasopressor and sympathetic responses to a novel endogenous peptide, adrenomedullin, in anesthetized rats, American Journal of Hypertension 7 (1994) 478–482.
[110] M.M. Taylor, S.L. Bagley, W.K. Samson, Intermedin/adrenomedullin-2 acts within central nervous system to elevate blood pressure and inhibit food and water intake, American Journal of Physiology. Regulatory, Integrative and Comparative Physiology 288 (2005) R919–R927.
[111] R. Serino, Y. Ueta, Y. Hara, M. Nomura, Y. Yamamoto, I. Shibuya, Y. Hattori, K. Kitamura, K. Kangawa, J.A. Russell, H. Yamashita, Centrally administered adrenomedullin increases plasma oxytocin level with induction of c-fos messenger ribonucleic acid in the paraventricular and supraoptic nuclei of the rat, Endocrinology 140 (1999) 2334–2342.
[112] Y. Ueta, R. Serino, I. Shibuya, K. Kitamura, K. Kangawa, J.A. Russell, H. Yamashita, A physiological role for adrenomedullin in rats; a potent hypotensive peptide in the hypothalamo-neurohypophysial system, Experimental Physiology 85 (2000) 163S–169S.
[113] H. Hashimoto, S. Hyodo, M. Kawasaki, T. Mera, L. Chen, A. Soya, T. Saito, H. Fujihara, T. Higuchi, Y. Takei, Y. Ueta, Centrally administered adrenomedullin 2 activates hypothalamic oxytocin-secreting neurons, causing elevated plasma oxytocin level in rats, American Journal of Physiology. Endocrinology and Metabolism 289 (2005) E753–E761.
[114] T.C. Murphy, W.K. Samson, The novel vasoactive hormone, adrenomedullin, inhibits water drinking in the rat, Endocrinology 136 (1995) 2459–2463.
[115] W.K. Samson, T.C. Murphy, Adrenomedullin inhibits salt appetite, Endocrinology 138 (1997) 613–616.
[116] G.M. Taylor, K. Meeran, D. O'Shea, D.M. Smith, M.A. Ghatei, S.R. Bloom, Adrenomedullin inhibits feeding in the rat by a mechanism involving calcitonin gene-related peptide receptors, Endocrinology 137 (1996) 3260–3264.
[117] H. Yokoi, H. Arima, T. Murase, K. Kondo, Y. Iwasaki, Y. Oiso, Intracerebroventricular injection of adrenomedullin inhibits vasopressin release in conscious rats, Neuroscience Letters 216 (1996) 65–67.
[118] H. Hashimoto, S. Hyodo, M. Kawasaki, M. Shibata, T. Saito, H. Suzuki, H. Otsubo, T. Yokoyama, H. Fujihara, T. Higuchi, Y. Takei, Y. Ueta, Adrenomedullin 2 (AM2)/intermedin is a more potent activator of hypothalamic oxytocin-secreting neurons than AM possibly through an unidentified receptor in rats, Peptides 28 (2007) 1104–1112.
[119] M. Ogoshi, K. Inoue, K. Naruse, Y. Takei, Evolutionary history of the calcitonin gene-related peptide family in vertebrates revealed by comparative genomic analyses, Peptides 27 (2006) 3154–3164.
[120] H. Otsubo, S. Hyodo, H. Hashimoto, M. Kawasaki, H. Suzuki, T. Saito, T. Ohbuchi, T. Yokoyama, H. Fujihara, T. Matsumoto, Y. Takei, Y. Ueta, Centrally administered adrenomedullin 5 activates oxytocin-secreting neurons in the hypothalamus and elevates plasma oxytocin level in rats, The Journal of Endocrinology 202 (2009) 237–247.
[121] Y. Ueta, K. Kitamura, T. Isse, I. Shibuya, N. Kabashima, S. Yamamoto, K. Kangawa, H. Matsuo, T. Eto, H. Yamashita, Adrenomedullin-immunoreactive neurons in the paraventricular and supraoptic nuclei of the rat, Neuroscience Letters 202 (1995) 37–40.
[122] B.F. O'Dowd, M. Heiber, A. Chan, H.H. Heng, L.C. Tsui, J.L. Kennedy, X. Shi, A. Petronis, S.R. George, T. Nguyen, A human gene that shows identity with the gene encoding the angiotensin receptor is located on chromosome 11, Gene 136 (1993) 355–360.
[123] K. Tatemoto, M. Hosoya, Y. Habata, R. Fujii, T. Kakegawa, M.X. Zou, Y. Kawamata, S. Fukusumi, S. Hinuma, C. Kitada, T. Kurokawa, H. Onda, M. Fujino, Isolation and characterization of a novel endogenous peptide ligand for the human APJ receptor, Biochemical and Biophysical Research Communications 251 (1998) 471–476.
[124] N. De Mota, Z. Lenkei, C. Llorens-Corte's, Cloning, pharmacological characterization and brain distribution of the rat apelin receptor, Neuroendocrinology 72 (2000) 400–407.
[125] D.K. Lee, R. Cheng, T. Nguyen, T. Fan, A.P. Kariyawasam, Y. Liu, D.H. Osmond, S.R. George, B.F. O'Dowd, Characterization of apelin, the ligand for the APJ receptor, Journal of Neurochemistry 74 (2000) 34–41.
[126] N. De Mota, A. Reaux-Le Goazigo, S. El Messari, N. Chartrel, D. Roesch, C. Dujardin, C. Kordon, H. Vaudry, F. Moos, C. Llorens-Cortes, Apelin, a potent diuretic neuropeptide counteracting vasopressin actions through inhibition of vasopressin neuron activity and vasopressin release, Proceedings of the National Academy of Sciences of the United States of America 101 (2004) 10464–10469.
[127] A. Reaux, N. De Mota, I. Skultetyova, Z. Lenkei, S. El Messari, K. Gallatz, P. Corvol, M. Palkovits, C. Llorens-Corte's, Physiological role of a novel neuropeptide, apelin, and its receptor in the rat brain, Journal of Neurochemistry 77 (2001) 1085–1096.
[128] A. Reaux-Le Goazigo, A. Morinville, A. Burlet, C. Llorens-Cortes, A. Beaudet, Dehydration-induced cross-regulation of apelin and vasopressin immunoreactivity levels in magnocellular hypothalamic neurons, Endocrinology 145 (2004) 4392–4400.
[129] A. Reaux, K. Gallatz, M. Palkovits, C. Llorens-Cortes, Distribution of apelin-synthesizing neurons in the adult rat brain, Neuroscience 113 (2002) 653–662.
[130] Y. Kawamata, Y. Habata, S. Fukusumi, M. Hosoya, R. Fujii, S. Hinuma, N. Nishizawa, C. Kitada, H. Onda, O. Nishimura, M. Fujino, Molecular properties of apelin: tissue distribution and receptor binding, Biochimica et Biophysica Acta 1538 (2001) 162–171.
[131] A.D. Medhurst, C.A. Jennings, M.J. Robbins, R.P. Davis, C. Ellis, K.Y. Winborn, K.W. Lawrie, G. Hervieu, G. Riley, J.E. Bolaky, N.C. Herrity, P. Murdock, J.G. Darker, Pharmacological and immunohistochemical characterization of the APJ receptor and its endogenous ligand apelin, Journal of Neurochemistry 84 (2003) 1162–1172.
[132] A.M. O'Carroll, T.L. Selby, M. Palkovits, S.J. Lolait, Distribution of mRNA encoding B78/apj, the rat homologue of the human APJ receptor, and its endogenous ligand apelin in brain and peripheral tissues, Biochimica et Biophysica Acta 1492 (2000) 72–80.
[133] D. Xi, K. Kusano, H. Gainer, Quantitative analysis of oxytocin and vasopressin messenger ribonucleic acids in single magnocellular neurons isolated from supraoptic nucleus of rat hypothalamus, Endocrinology 140 (1999) 4677–4682.
[134] G.C. Brailoiu, S.L. Dun, J. Yang, M. Ohsawa, J.K. Chang, N.J. Dun, Apelin-immunoreactivity in the rat hypothalamus and pituitary, Neuroscience Letters 327 (2002) 193–197.
[135] V.A. Tobin, P.M. Bull, S. Arunachalam, A.M. O'Carroll, Y. Ueta, M. Ludwig, The effects of apelin on the electrical activity of hypothalamic magnocellular vasopressin and oxytocin neurons and somatodendritic peptide release, Endocrinology 149 (2008) 6136–6145.
[136] L. Bodineau, C. Taveau, H.H. Lê Quan Sang, G. Osterstock, I. Queguiner, F. Moos, A. Frugière, C. Llorens-Cortes, Data supporting a new physiological role for brain apelin in the regulation of hypothalamic oxytocin neurons in lactating rats, Endocrinology 152 (2011) 3492–3503.

[137] T.H. Moran, Cholecystokinin and satiety: current perspectives, Nutrition 16 (2000) 858–865.
[138] J.G. Verbalis, M.J. McCann, C.M. McHale, E.M. Stricker, Oxytocin secretion in response to cholecystokinin and food: differentiation of nausea from satiety, Science 232 (1986) 1417–1419.
[139] L.P. Renaud, M. Tang, M.J. McCann, E.M. Stricker, J.G. Verbalis, Cholecystokinin and gastric distension activate oxytocinergic cells in rat hypothalamus, American Journal of Physiology 253 (1987) R661–R665.
[140] M.J. McCann, J.G. Verbalis, E.M. Stricker, LiCl and CCK inhibit gastric emptying and feeding and stimulate OT secretion in rats, American Journal of Physiology 256 (1989) R463–R468.
[141] Y. Ueta, H. Kannan, T. Higuchi, H. Negoro, K. Yamaguchi, H. Yamashita, Activation of gastric afferents increases noradrenaline release in the paraventricular nucleus and plasma oxytocin level, Journal of the Autonomic Nervous System 78 (2000) 69–76.
[142] K. Kawano, T. Hirashima, S. Mori, Y. Saitoh, M. Kurosumi, T. Natori, Spontaneous long-term hyperglycemic rat with diabetic complications. Otsuka Long-Evans Tokushima Fatty (OLETF) strain, Diabetes 41 (1992) 1422–1428.
[143] K. Kawano, T. Hirashima, S. Mori, T. Natori, OLETF (Otsuka Long-Evans Tokushima Fatty) rat: a new NIDDM rat strain, Diabetes Research and Clinical Practice 24 (Suppl.) (1994) S317–S320.
[144] A. Funakoshi, K. Miyasaka, H. Shinozaki, M. Masuda, T. Kawanami, Y. Takata, A. Kono, An animal model of congenital defect of gene expression of cholecystokinin (CCK)-A receptor, Biochemical and Biophysical Research Communications 210 (1995) 787–796.
[145] H. Hashimoto, T. Onaka, M. Kawasaki, L. Chen, T. Mera, A. Soya, T. Saito, H. Fujihara, H. Sei, Y. Morita, Y. Ueta, Effects of cholecystokinin (CCK)-8 on hypothalamic oxytocin-secreting neurons in rats lacking CCK-A receptor, Autonomic Neuroscience: Basic & Clinical 121 (2005) 16–25.
[146] J.G. Verbalis, C.M. McHale, T.W. Gardiner, E.M. Stricker, Oxytocin and vasopressin secretion in response to stimuli producing learned taste aversions in rats, Behavioral Neuroscience 100 (1986) 466–475.
[147] M.J. McCann, J.G. Verbalis, E.M. Stricker, Capsaicin pretreatment attenuates multiple responses to cholecystokinin in rats, Journal of the Autonomic Nervous System 23 (1988) 265–272.
[148] S.M. Luckman, M. Hamamura, I. Antonijevic, S. Dye, G. Leng, Involvement of cholecystokinin receptor types in pathways controlling oxytocin secretion, British Journal of Pharmacology 110 (1993) 378–384.
[149] H.E. Raybould, R.J. Gayton, G.J. Dockray, Mechanisms of action of peripherally administered cholecystokinin octapeptide on brain stem neurons in the rat, Journal of Neuroscience 8 (1988) 3018–3024.
[150] L. Rinaman, J.G. Verbalis, E.M. Stricker, G.E. Hoffman, Distribution and neurochemical phenotypes of caudal medullary neurons activated to express cFos following peripheral administration of cholecystokinin, The Journal of Comparative Neurology 338 (1993) 475–490.
[151] K.M. Kendrick, E.B. Keverne, M.R. Hinton, J.A. Goode, Cerebrospinal fluid and plasma concentrations of oxytocin and vasopressin during parturition and vaginocervical stimulation in the sheep, Brain Research Bulletin 26 (1991) 803–807.
[152] J.N. Crawley, J.S. Schwaber, Abolition of the behavioral effects of cholecystokinin following bilateral radiofrequency lesions of the parvocellular subdivision of the nucleus tractus solitarius, Brain Research 295 (1984) 289–299.
[153] G. Leng, C.H. Brown, J.A. Russel, Physiological pathways regulating the activity of magnocellular neurosecretory cells, Progress in Neurobiology 57 (1999) 625–655.
[154] T. Onaka, Catecholaminergic mechanisms underlying neurohypophysial hormone responses to unconditioned or conditioned aversive stimuli in rats, Experimental Physiology 85S (2000) 101S–110S.
[155] Y. Ueta, H. Kannan, T. Higuchi, H. Negoro, H. Yamashita, CCK-8 excites oxytocin-secreting neurons in the paraventricular nucleus in rats: possible involvement of noradrenergic pathway, Brain Research Bulletin 32 (1993) 453–459.
[156] L. Zhu, T. Onaka, Involvement of medullary A2 noradrenergic neurons in the activation of oxytocin neurons after conditioned fear stimuli, The European Journal of Neuroscience 16 (2002) 2186–2198.
[157] T. Onaka, T. Yamashita, X. Liu, K. Honda, T. Saito, K. Yagi, Medullary A1 noradrenergic neurons may mediate oxytocin release after noxious stimuli, Neuroreport 12 (2001) 2499–2502.
[158] B.R. Olson, M.D. Drutarosky, E.M. Stricker, J.G. Verbalis, Oxytocin and oxytocin agonist administered centrally decrease food intake in rats, Peptides 12 (1991) 113–118.
[159] B.R. Olson, M.D. Drutarosky, E.M. Stricker, J.G. Verbalis, Brain oxytocinreceptor antagonism blunts the effects of anorexigenic treatments in rats: evidence for central oxytocin inhibition of food intake, Endocrinology 129 (1991) 785–791.
[160] B.R. Olson, M.D. Drutarosky, E.M. Stricker, J.G. Vebalis, Brain oxytocin receptors mediate corticotropin-releasing hormone-induced anorexia, American Journal of Physiology 260 (1991) R448–R452.
[161] M.L. Gottsch, M.J. Cunningham, J.T. Smith, S.M. Popa, B.V. Acohido, W.F. Crowley, S. Seminara, D.K. Clifton, R.A. Steiner, A role for kisspeptins in the regulation of gonadotrophin secretion in the mouse, Endocrinology 145 (2004) 4073–4077.
[162] M.S. Irwig, G.S. Fraley, J.T. Smith, B.V. Acohido, S.M. Popa, M.J. Cunningham, M.L. Gottsch, D.K. Clifton, R.A. Steiner, Kisspeptin activation of gonadotrophin releasing hormone neurons and regulation of KiSS-1 mRNA in the male rat, Neuroendocrinology 80 (2004) 264–272.
[163] S. Messager, E.E. Chatzidaki, D. Ma, A.G. Hendrick, D. Zahn, J. Dixon, R.R. Thresher, I. Malinge, D. Lomet, M.B. Carlton, W.H. Colledge, A. Caraty, S.A. Aparicio, Kisspeptin directly stimulates gonadotrophin-releasing hormone release via G protein-coupled receptor 54, Proceedings of the National Academy of Sciences of the United States of America 102 (2005) 1761–1766.
[164] V.M. Navarro, J.M. Castellano, R. Fernandez-Fernandez, S. Tovar, J. Roa, A. Mayen, R. Nogueiras, M.J. Vazquez, M.L. Barreiro, P. Magni, E. Aguilar, C. Dieguez, L. Pinilla, M. Tena-Sempere, Characterization of the potent luteinizing hormone releasing activity of KiSS-1 peptide, the natural ligand of GPR54, Endocrinology 146 (2005) 156–163.
[165] M. Shahab, C. Mastronardi, S.B. Seminara, W.F. Crowley, S.R. Ojeda, T.M. Plant, Increased hypothalamic GPR54 signaling: a potential mechanism for initiation of puberty in primates, Proceedings of the National Academy of Sciences of the United States of America 102 (2005) 2129–2134.
[166] V.M. Navarro, R. Fernandez-Fernandez, J.M. Castellano, J. Roa, A. Mayen, M.L. Barreiro, F. Gaytan, E. Aguilar, L. Pinilla, C. Dieguez, M. Tena-Sempere, Advanced vaginal opening and precocious activation of the reproductive axis by KiSS-1 peptide, the endogenous ligand of GPR54, The Journal of Physiology 561 (2004) 379–386.
[167] T. Ohtaki, Y. Shintani, S. Honda, H. Matsumoto, A. Hori, K. Kanehashi, Y. Terao, S. Kumano, Y. Takatsu, Y. Masuda, Y. Ishibashi, T. Watanabe, M. Asada, T. Yamada, M. Suenaga, C. Kitada, S. Usuki, T. Kurokawa, H. Onda, O. Nishimura, M. Fujino, Metastasis suppressor gene KiSS-1 encodes peptide ligand of a G-protein-coupled receptor, Nature 411 (2001) 613–617.
[168] N. Richard, G. Galmiche, S. Corvaisier, A. Caraty, M.L. Kottler, KiSS-1 and GPR54 genes are co-expressed in rat gonadotrophs and differentially regulated in vivo by oestradiol and gonadotrophin-releasing hormone, Journal of Neuroendocrinology 2008 (20) (2008) 381–393.
[169] F. Gaytán, M. Gaytán, J.M. Castellano, M. Romero, J. Roa, B. Aparicio, N. Garrido, J.E. Sánchez-Criado, R.P. Millar, A. Pellicer, H.M. Fraser, M. Tena-Sempere, KiSS-1 in the mammalian ovary: distribution of kisspeptin in human and marmoset and alterations in KiSS-1 mRNA levels in a rat model of ovulatory dysfunction, American Journal of Physiology. Endocrinology and Metabolism 296 (2009) E520–E531.

[170] A.I. Muir, L. Chamberlain, N.A. Elshourbagy, D. Michalovich, D.J. Moore, A. Calamari, P.G. Szekeres, H.M. Sarau, J.K. Chambers, P. Murdock, K. Steplewski, U. Shabon, J.E. Miller, S.E. Middleton, J.G. Darker, C.G. Larminie, S. Wilson, D.J. Bergsma, P. Emson, R. Faull, K.L. Philpott, D. Harrison, AXOR12, a novel human G protein-coupled receptor, activated by the peptide KiSS-1.C, The Journal of Biological Chemistry 276 (2001) 28969–28975.

[171] Y. Horikoshi, H. Matsumoto, Y. Takatsu, T. Ohtaki, C. Kitada, S. Usuki, M. Fujino, Dramatic elevation of plasma metastin concentrations in human pregnancy: metastin as a novel placenta-derived hormone in humans, Journal of Clinical Endocrinology and Metabolism 88 (2003) 914–919.

[172] A.M. Rometo, S.J. Krajewski, M.L. Voytko, N.E. Rance, Hypertrophy and increased kisspeptin gene expression in the hypothalamic infundibular nucleus of postmenopausal women and ovariectomized monkeys, Journal of Clinical Endocrinology and Metabolism 92 (2007) 2744–2750.

[173] M. Kotani, M. Detheux, A. Vandenbogaerde, D. Communi, J.M. Vanderwinden, E. Le Poul, S. Bre'zillon, R. Tyldesley, N. Suarez-Huerta, F. Vandeput, C. Blanpain, S.N. Schiffmann, G. Vassart, M. Parmentier, The metastasis suppressor gene KiSS-1 encodes kisspeptins, the natural ligands of the orphan G protein-coupled receptor GPR54, The Journal of Biological Chemistry 276 (2001) 34631–34636.

[174] S.C. Ten, S.Y. Gu, Y.F. Niu, X.F. An, M. Yan, M. He, Central administration of kisspeptin-10 inhibits water and sodium excretion of anesthetized male rats and the involvement of arginine vasopressin, Endocrine Research 35 (2010) 128–136.

[175] V. Scott, C.H. Brown, Kisspeptin activation of supraoptic nucleus neurons in vivo, Endocrinology 152 (2011) 3862–3870.

[176] S. Oh-I, H. Shimizu, T. Satoh, S. Okada, S. Adachi, K. Inoue, H. Eguchi, M. Yamamoto, T. Imaki, K. Hashimoto, T. Tsuchiya, T. Monden, K. Horiguchi, M. Yamada, M. Mori, Identification of nesfatin-1 as a satiety molecule in the hypothalamus, Nature 443 (2006) 709–712.

[177] K. Foo, H. Brismar, C. Broberger, Distribution and neuropeptide coexistence of nucleobindin-2 mRNA/nesfatin-like immunoreactivity in the rat CNS, Neuroscience 156 (2008) 563–579.

[178] D. Kohno, M. Nakata, Y. Maejima, H. Shimizu, U. Sedbazar, N. Yoshida, K. Dezaki, T. Onaka, M. Mori, T. Yada, Nesfatin-1 neurons in paraventricular and supraoptic nuclei of the rat hypothalamus coexpress oxytocin and vasopressin and are activated by refeeding, Endocrinology 149 (2008) 1295–1301.

[179] G.C. Brailoiu, S.L. Dun, E. Brailoiu, S. Inan, J. Yang, J.K. Chang, N.J. Dun, Nesfatin-1: distribution and interaction with a G protein-coupled receptor in the rat brain, Endocrinology 148 (2007) 5088–5094.

[180] G.L. Yosten, W.K. Samson, The anorexigenic and hypertensive effects of nesfatin-1 are reversed by pretreatment with an oxytocin receptor antagonist, American Journal of Physiology. Regulatory, Integrative and Comparative Physiology 298 (2010) R1642–R1647.

[181] Y. Maejima, U. Sedbazar, S. Suyama, D. Kohno, T. Onaka, E. Takano, N. Yoshida, M. Koike, Y. Uchiyama, K. Fujiwara, T. Yashiro, T.L. Horvath, M.O. Dietrich, S. Tanaka, K. Dezaki, S. Oh-I, K. Hashimoto, H. Shimizu, M. Nakata, M. Mori, T. Yada, Nesfatin-1 regulated oxytocinergic signaling in the paraventricular nucleus causes anorexia through a leptin-independent melanocortin pathway, Cell Metabolism 10 (2009) 355–365.

[182] K. Atsuchi, A. Asakawa, M. Ushikai, K. Ataka, M. Tsai, K. Koyama, Y. Sato, I. Kato, M. Fujimiya, A. Inui, Centrally administered nesfatin-1 inhibits feeding behaviour and gastroduodenal motility in mice, Neuroreport 21 (2010) 1008–1011.

[183] S. Hinuma, Y. Habata, R. Fujii, M. Hosoya, S. Fukusumi, C. Kitada, Y. Masuo, T. Asano, H. Matsumoto, M. Sekiguchi, T. Kurokawa, O. Nishimura, H. Onda, M. Fujino, A prolactin-releasing peptide in the brain, Nature 393 (1998) 272–276.

[184] H. Matsumoto, J. Noguchi, Y. Horikoshi, Y. Kawamata, C. Kitada, S. Hinuma, H. Onda, O. Nishimura, M. Fujino, Stimulation of prolactin release by prolactin-releasing peptide in rats, Biochemical and Biophysical Research Communications 259 (1999) 321–324.

[185] R. Tokita, T. Nakata, H. Katsumata, S. Konishi, H. Onodera, J. Imaki, S. Minami, Prolactin secretion in response to prolactin-releasing peptide and the expression of the prolactin-releasing peptide gene in the medulla oblongata are estrogen dependent in rats, Neuroscience Letters 276 (1999) 103–106.

[186] P.J. Havel, T.M. Hahn, D.K. Sindelar, D.G. Baskin, M.F. Dallman, D.S. Weigle, M.W. Schwartz, Effect of streptozotocin-induced diabetes and insulin treatment on hypothalamic melanocortin system and muscle uncoupling protein 3 expression in rats, Diabetes 49 (2000) 244–252.

[187] H. Jarry, H. Heuer, L. Schomburg, K. Bauer, Prolactin-releasing peptide do not stimulate prolactin release in vivo, Neuroendocrinology 71 (2000) 262–267.

[188] W.K. Samson, Z.T. Resch, T.C. Murphy, J.K. Chang, Gender-biased activity of the novel prolactin releasing peptide. Comparison with thyrotropin releasing hormone reveals only pharmacologic effects, Endocrine 9 (1998) 289–291.

[189] T. Sakamoto, M. Fujimoto, M. Ando, Fishy tales of prolactin-releasing peptide, International Review of Cytology 225 (2003) 91–130.

[190] M.M. Taylor, W.K. Samson, Prolactin-releasing peptide: RF-amide peptide, Cellular and Molecular Life Sciences 58 (2001) 1206–1215.

[191] S. Minami, T. Nakata, R. Tokita, H. Onodera, Y. Ibata, Cellular localization of prolactin-releasing peptide messenger RNA in the rat brain, Neuroscience Letters 266 (1999) 73–76.

[192] C.T. Chen, S.L. Dun, J.K. Chang, Prolactin-releasing peptide immunoreactivity in A1 and A2 noradrenergic neurons of the rat medulla, Brain Research 822 (1999) 276–279.

[193] B.L. Roland, S.W. Sutton, S.J. Wilson, L. Luo, J. Pyati, R. Huvar, M.G. Erlander, T.W. Lovenberg, Anatomical distribution of prolactin-releasing peptide and its receptor suggests additional functions in the central nervous system and periphery, Endocrinology 140 (1999) 5736–5745.

[194] Y. Ibata, N. Iijima, Y. Kataoka, K. Kakihara, M. Tanaka, M. Hosoya, S. Hinuma, Morphological survey of prolactin-releasing peptide and its receptor with special reference to their functional roles in the brain, Neuroscience Research 38 (2000) 223–230.

[195] H. Matsumoto, M. Maruyama, J. Noguchi, Y. Horikoshi, K. Fujisawa, C. Kitada, S. Hinuma, H. Onda, O. Nishimura, K. Inoue, M. Fujino, Stimulation of corticotropin-releasing hormone-mediated adrenocorticotropin secretion by central administration of prolactin-releasing peptide in rats, Neuroscience Letters 285 (2000) 234–238.

[196] M. Maruyama, H. Matsumoto, K. Fujisawa, J. Noguchi, C. Kitada, M. Fujino, K. Inoue, Prolactin-releasing peptide as a novel stress mediator in the central nervous system, Endocrinology 142 (2001) 2032–2038.

[197] L. Zhu, T. Onaka, Facilitative role of prolactin-releasing peptide neurons in oxytocin cell activation after conditioned fear stimuli, Neuroscience 118 (2003) 1045–1053.

[198] C.B. Lawrence, K.L. Ellacott, S.M. Luckman, PRL-releasing peptide reduces food intake and may mediate satiety signaling, Endocrinology 143 (2000) 360–367.

[199] D.A. Bechtold, S.M. Luckman, Prolactin-releasing peptide mediates CCK-induced satiety in mice, Endocrinology 147 (2006) 4723–4729.

[200] T. Mera, H. Fujihara, M. Kawasaki, H. Hashimoto, T. Saito, M. Shibata, J. Saito, T. Oka, S. Tsuji, T. Onaka, Y. Ueta, Prolactin-releasing peptide is a potent mediator of stress responses in the brain through the hypothalamic paraventricular nucleus, Neuroscience 6141 (2006) 1069–1086.

[201] C.B. Lawrence, F. Celsi, J. Brennand, S.M. Luckman, Alternative role for prolactin-releasing peptide in the regulation of food intake, Nature Neuroscience 3 (2000) 645–646.

[202] L.J. Seal, C.J. Small, W.S. Dhillo, S.A. Stanley, C.R. Abbott, M.A. Ghatei, S.R. Bloom, PRL-releasing peptide inhibits food intake in male rats via the dorsomedial hypothalamic nucleus and not the

paraventricular hypothalamic nucleus, Endocrinology 142 (2001) 4236–4243.
[203] K.L. Ellacott, C.B. Lawrence, N.J. Rothwell, S.M. Luckman, PRL-releasing peptide interacts with leptin to reduce food intake and body weight, Endocrinology 143 (2002) 368–374.
[204] C.B. Lawrence, Y.L. Liu, M.J. Stock, S.M. Luckman, Anorectic actions of prolactin-releasing peptide are mediated by corticotropin-releasing hormone receptors, American Journal of Physiology. Regulatory, Integrative and Comparative Physiology 286 (2004) 101–107.
[205] W. Gu, B.J. Geddes, C. Zhang, K.P. Foley, A. Stricker-Krongrad, The prolactin-releasing peptide receptor (GPR 10) regulates body weight homeostasis in mice, Journal of Molecular Neuroscience 22 (2003) 93–103.
[206] M. Kaewwongse, Y. Takayanagi, T. Onaka, Effects of RFamide-related peptide (RFRP)-1 and RFRP-3 on oxytocin release and anxiety-related behaviour in rats, Journal of Neuroendocrinology 23 (2011) 20–27.
[207] W.Y. Chey, T. Chang, Neural hormonal regulation of exocrine pancreatic secretion, Pancreatology 1 (2001) 320–335.
[208] Y. Li, X. Wu, H. Yao, C. Owyang, Secretin activates vagal primary afferent neurons in the rat: evidence from electrophysiological and immunohistochemical studies, American Journal of Physiology. Gastrointestinal and Liver Physiology 289 (2005) G745–G752.
[209] H. Yang, L. Wang, S.V. Wu, J. Tay, M. Goulet, R. Boismenu, J. Czimmer, Y. Wang, S. Wu, Y. Ao, Y. Taché, Peripheral secretin-induced Fos expression in the rat brain is largely vagal dependent, Neuroscience 128 (2004) 131–141.
[210] M.G. Welch, J.D. Keune, T.B. Welch-Horan, N. Anwar, M. Anwar, R.J. Ludwig, D.A. Ruggiero, Secretin: hypothalamic distribution and hypothesized neuroregulatory role in autism, Cellular and Molecular Neurobiology 24 (2004) 219–241.
[211] K. Fuxe, K. Andersson, T. Hokfelt, V. Mutt, L. Ferland, L.F. Agnati, D. Ganten, S. Said, P. Eneroth, J.A. Gustafsson, Localization and possible function of peptidergic neurons and their interactions with central catecholamine neurons, and the central actions of gut hormones, Federation Proceedings 38 (1979) 2333–2340.
[212] N. Itoh, T. Furuya, K. Ozaki, M. Ohta, T. Kawasaki, The secretin precursor gene. Structure of the coding region and expression in the brain, The Journal of Biological Chemistry 266 (1991) 12595–12598.
[213] W.K. Samson, M.D. Lumpkin, S.M. McCann, Presence and possible site of action of secretin in the rat pituitary and hypothalamus, Life Sciences 34 (1984) 155–163.
[214] M. Goulet, P.J. Shiromani, C.M. Ware, R.A. Strong, R. Boismenu, J.R. Rusche, A secretin iv infusion activates gene expression in the central amygdala of rats, Neuroscience 118 (2003) 881–888.
[215] J.Y. Chu, W.H. Yung, B.K. Chow, Endogenous release of secretin from the hypothalamus, Annals of the New York Academy of Sciences 1070 (2006) 196–200.
[216] I. Nishijima, T. Yamagata, C.M. Spencer, E.J. Weeber, O. Alekseyenko, J.D. Sweatt, M.Y. Momoi, M. Ito, D.L. Armstrong, D.L. Nelson, R. Paylor, A. Bradley, Secretin receptor-deficient mice exhibit impaired synaptic plasticity and social behavior, Human Molecular Genetics 15 (2006) 3241–3250.
[217] K. Horvath, G. Stefanatos, K.N. Sokolski, R. Wachtel, L. Nabors, J.T. Tildon, Improved social and language skills after secretin administration in patients with autistic spectrum disorders, Journal of the Association for Academic Minority Physicians 9 (1998) 9–15.
[218] K.W. Williams, J.J. Wray, D.M. Wheeler, Intravenous secretin for autism spectrum disorder, Cochrane Database of Systematic Reviews 3 (2005) CD003495.
[219] M.G. Welch, M. Anwar, C.Y. Chang, K.J. Gross, D.A. Ruggiero, H. Tamir, M.D. Gershon, Combined administration of secretin and oxytocin inhibits chronic colitis and associated activation of forebrain neurons, Neurogastroenterology and Motility 22 (2010) 654-e202.
[220] T.O. Bruhn, S.W. Sutton, P.M. Plotsky, W.W. Vale, Central administration of corticotropin-releasing factor modulates oxytocin secretion in the rat, Endocrinology 119 (1986) 1558–1563.
[221] P.J. Larsen, M. Tang-Christensen, D.S. Jessop, Central administration of glucagon-like peptide-1 activates hypothalamic neuroendocrine neurons in the rat, Endocrinology 138 (1997) 4445–4455.
[222] J.A. Zueco, A.I. Esquifino, J.A. Chowen, E. Alvarez, P.O. Castrillón, E. Blázquez, Coexpression of glucagon-like peptide-1 (GLP-1) receptor, vasopressin, and oxytocin mRNAs in neurons of the rat hypothalamic supraoptic and paraventricular nuclei: effect of GLP-1(7-36) amide on vasopressin and oxytocin release, Journal of Neurochemistry 72 (1999) 10–16.
[223] E. Bojanowska, B. Stempniak, Effects of centrally or systemically injected glucagon-like peptide-1 (7-36) amide on release of neurohypophysial hormones and blood pressure in the rat, Regulatory Peptides 91 (2000) 75–81.
[224] C. Caquineau, G. Leng, X.M. Guan, M. Jiang, L. Van der Ploeg, A.J. Douglas, Effects of alpha-melanocyte-stimulating hormone on magnocellular oxytocin neurones and their activation at intromission in male rats, Journal of Neuroendocrinology 18 (2006) 685–691.
[225] N. Sabatier, C. Caquineau, G. Dayanithi, P. Bull, A.J. Douglas, X.M. Guan, M. Jiang, L. Van der Ploeg, G. Leng, Alpha-melanocyte-stimulating hormone stimulates oxytocin release from the dendrites of hypothalamic neurons while inhibiting oxytocin release from their terminals in the neurohypophysis, The Journal of Neuroscience 23 (2003) 10351–10358.
[226] Y. Ozaki, T. Onaka, M. Nakazato, J. Saito, K. Kanemoto, T. Matsumoto, Y. Ueta, Centrally administered neuromedin U activates neurosecretion and induction of c-fos messenger ribonucleic acid in the paraventricular and supraoptic nuclei of rat, Endocrinology 143 (2002) 4320–4329.
[227] J.E. Coombes, I.C. Robinson, F.A. Antoni, J.A. Russell, Release of oxytocin into blood and into cerebrospinal fluid induced by naloxone in anaesthetized morphine-dependent rats: the role of the paraventricular nucleus, Journal of Neuroendocrinology 3 (1991) 551–561.
[228] A.J. Douglas, I. Neumann, H.K. Meeren, G. Leng, L.E. Johnstone, G. Munro, J.A. Russell, Central endogenous opioid inhibition of supraoptic oxytocin neurons in pregnant rats, The Journal of Neuroscience 15 (1995) 5049–5057.
[229] J. Ciosek, K. Izdebska, Thyrotropin-releasing hormone modulates vasopressin and oxytocin synthesis and release from the hypothalamo-neurohypophysial system of different age male rats, Journal of Physiology and Pharmacology 60 (2009) 63–70.
[230] B. Bardrum, B. Ottesen, J. Fahrenkrug, A.R. Fuchs, Release of oxytocin and vasopressin by intracerebroventricular vasoactive intestinal polypeptide, Endocrinology 123 (1988) 2249–2254.

Peptides 46 (2013) 13–19

Contents lists available at SciVerse ScienceDirect

Peptides

journal homepage: www.elsevier.com/locate/peptides

The gene expression of the hypothalamic feeding-regulating peptides in cisplatin-induced anorexic rats

Mitsuhiro Yoshimura[a], Takanori Matsuura[a], Junichi Ohkubo[a], Motoko Ohno[a], Takashi Maruyama[a], Toru Ishikura[a], Hirofumi Hashimoto[a], Tetsuya Kakuma[b], Hironobu Yoshimatsu[b], Kiyoshi Terawaki[c], Yasuhito Uezono[c], Yoichi Ueta[a,*]

[a] Department of Physiology, School of Medicine, University of Occupational and Environmental Health, Kitakyushu 807-8555, Japan
[b] Department of Internal Medicine 1, Faculty of Medicine, Oita University, Oita 879-5503, Japan
[c] Division of Cancer Pathophysiology, Group for Development of Molecular Diagnostics and Individualized Therapy, National Cancer Center Research Institute, Tokyo 104-0045, Japan

ARTICLE INFO

Article history:
Received 22 November 2012
Received in revised form 30 April 2013
Accepted 30 April 2013
Available online 15 May 2013

Keywords:
Anorexia
Cisplatin
Feeding
Neuropeptides
Hypothalamus
Rikkunshito

ABSTRACT

Cisplatin has been widely used; however, various disadvantageous side effects afflict patients. Rikkunshito (RKT), a traditional Japanese herbal medicine, has been widely prescribed in Japan to improve anorexia; but the mechanisms are unknown. Here we studied whether RKT could improve anorexia induced by cisplatin and changes in feeding-regulating peptides in the hypothalamus in rats. Adult male rats were divided into 4 groups: water + saline (WS), water + cisplatin (WC), RKT + saline (RS), and RKT + cisplatin (RC) groups. Water or RKT (1 g/kg) was intragastrically administered for 4 days, from day −1 to day 2, and saline or cisplatin (6 mg/kg) was intraperitoneally (i.p.) administered at day 0. After i.p. administration, cumulative food intake, water intake, urine volume and body weight were measured. The rats were then decapitated, followed by removal of the brain, and feeding-regulating peptides in the hypothalamus were measured by *in situ* hybridization histochemistry. In the three-day measurements, there were no significant changes in cumulative water intake and urine volume. The body weight and cumulative food intake in WC significantly decreased compared to WS, whereas these were not observed in RC. *Pro-opiomelanocortin (POMC)* and *cocaine and amphetamine-regulated transcript (CART)* in the arcuate nucleus (ARC) in WC significantly increased, and *neuropeptide Y (NPY)* in the ARC decreased compared to WS, whereas those in RS and RC were comparable to WS. These results suggest that RKT may have therapeutic potential for anorexia induced by cisplatin.

1. Introduction

Cisplatin is widely used for a variety of malignant tumors. It demonstrates anti-tumor effects by inhibiting the replication of DNA [7], but it has various side effects, such as loss of appetite, nausea and vomiting. It has been suggested that serotonin receptors are involved in the occurrence of nausea and vomiting from the use of cisplatin [17]. 5-HT3 receptor antagonist, steroids and metoclopramide have been used for the treatment of nausea and vomiting caused by cisplatin. However, in Japan, Rikkunshito (RKT) has also been used empirically for patients who suffer from anorexia caused by cisplatin.

RKT, a traditional Japanese herbal medicine, or "kampo", is widely prescribed in Japan for the treatment of the various disorders, such as upper gastrointestinal symptoms in patients with functional dyspepsia, gastroesophageal reflux disease, dyspeptic symptoms in postgastrointestinal surgery patients, and chemotherapy-induced dyspepsia in cancer patients [6,15,16,20,23]. The largest component of RKT is "Hesperidin" [22], which is a polyphenol that is contained in the peels of some kinds of oranges. It has been reported in *in vitro* experiments that RKT could act as an antioxidant [4].

Recent studies have revealed that RKT administration stimulates peripheral ghrelin secretion [3] or selective serotonin reuptake inhibitor [3,19] in rats with anorexia induced by cisplatin. Yakabi et al. demonstrated that cisplatin-induced anorexia is due to reduced ghrelin secretion in the hypothalamus of rats [26]. However, there are few studies about the mechanism of RKT for cisplatin-induced anorexia, and details of its actions have not been elucidated.

Here we studied the effects of RKT on cisplatin-induced anorexia in rats. We also assessed the impact of RKT and cisplatin on the feeding-regulating peptides in the hypothalamus.

* Corresponding author at: Department of Physiology, School of Medicine, University of Occupational and Environmental Health, 1-1 Iseigaoka, Yahatanishi-ku, Kitakyushu 807-8555, Japan. Tel.: +81 93 691 7420; fax: +81 93 692 1711.
E-mail address: yoichi@med-uoeh-u.ac.jp (Y. Ueta).

0196-9781/$ – see front matter © 2013 Published by Elsevier Inc.
http://dx.doi.org/10.1016/j.peptides.2013.04.019

2. Materials and methods

2.1. Animals

Adult male Wistar rats (260–290 g body weight) were individually housed and maintained in temperature controlled (23–25 °C) conditions under a 12.12 h light/dark cycle (lights on 07.00 h). All experiments were performed in strict accordance with guidelines on the use and care of laboratory animals issued by the Physiological Society of Japan, and were approved by the Ethics Committee of Animal Care and Experimentation of University of Occupational and Environmental Health.

2.2. Test substance

RKT (Tsumura & Co., Tokyo, Japan) includes eight crude herbs (*Atractylodis lanceae rhizome*, *Ginseng radix*, *Pinelliae tuber*, *Hoelen*, *Zizyphi fructus*, *Aurantii nobilis pericarpium*, *Glycyrrhizae radix* and *Zingiberis rhizoma*). These were mixed and extracted with hot water and then spray-dried to make a RKT powdered extract. The RKT was dissolved in tap water (0.1 g/mL) for intragastrical administration. Cisplatin (Sigma–Aldrich Japan Co. LLC., Tokyo, Japan) was dissolved in 0.9% sterile physiological saline (Otsuka Pharmaceutical Co. Ltd., Tokyo, Japan) (0.6 mg/1 mL).

2.3. Experimental procedure

All the rats had access to food and water *ad libitum* throughout the experiments. The rats were divided into four groups: water + saline (WS, $n = 7$), water + cisplatin (WC, $n = 7$), RKT + saline (RS, $n = 8$), and RKT + cisplatin (RC, $n = 8$). Water (1 mL/100 g bode weight) or RKT (0.1 g/1 mL/100 g body weight) were administered directly to the stomach using sondes per os. These were administered from day −1 to day 2 (16.00–18.00 h). Saline (1 mL/100 g body weight) or cisplatin (0.6 mg/1 mL/100 g body weight) were administered intraperitoneally only one time 1 h after administration of water or RKT on day 0. Body weights were measured from day −1 to day 3 every 24 h. Food and water intake were measured from day 0 to day 3 every 24 h.

After the treatment, at day 3, the rats were decapitated immediately without being anesthetized, followed by removal of the brain promptly onto dry ice, then storing at −80 °C. Trunk blood samples were taken during decapitation, and were collected into chilled reaction tubes (Greiner Bio-One) containing an aprotinin/EDTA mixture. Blood samples were centrifuged for 10 min at 4 °C, 3000 rpm. After the blood was centrifuged, a 15 μL sample of plasma was taken for measuring plasma osmolality (P-Osm) using a ONE-TEN osmometer (FISKE, Norwood, MA, USA), 10 μL for measuring plasma glucose using a Medisafe Reader GR-101 (TERUMO, Tokyo, Japan), 500 μL for measuring plasma corticotrophin (SRL, Tokyo, Japan), and 500 μL for measuring plasma active and desasyl ghrelin.

2.4. In situ hybridization histochemistry

The removed brains were cut into 12 μm thickness, and thaw mounted on gelatin/chrome alum-coated slides. The locations of the hypothalamic areas, including the paraventricular nucleus (PVN), arcuate nucleus (ARC) and lateral hypothalamic area (LHA), were determined according to coordinates of the rat brain atlas. ^{35}S 3′-end-labeled deoxyoligonucleotide complementary to transcripts encoding *oxytocin*, *corticotrophin releasing hormone* (*CRH*), *pro-opiomelanocortin* (*POMC*), *cocaine and amphetamine-regulated transcript* (*CART*), *neuropeptide Y* (*NPY*), *agouti-related protein* (*AgRP*), *melanin-concentrating hormone* (*MCH*) and *orexin* were used (*oxytocin* probe sequence, 5′-CTC GGA GAA GGC AGA CTC AGG GTC GCA GGC-3′; *CRH* probe sequence, 5′-CAG TTT CCT GTT GCT GTG AGC TTG CTG AGC TAA CTG CTC TGC CCT GGC-3′; *POMC* probe sequence, 5′-TGG CTG CTC TCC AGG CAC CAG CTC CAC ACA TCT ATG GAG G-3′; *CART* probe sequence, 5′-TCC TTC TCG TGG GAC GCA TCA TCC ACG GCA GAG TAG ATG TCC AGG-3′; *NPY* probe sequence, 5′-CAA ATG GAT GAT TGG TCA TTT CAA CAT AGA GTT GGG GGC TTG CT-3′; *AgRP* probe sequence, 5′-CGA CGC GGA GAA CGA GAC TCG CGG TTC TGT GGA TCT AGC ACC TCT GCC-3′; *MCH* probe sequence, 5′-CCA ACA GGG TCG GTA GAC TCG TCC CAG CAT-3′; and *orexin* probe sequence, 5′-TCC TCA TAG TCT GGA GGC AGG TGG AAG GGT TCC CCA CTG CTA GTG-3′).

The probe was 3′-end-labeled using terminal deoxynucleotidyl transferase and [^{35}S] dATP. The *in situ* hybridization protocol has been previously described in detail [24]. Briefly, sections were fixed in 4% (w/v) formaldehyde for 5 min and incubated in saline containing 0.25% (v/v) acetic anhydride and 0.1 M triethanolamine for 10 min and then dehydrated, delipidated in chloroform, and partially rehydrated. Hybridization was carried out overnight at 37 °C in 45 μL of hybridization buffer under a Nescofilm (Bando Kagaku, Osaka, Japan) cover slip. A total count of 1×10^5 c.p.m. for oxytocin transcripts and 1×10^6 c.p.m. for CRH, POMC, CART, NPY, AgRP, MCH and orexin transcripts and per slide were used. After hybridization, sections were washed 4 times with SSC (150 mM NaCl and 15 mM sodium citrate) for 1 h at 55 °C and for an additional hour with two changes of SSC at room temperature. Hybridized sections containing hypothalamus were exposed for autoradiography (Hyperfilm, Amersham, Bucks, UK) for 6 h for oxytocin probe, 5 days for MCH and orexin probe, and 1 week for CRH, POMC, CART, NPY and AgRP probe. The resulting images were analyzed by computerized densitometry using a MCID imaging analyzer (Imaging Research Inc., Ontario, Canada). The mean optical densities (OD) of the autoradiographs were measured by comparison with simultaneously exposed ^{14}C-labeled microscale samples (Amersham, Bucks, UK) and represented in arbitrary units setting the mean OD obtained from control rats.

2.5. Statistical analysis

The mean ± SEM was calculated from the results of the body weight change, cumulative water and food intake, cumulative urine volume, and *in situ* hybridization histochemistry studies. In the results of *in situ* hybridization, the expression levels of the genes were expressed as a percentage of WS. All data were analyzed by one-way ANOVA followed by a Bonferroni-type adjustment for multiple comparisons (Origin Pro version 8.5J, Lightstone, Tokyo, Japan). Statistical significance was set at $P < 0.05$.

3. Results

3.1. Changes in body weight

The body weight of each group was measured from day 0 to day 3 (Fig. 1A). The body weight gradually increased during the experiments, except for WC (Fig. 1A). The body weight in WC at day 3 was significantly difference in comparison with all the other groups. Data are also presented as percentage from day 0 (Fig. 1B). The results of body weight in WC presented as percentage was also significantly different in comparison with all the other groups.

3.2. Water intake, urine volume, food intake

Cumulative water intake and cumulative urine volume were measured from day −1 to day 3 (Fig. 2A and B). There were no significant differences in cumulative water intake (Fig. 2A) or cumulative urine volume (Fig. 2B) among all the experimental groups.