0021-972X/01/$03.00/0
The Journal of Clinical Endocrinology & Metabolism

Vol. 86, No. 2
Printed in U.S.A.

Orexin A Stimulates Cortisol Secretion from Human Adrenocortical Cells through Activation of the Adenylate Cyclase-Dependent Signaling Cascade

G. MAZZOCCHI, L. K. MALENDOWICZ, L. GOTTARDO, F. ARAGONA, AND G. G. NUSSDORFER

Departments of Human Anatomy and Physiology, Sections of Anatomy (G.M., L.K.M., L.G., G.G.N.) and Urology (F.A.), University of Padua, I-35121 Padua, Italy

ABSTRACT

Orexins A and B are two hypothalamic peptides that increase food intake and body weight and probably play a role in the sleep regulation. They act through two subtypes of G protein-coupled receptors, called OX1-R and OX2-R. OX1-R selectively binds orexin-A, whereas OX2-R is nonselective for both orexins. Orexins did not affect the *in vitro* secretion of either catecholamine or aldosterone from human adrenals. Conversely, orexin A, but not orexin B, concentration dependently increased basal cortisol secretion from dispersed adrenocortical cells; the maximal effective concentration was 10^{-8} mol/L. Orexin A (10^{-8} mol/L) enhanced the cortisol response to maximal effective concentrations (10^{-9} mol/L) of angiotensin II and endothelin-1, but only to low concentrations of ACTH (10^{-12}/10^{-11} mol/L). Orexin A (10^{-8} mol/L) increased basal cAMP release by dispersed adrenocortical cells, and the effect was blocked by the adenylate cyclase inhibitor SQ-22536. The cortisol response to 10^{-8} mol/L orexin A was unaffected by the ACTH receptor antagonist corticotropin-inhibiting peptide, but was abolished by either SQ-22536 or the protein kinase A inhibitor H-89. RT-PCR demonstrated high levels of OX1-R messenger ribonucleic acid and very low levels of OX2-R messenger ribonucleic acid in human adrenal zona fasciculata-reticularis and adrenal medulla. Collectively, our findings suggest that orexins selectively stimulate glucocorticoid secretion from human adrenocortical cells, acting through OX1-R coupled with the adenylate cyclase-dependent signaling pathway. (*J Clin Endocrinol Metab* **86:** 778–782, 2001)

MANY LINES OF evidence indicate that close interrelationships occur between body weight homeostasis and adrenal secretory activity (1–4). Accordingly, neuropeptide Y (NPY) and leptin, two peptides that are involved in the central regulation of feeding (for review, see Refs. 5–7), are known to control the function of the hypothalamo-pituitary-adrenal axis, acting on both central (8–11) and peripheral (12–15) branches.

Orexins A and B are two recently identified hypothalamic peptides that, like leptin and NPY, play a role in the central control of food intake (for review, see Refs. 16–18). They originate from the posttranslational proteolytic cleavage of a common precursor, the prepro-orexin, and act through two subtypes of G protein-coupled receptors, named OX1-R and OX2-R (16, 19).

Recent findings indicate that orexins, through the OX2-R, may be involved in sleep regulation; their deficit provokes a narcolepsy-like condition in dogs and mice (20, 21). Although these observations could suggest a role for orexins in the control of circadian rhythms of glucocorticoid secretion, few studies are available on the possible actions of these peptides on adrenal function. The expression of OX1-R and OX2-R messenger ribonucleic acids (mRNAs) has been recently detected in rat adrenal medulla (22), and orexins were reported to stimulate corticosterone secretion from rat adrenals both *in vivo* and *in vitro* (23). Hence, the present study was designed to investigate whether orexins affect *in vitro* adrenal function in humans by using both dispersed adrenocortical cells and adrenal slices containing adrenomedullary tissue.

Received January 22, 2000. Revision received July 31, 2000. Rerevision received October 4, 2000. Accepted October 19, 2000.

Address all correspondence and requests for reprints to: Prof. G. Mazzocchi, Department of Human Anatomy and Physiology, Section of Anatomy, Via Gabelli 65, I-35121 Padova, Italy. E-mail: mazzocch@ux1.unipd.it.

Materials and Methods

Reagents

All chemicals were obtained from Sigma (St. Louis, MO) with the following exceptions: orexins, corticotropin-inhibiting peptide (CIP), and endothelin-1 (ET-1) were supplied by Peninsula Laboratories, Inc. (St. Helens, UK); H-89 was provided by BIOMOL Research Laboratories, Inc. (Plymouth Meeting, PA); and medium 199 was supplied by Difco (Detroit, MI). Aldosterone and cortisol RIA kits were purchased from IRE-Sorin (Vercelli, Italy), and the cAMP RIA kit was obtained from Amersham Pharmacia Biotech (Aylesbury, UK). Moloney murine leukemia virus reverse transcriptase (Gene Amp RNA PCR core kit) and *Taq* polymerase (Ampli-Taq) were provided by Perkin-Elmer Corp./Cetus (Norwalk, CT).

Incubation experiments

Adrenal glands were obtained from adult patients (40–50 yr old) undergoing unilateral nephrectomy/adrenalectomy for kidney cancer. The criteria for patient recruitment were described previously (24), and each patient gave written consensus. The study protocol was approved by the local ethical committee for human studies.

Portions of the head and tail of each adrenal, which are known to, respectively, contain and not contain adrenal medulla (for references, see Ref. 25), were removed, placed in Krebs-Ringer bicarbonate buffer with 0.2% glucose at 4 C, and immediately taken to our laboratory. Head fragments were cut into slices, always including the gland capsule and medulla; tail fragments were employed to obtain dispersed adrenocortical cell preparations by sequential collagenase digestion and mechanical disaggregation (25). Dispersed cells were actually a mixture of zona

glomerulosa (ZG) aldosterone-secreting and zona fasciculata-reticularis (ZF/R) cortisol-secreting cells; the percentages of the two cell types (as evaluated by phase microscopy) were about 15% and 85%, respectively.

Dispersed adrenocortical cells and adrenal slices were placed in medium 199 and Krebs-Ringer bicarbonate buffer with 0.2% glucose containing 5 mg/mL human serum albumin. The samples were incubated (10^5 cells or 4–5 mg/mL) as follows: 1) orexins (from 10^{-12}–10^{-6} mol/L); 2) orexin A (10^{-8} mol/L) alone or in the presence of 10^{-9} mol/L ACTH, angiotensin II (Ang-II), or ET-1; 3) ACTH (from 10^{-12}–10^{-8} mol/L) alone or in the presence of 10^{-8} mol/L orexin A; and 4) CIP (10^{-6} mol/L), SQ-22536 (10^{-4} mol/L), and H-89 (10^{-5} mol/L) alone or in the presence of 10^{-8} mol/L orexin A. When cAMP production was assayed (see below), 10^{-4} mol/L of the phosphodiesterase inhibitor 3-isobutyl-1-methylxanthine was added to prevent cAMP metabolism. Incubation was carried out for 60 min (steroid hormone production), 30 min (catecholamine production), or 10 min (cAMP production) in a shaking bath at 37 C in an atmosphere of 95% air-5% CO_2. At the end of the experiments, the incubation tubes were centrifuged at 4 C, and media were collected and kept frozen at −80 C.

Hormonal and cAMP assays

Aldosterone and cortisol were extracted from supernatants and purified by high performance liquid chromatography (25); their concentrations were measured by RIA with the following commercial kits: 1) aldosterone CTK2 (sensitivity, 5 pg/mL; intra- and interassay variations, 5.5% and 7.1%, respectively), and 2) cortisol RIA kit (sensitivity, 30 pg/mL; intra- and interassay variations, 6.7% and 8.2%, respectively).

Catecholamine concentrations were assayed, without previous allumina purification and concentration, by high performance liquid chromatography with a reverse phase column and a glassy carbon electrochemical detector, as described previously (26). Intra- and interassay variations were 7.3% and 8.4% for norepinephrine, and 6.8% and 8.1% for epinephrine, respectively.

cAMP was extracted by incubating the medium with 0.1 N HCl for 20 min at 4 C. The HCl extract was then neutralized, and the cAMP concentration was determined using the acetylation protocol of the Amersham Pharmacia Biotech Biotrak cAMP RIA system (sensitivity, 14 pg/mL; intra- and interassay variations, 5.3% and 6.6%, respectively).

RT-PCR of orexin receptors

Head fragments of two adrenals were cut into slices of about 5 mm. The capsule was stripped, and adherent parenchymal tissue (*bona fide* ZG) was scraped off. Adrenal medulla was then separated from ZF/R under the dissecting microscope.

RNA was extracted from adrenal samples, using the guanidium isothiocyanate method, and total RNA was reverse transcribed to complementary DNA (cDNA) with random hexamers (2.5 μmol/L) and 50 U cloned Moloney murine leukemia virus reverse transcriptase, as previously detailed (27). For amplification of the resulting cDNA, 10 μL of the RT mixture were used. The sample volume was increased to 50 μL with a solution containing 50 mmol/L KCl, 10 mmol/L Tris (pH 8.3), 2 mmol/L $MgCl_2$, 0.1 μmol/L of up- and downstream primers, and 1 U *Taq* polymerase. The primers used for human OX1-R and OX2-R mRNAs were selected according to the procedure of Sakurai *et al.* (16): OX1-R, 5′-CCT TCC TGG CTG AAG TGA AG-3′ and 5′-AGT GGG AGA AGG TGA AGC AG-3′; and OX2-R, 5′-ACA TGG CAC CAC TGT GTC TC-3′ and 5′-TGG CTC GGA TCT GCT TTA TT-3′. In a Delphi 100 Thermal Cycler (Oracle Biosystem, MJ Research, Inc., Waterston, MA), we used a denaturation step at 95 C for 1 min, an annealing step at 59 C for 1 min, and an extension step at 72 C for 1 min for a total of 38 cycles. An additional extension step at 72 C for 7 min was then carried out. The sizes of the amplification products were: OX1-R, 189 bp; and OX2-R, 201 bp. Detection of the PCR amplification products was carried out by size fractionation on 2% agarose gel electrophoresis.

Statistics

Each incubation experiment was performed in triplicate or quadruplicate (three or four adrenals from three or four patients), and results were expressed as the mean ± SEM. They statistical comparison was performed using ANOVA, followed by the multiple range test of Duncan.

Results

Orexins did not affect basal catecholamine release from adrenal slices and aldosterone production from dispersed adrenocortical cells (Fig. 1). Conversely, orexin A, but not orexin B, concentration dependently increased basal cortisol secretion from dispersed cells. Minimal and maximal effective concentrations were 10^{-10} and 10^{-8} mol/L and elicited about 1.5- and 2.4-fold increases, respectively (Fig. 2).

Orexin A (10^{-8} mol/L) did not enhance the cortisol response of dispersed adrenocortical cells to a maximal effective concentration (10^{-9} mol/L) of ACTH, but increased the response to maximal effective concentrations (10^{-9} mol/L) of both Ang-II and ET-1 (Fig. 3) by about 60% and 45%, respectively. However, it enhanced the cortisol response to submaximal concentrations of ACTH (10^{-12} and 10^{-11} mol/L) by about 55–60% (Fig. 4).

Orexin A (10^{-8} mol/L) markedly stimulated basal cAMP release by dispersed adrenocortical cells (~5.5-fold rise), and the effect was annulled by 10^{-4} mol/L SQ-22536 (Fig. 5). The cortisol response to 10^{-8} mol/L orexin A was unaffected by

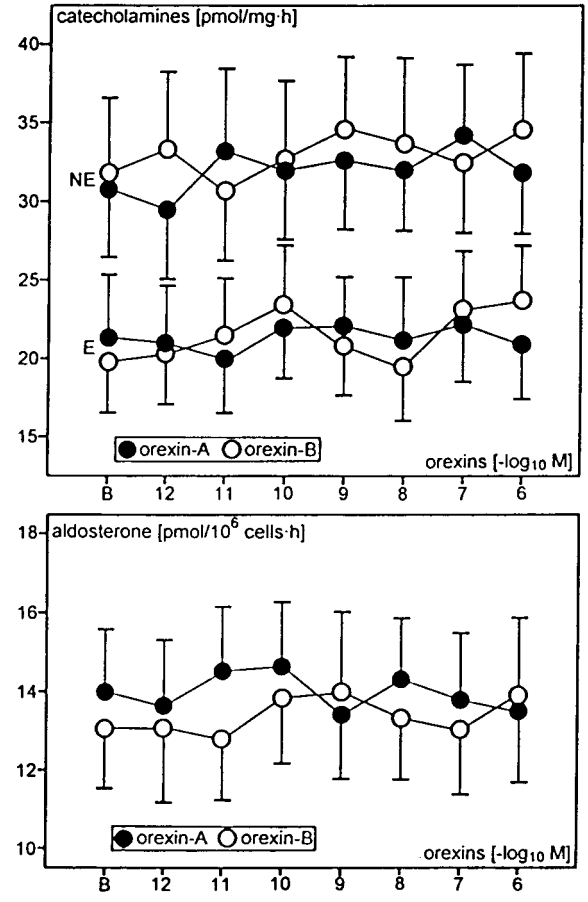

FIG. 1. Lack of effect of orexins on epinephrine (E) and norepinephrine (NE) release by human adrenal slices containing medullary chromaffin tissue (*upper panel*), and on aldosterone production by dispersed human adrenocortical cells (*lower panel*). The mean ± SEM of three separate experiments are shown.

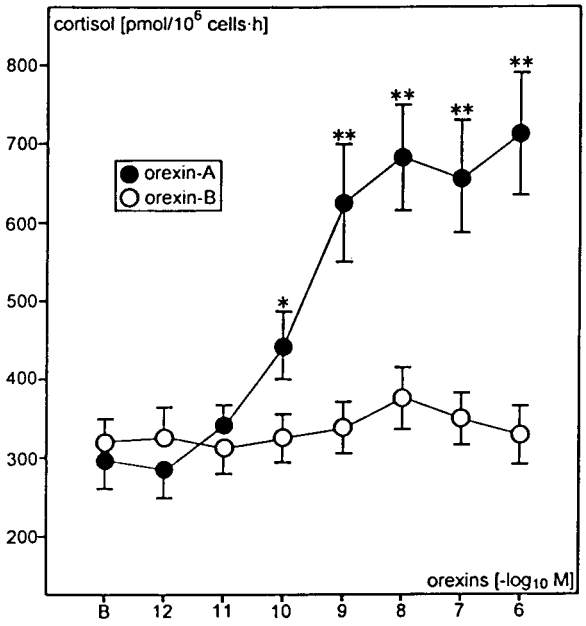

FIG. 2. Effects of orexins on basal cortisol production by dispersed human adrenocortical cells. The mean ± SEM of three separate experiments are shown. *, $P < 0.05$; **, $P < 0.01$ [*vs.* the respective baseline value (B)].

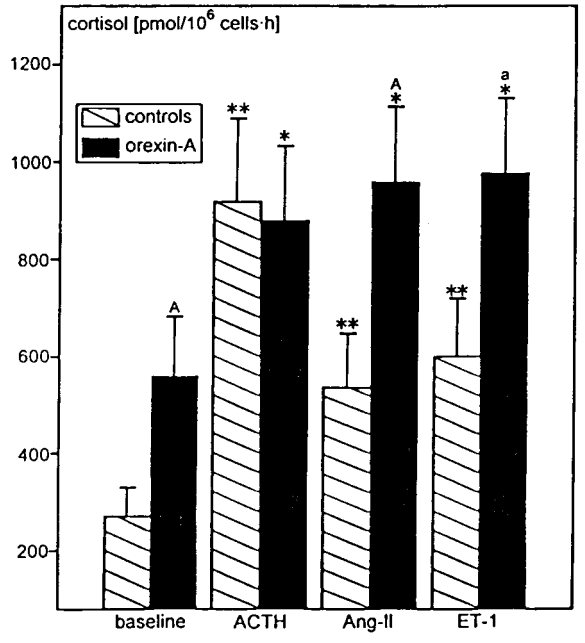

FIG. 3. Effects of orexin A (10^{-8} mol/L) on basal and agonist (10^{-9} mol/L)-stimulated cortisol production by dispersed human adrenocortical cells. The mean ± SEM of four separate experiments are shown. a, $P < 0.05$; A, $P < 0.01$ (*vs.* the respective control value). *, $P < 0.05$; **, $P < 0.01$ (*vs.* the respective baseline value).

10^{-6} mol/L CIP, but was abolished by either 10^{-4} mol/L SQ-22536 or 10^{-5} mol/L H-89 (Fig. 6).

RT-PCR demonstrated the presence of high levels of OX1-R mRNA and very low levels of OX2-R mRNA in both ZF/R and adrenal medulla. ZG displayed a weak expression of the OX1-R gene, and no expression of the OX2-R gene (Fig. 7).

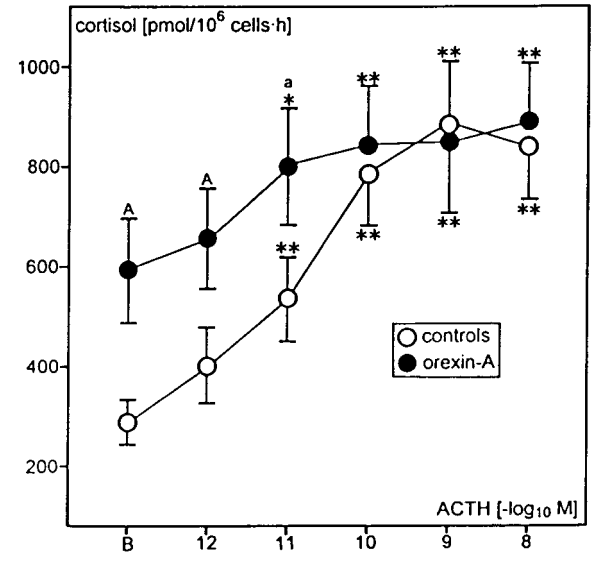

FIG. 4. Effect of orexin A (10^{-8} mol/L) on the cortisol response of dispersed human adrenocortical cells to increasing concentrations of ACTH. The mean ± SEM of three separate experiments are shown. a, $P < 0.05$; A, $P < 0.01$ (*vs.* the respective control value). *, $P < 0.05$; **, $P < 0.01$ [*vs.* the respective baseline value (B)].

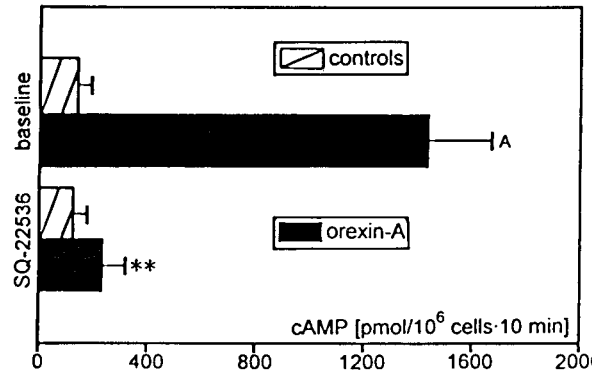

FIG. 5. Effect of orexin A (10^{-8} mol/L) on basal cAMP release from dispersed human adrenocortical cells. The adenylate cyclase inhibitor SQ-22536 (10^{-4} mol/L) abolishes the cAMP response to orexin A. The mean ± SEM of four separate experiments are shown. A, $P < 0.01$ *vs.* the respective control value; **, $P < 0.01$ *vs.* the respective baseline value.

Discussion

Our findings provide evidence that orexin A, but not orexin B, selectively stimulates glucocorticoid secretion from dispersed human adrenocortical cells. As OX1-R selectively binds orexin A, and OX2-R is a nonselective receptor for both orexins (16, 19), our data suggest that the cortisol secretagogue effect of orexin A involves the activation of OX1-R located on ZF/R cells. The lack of effect of orexins on both aldosterone and catecholamine secretion may be explained by assuming that human ZG and medullary cells are not provided with orexin receptors. However, this contention conflicts with our RT-PCR findings showing low and high OX1-R gene expression in ZG and adrenal medulla, respectively. It may be that these receptors located on ZG and chromaffin cells mediate orexin functions other than stimulation of aldosterone and catecholamine secretion. In this

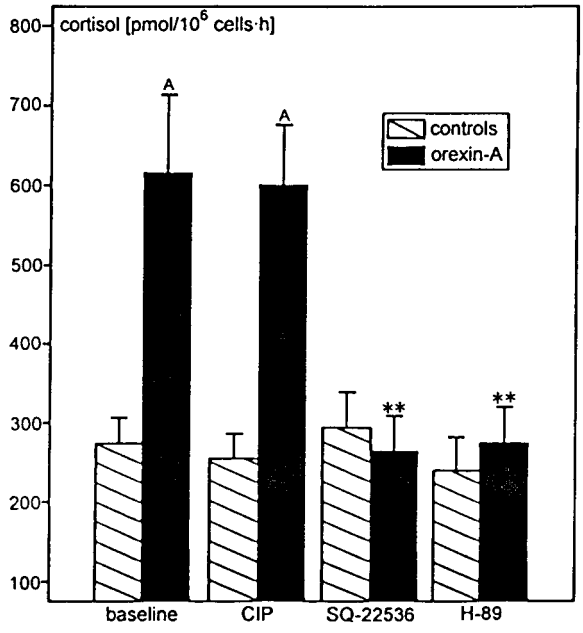

FIG. 6. Effects of the ACTH receptor antagonist CIP (10^{-6} ml/L), the adenylate cyclase inhibitor SQ-22536 (10^{-4} mol/L), and the PKA inhibitor H-89 (10^{-5} mol/L) on the cortisol response of dispersed human adrenocortical cells to 10^{-8} mol/L orexin A. the mean ± SEM of four separate experiments are shown. A, $P < 0.01$ *vs.* the respective control value; **, $P < 0.01$ *vs.* the respective baseline value.

connection, we want to stress that the same discrepancy occurs as far as leptin and its receptors (Ob-R) are concerned. Ob-R mRNA was found in human adrenomedullary cells, but leptin, although able to inhibit glucocorticoid production, does not affect catecholamine secretion (14).

As expected, human ZF/R cells respond not only to ACTH, but also to Ang-II and ET-1, because they are provided with both AT1 (28) and ET-1 receptors of A and B subtypes (29). Orexin A enhances the cortisol response to maximal effective concentrations of Ang-II and ET-1, but only to low concentrations of ACTH, thereby making it likely that orexin A and ACTH share the same mechanism of signal transduction. The possibility that orexin A binds and activates ACTH receptors is ruled out by the demonstration that the selective ACTH receptor antagonist CIP (30), at a concentration that was previously found to abolish the secretagogue effect of ACTH (31), does not affect the stimulatory action of orexin A on human ZF/R cells. Moreover, ACTH is a powerful aldosterone secretagogue (32), but orexin A does not enhance aldosterone production by dispersed adrenocortical cells.

It is current knowledge that the signaling mechanism of ACTH mainly involves the activation of the adenylate cyclase/protein kinase A (PKA)-dependent cascade (32), and our findings indicate that the same occurs for orexin A. In fact, 1) orexin A enhances cAMP release by dispersed human adrenocortical cells; and 2) the cortisol response to orexin A is abolished by either the adenylate cyclase inhibitor SQ-22536 (33) or the PKA-selective inhibitor H-89 (34). Neither inhibitor *per se* alters basal cortisol secretion, which rules out the possibility that their effect may be due to a nonspecific toxic lesion of the steroidogenic machinery.

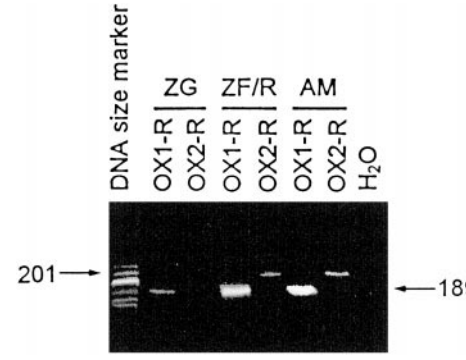

FIG. 7. Ethidium bromide-stained 2% agarose gel showing cDNA amplified with human OX1-R- and OX2-R-specific primers from RNA of human ZG, ZF/R, and adrenal medulla (AM). Lane 1 was loaded with 200 ng of a size marker (Marker VIII, Roche Molecular Biochemicals). The amplified fragments were of the expected sizes, which was 189 bp for OX1-R and 201 bp for OX2-R. No amplification with water instead of RNA, as a negative control, is shown in lane 8.

The physiological relevance of our findings remains to be demonstrated, also because it is not known whether orexins circulate in the periphery at a concentration sufficient to produce the adrenocortical effects herein described. However, other hypothalamic peptides (*e.g.* CRH and NPY) that are able to modulate steroid hormone secretion through specific receptors located on adrenocortical cells act in a paracrine manner, being synthesized in adrenal medulla (for review, see Ref. 12). Hence, the possibility that orexins may be locally produced in the adrenal glands remains to be explored.

Leptin is an adipostatic hormone secreted by adipocytes that decreases food intake and body weight (6, 7), displays increased plasma concentrations in obesity (35) and in patients with Cushing's syndrome (36, 37), and has been found to inhibit glucocorticoid secretion from bovine (13) and human adrenocortical cells (14). Conversely, orexins, hypothalamic peptides that are overexpressed in fasted and hypoglycemic animals (16, 38), increase food intake and body weight (18) and have been found to stimulate glucocorticoid secretion from rat (23) and human adrenocortical cells. Hence, our study suggests that orexins together with leptin may take part in counterregulatory mechanisms that, through the modulation of adrenal-cortex secretory activity, are involved in the maintenance of glycemia and body weight homeostasis.

References

1. **Debons AF, Zurek LD, Tse CS, Abrahamsen S.** 1986 Central nervous system control of hyperphagia in hypothalamic obesity: dependence on adrenal glucocorticoids. Endocrinology. 118:1678–1681.
2. **Freedman MR, Horwitz BA, Stern JS.** 1986 Effect of adrenalectomy and glucocorticoid replacement on development of obesity. Am J Physiol. 250:R595–R607.
3. **Akana SF, Strack AM, Hanson ES, Dallman MF.** 1994 Regulation of activity in the hypothalamo-pituitary-adrenal axis is integral to a larger hypothalamic system that determines caloric flow. Endocrinology. 135:1125–1134.
4. **Bjorntorp P.** 1995 Endocrine abnormalities of obesity. Metabolism. 44:21–23.
5. **Rohner-Jeanrenaud F, Cusin I, Sainsbury A, Zakrzewska KE.** 1996 The loop system between neuropeptide Y and leptin in normal and obese rodents. Horm Metab Res. 28:642–648.
6. **Remesar X, Rafecas I, Fernandez-López JA, Alemany M.** 1997 Leptin. Med Res Rev. 17:225–234.
7. **Mantzoros CS, Moschos SJ.** 1998 Leptin: in search of role(s) in human physiology and pathophysiology. Clin Endocrinol (Oxf). 49:551–567.

8. **Heiman ML, Ahima RS, Craft LS, Schoner B, Stephens TW, Flier JS.** 1997 Leptin inhibition of the hypothalamic-pituitary-adrenal axis in response to stress. Endocrinology. 138:3859–3863.
9. **Malendowicz LK, Macchi C, Nussdorfer GG, Nowak KW.** 1998 Acute effects of recombinant murine leptin on rat pituitary-adrenocortical function. Endocr Res. 24:235–246.
10. **Spinedi E, Gaillard RC.** 1998 A regulatory loop between the hypothalamo-pituitary-adrenal (HPA) axis and circulating leptin: a physiological role of ACTH. Endocrinology. 139:4016–4020.
11. **Krysiak R, Obuchowicz E, Herman ZS.** 1999 Interactions between the neuropeptide Y system and the hypothalamic-pituitary-adrenal axis. Eur J Endocrinol. 140:130–136.
12. **Nussdorfer GG.** 1996 Paracrine control of adrenal cortical function by medullary chromaffin cells. Pharmacol Rev. 48:495–530.
13. **Bornstein SR, Uhlmann K, Haidan A, Ehrhart-Bornstein M, Scherbaum WA.** 1997 Evidence for a novel peripheral action of leptin as a metabolic signal to adrenal gland. Leptin inhibits cortisol release directly. Diabetes. 46:1235–1238.
14. **Glasow A, Haidan A, Hilbers U, Breidert M, Gillespie J, Scherbaum WA, Chrousos GP, Bornstein SR.** 1998 Expression of Ob receptor in normal human adrenals: differential regulation of adrenocortical and adrenomedullary function by leptin. J Clin Endocrinol Metab. 83:4459–4466.
15. **Nussdorfer GG, Gottardo G.** 1998 Neuropeptide-Y family of peptides in the autocrine-paracrine regulation of adrenocortical function. Horm Metab Res. 30:368–373.
16. **Sakurai T, Ameniya A, Ishii M, et al.** 1998 Orexins and orexin receptors: a family of hypothalamic neuropeptides and G protein-coupled receptors that regulate feeding behaviour. Cell. 92:573–585.
17. **Van den Pol AN, Gao XB, Obrietan K, Kilduff TS, Belousov AB.** 1998 Presynaptic and postsynaptic actions and modulation of neuroendocrine neurons by a new hypothalamic peptide, hypocretin/orexin. J Neurosci. 18:7962–7971.
18. **Wolf G.** 1998 Orexins: a newly discovered family of hypothalamic regulators of food intake. Nutrit Rev. 56:172–189.
19. **De Lecea L, Kilduff TS, Peyron C, et al.** 1998 The hypocretins: hypothalamus-specific peptides with neuroexcitatory activity. Proc Natl Acad Sci USA. 95:322–327.
20. **Chemelli R, Willie J, Sinton C, et al.** 1999 Narcolepsy in orexin knockout mice: molecular genetics of sleep regulation. Cell. 98:437–451.
21. **Lin L, Faraco J, Li R, et al.** 1999 The sleep disorder canine narcolepsy is caused by a mutation in the hypocretin (orexin) receptor 2 gene. Cell. 98:365–376.
22. **López M, Señaris R, Gallego R, et al.** 1999 Orexin receptors are expressed in the adrenal medulla of the rat. Endocrinology. 140:5991–5994.
23. **Malendowicz LK, Tortorella C, Nussdorfer GG.** 1999 Orexins stimulate corticosterone secretion of rat adrenocortical cells, through the activation of the adenylate cyclase-dependent signaling cascade. J Steroid Biochem Mol Biol. 70:185–188.
24. **Mazzocchi G, Andreis PG, De Caro R, Aragona F, Gottardo L, Nussdorfer GG.** 1999 Cerebellin enhances *in vitro* secretory activity of human adrenal gland. J Clin Endocrinol Metab. 84:632–635.
25. **Neri G, Andreis PG, Prayer-Galetti T, Rossi GP, Malendowicz LK, Nussdorfer GG.** 1996 Pituitary adenylate cyclase-activating peptide (PACAP) enhances aldosterone secretion of human adrenal gland: evidence for an indirect mechanism probably involving the local release of catecholamines. J Clin Endocrinol Metab. 81:169–173.
26. **Andreis PG, Neri G, Prayer-Galetti T, Rossi GP, Gottardo G, Malendowicz LK, Nussdorfer GG.** 1997 Effects of adrenomedullin on the human adrenal glands: an *in vitro* study. J Clin Endocrinol Metab. 82:1167–1170.
27. **Rossi GP, Albertin G, Belloni AS, et al.** 1994 Gene expression, localization and characterization of endothelin A and B receptors in the human adrenal cortex. J Clin Invest. 94:1126–1234.
28. **Lebrethon MC, Jaillard C, Defayes G, Begeot M, Saez JM.** 1994 Human cultured adrenal fasciculata-reticularis cells are targets for angiotensin II: effects on cytochrome P450 cholesterol side-chain cleavage, cytochrome P450 17α-hydroxylase and 3β-hydroxysteroid dehydrogenase messenger ribonucleic acid and proteins and on steroidogenic responsiveness to corticotropin and angiotensin II. J Clin Endocrinol Metab. 78:1212–1219.
29. **Rossi GP, Albertin G, Neri G, Andreis PG, Hofmann S, Pessina AC, Nussdorfer GG.** 1997 Endothelin-1 stimulates steroid secretion of human adrenocortical cells *ex vivo* via both ETA and ETB receptor subtypes. J Clin Endocrinol Metab. 82:3445–3449.
30. **Li CH, Chung D, Yamashiro D, Lee CY.** 1978 Isolation, characterization and synthesis of a corticotropin-inhibiting peptide from human pituitary glands. Proc Natl Acad Sci USA. 75:4306–4309.
31. **Andreis PG, Neri G, Belloni AS, Mazzocchi G, Kasprzak A, Nussdorfer GG.** 1991 Interleukin-1β enhances corticosterone secretion by acting directly on the rat adrenal gland. Endocrinology. 129:53–57.
32. **Ganguly A, Davis JS.** 1994 Role of calcium and other mediators in aldosterone secretion from adrenal glomerulosa cells. Pharmacol Rev. 46:417–447.
33. **Goldsmith BA, Abrams TW.** 1991 Reversal of synaptic depression by serotonin at *Aplysia* sensory neuron synapses involves activation of adenyl cyclase. Proc Natl Acad Sci USA. 88:9021–9025.
34. **Chijiwa T, Mishima A, Hagiwara M, et al.** 1990 Inhibition of forskolin-induced neurite outgrowth and protein phosphorylation by a newly synthesized selective inhibitor of cyclic AMP-dependent protein kinase, *N*-[2-(*p*-bromocinnamylamino)ethyl]-5-iso-quinolinesulfonamide (H-89), of PC12D pheochromocytoma cells. J Biol Chem. 265:5267–5272.
35. **Blum WF, Englaro P, Hanitsch S, et al.** 1997 Plasma leptin levels in healthy children and adolescents: dependence on body mass index, body fat mass, gender, pubertal stage, and testosterone. J Clin Endocrinol Metab. 82:2904–2910.
36. **Leal-Cerro A, Considine RV, Peino R, Venegas E, Astorga R, Casanueva FF, Diéguez C.** 1996 Serum immunoreactive-leptin levels are increased in patients with Cushing's syndrome. Horm Metab Res. 28:711–713.
37. **Weise M, Abad V, Considine RV, Nieman L, Rother KI.** 1999 Leptin secretion in Cushing's syndrome: preservation of diurnal rhythm and absent response to corticotropin-releasing hormone. J Clin Endocrinol Metab. 84:2075–2079.
38. **Cai XJ, Widdowson PS, Harrold J, et al.** 1999 Hypothalamic orexin expression. Modulation by blood glucose and feeding. Diabetes. 48:2132–2137.