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Orexins stimulate corticosterone secretion of rat adrenocortical cells, through the activation of the adenylate cyclase-dependent signaling cascade

Ludwik K. Malendowicz[a], Cinzia Tortorella[b], Gastone G. Nussdorfer[b,*]

[a]*Department of Histology and Embryology, School of Medicine, Poznan, Poland*
[b]*Department of Human Anatomy and Physiology (Section of Anatomy), University of Padua, via Gabelli 65, I-35121, Padova, Italy*

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Abstract

Orexins-A and B are two novel hypothalamic peptides, which, like leptin and neuropeptide-Y (NPY), are involved in the central regulation of feeding. Since leptin and NPY were found to modulate adrenal function, we have examined whether orexins are able to directly affect rat adrenal steroid secretion. Both orexin-A and orexin-B raised basal corticosterone secretion of dispersed rat zona fasciculata–reticularis (ZF/R) cells, their maximal effective concentration being 10^{-8} M. In contrast, orexins did not affect either maximally ACTH (10^{-9} M)-stimulated corticosterone production by ZF/R cells or the basal and agonist-stimulated aldosterone secretion of dispersed zona glomerulosa cells. The ACTH-receptor antagonist corticotropin-inhibiting peptide (10^{-6} M) annulled corticosterone response of ZF/R cells to ACTH (10^{-9} M), but not to orexins (10^{-8} M). Orexins (10^{-8} M) enhanced cyclic-AMP release by ZF/R cells, and the selective inhibitor of protein-kinase A (PKA) H-89 (10^{-5} M) abolished corticosterone responses to both ACTH (10^{-9} M) and orexins (10^{-8} M). A subcutaneous injection of both orexins (5 or 10 nmol/kg) evoked a clear-cut increase in the plasma concentration of corticosterone (but not aldosterone), the effect of orexin-A being significantly more intense than that of orexin-B. Collectively, these findings suggest that orexins exert a selective and direct glucocorticoid secretagogue action on the rat adrenals, acting through a receptor-mediated activation of the adenylate cyclase/PKA-dependent signaling pathway.

1. Introduction

Orexin-A and orexin-B are two recently identified hypothalamic peptides, which originate from the post-translational proteolytic cleavage of a common precursor, named prepro-orexin. Orexins do not display structural similarities with regulatory neuropeptides of other families, and act through two subtypes of receptors (OX_1-R and OX_2-R), belonging to the G protein-coupled receptor superfamily (see ref. [1] for review). Hypothalamic prepro-orexin mRNA was found to undergo a 2.4-fold increase after 48 h of fasting [1], and orexins were reported to regulate the synaptic activity of hypothalamic neuroendocrine neurons and the axonal input to the hypothalamus [2]. Hence, these findings indicate that orexins belong to that group of neuropeptides, including leptin and neuropeptide-Y (NPY), involved in the central regulation of feeding [3–5].

Both leptin and NPY, apart form their central effects, also directly modulate corticosteroid secretion of rat adrenocortical cells [6–9]. Therefore, it seemed worthwhile to investigate whether orexins are able to affect the secretory activity of the rat adrenal cortex.

2. Experimental

Orexins, ACTH(1–24) and corticotropin-inhibiting peptide (CIP) were purchased from Peninsula

* Corresponding author. Tel.: +39-049-827-2317; fax: +39-049-827-2319.
E-mail address: ggnanat@ipdunidx.unipd.it (G.G. Nussdorfer)

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Laboratories (St. Helene, UK), and H-89 was from Biomol Research (DBA, Milan, Italy). The phosphodiesterase inhibitor 3-isobutyl-1-methylxantine (IBMX), bovine serum albumin (BSA) and other laboratory reagents were obtained from Sigma Chemical Company (St. Louis, MO).

Adrenal glands of adult female Wistar rats were gently decapsulated to separate zona glomerulosa (ZG) from the zonae fasciculata and reticularis (ZF/R). Dispersed capsular (ZG) and inner (ZF/R) cells were obtained by sequential collagenase digestion and mechanical disaggregation [10]. Inner-cell contamination of capsular-cell preparation, checked by phase microscopy, proved to be less than 10%. The viability of isolated cells, as assayed by the trypan-blue exclusion test, was higher than 92%. Dispersed cells obtained from 6–8 rats were pooled to obtain a single cell suspension, and six cell suspensions for each incubation experiment were employed. Aliquots of each cell suspension (10^5 cells/ml) in Krebs–Ringer bicarbonate buffer with 0.3% glucose and 0.2% BSA were incubated as follows: (i) orexin-A and orexin-B (from 10^{-12} to 10^{-6} M) alone or in the presence of 10^{-9} M ACTH; and (ii) orexins (10^{-8} M) and ACTH (10^{-9} M) in the presence or absence of CIP (10^{-6} M) and H-89 (10^{-5} M). The incubation was carried out in a shaking bath at 37°C for 60 min (hormone production) or 10 min (cyclic-AMP production), in an atmosphere of 95% air–5% CO_2. In the case of cyclic-AMP assay, 10^{-4} M IBMX was added to prevent phosphodiesterase activity and cyclic-AMP metabolism. At the end of the experiments, the incubation tubes were centrifuged at 4°C, and supernatants were stored at −30°C.

Other rats were kept under a 12:12 h light–dark cycle at 22°C, and given daily subcutaneous (sc) injections of 0.2 ml 0.9% NaCl for 7 days, in order to dampen the response of their hypothalamo–pituitary–adrenal axis to handling stress. On the 8th day, rats were divided into equal groups ($n = 6$), which were given a sc injection of 5 or 10 nmol/kg of orexin-A or orexin-B dissolved in 0.2 ml saline vehicle. Rats were decapitated at 10:00 am, 60 min after the injection. The trunk blood was collected, plasma separated and stored at −30°C.

Aldosterone and corticosterone were extracted from incubation media and plasma, and purified by HPLC [11], and their concentrations estimated by specific RIA, as previously detailed [12]. Intra- and interassay variation coefficients were: aldosterone, 5.2 and 7.1%; and corticosterone, 7.5 and 9.4%, respectively. Cyclic-AMP was extracted by incubating the medium with 0.1 N HCl for 20 min at 4°C. The HCl extract was then neutralized and cyclic-AMP concentration determined using the acetylation protocol of the Amersham's Biotrak cAMP RIA system (Amersham Laboratories, UK). Intra- and interassay coefficient of variations were 5.3 and 6.1%, respectively.

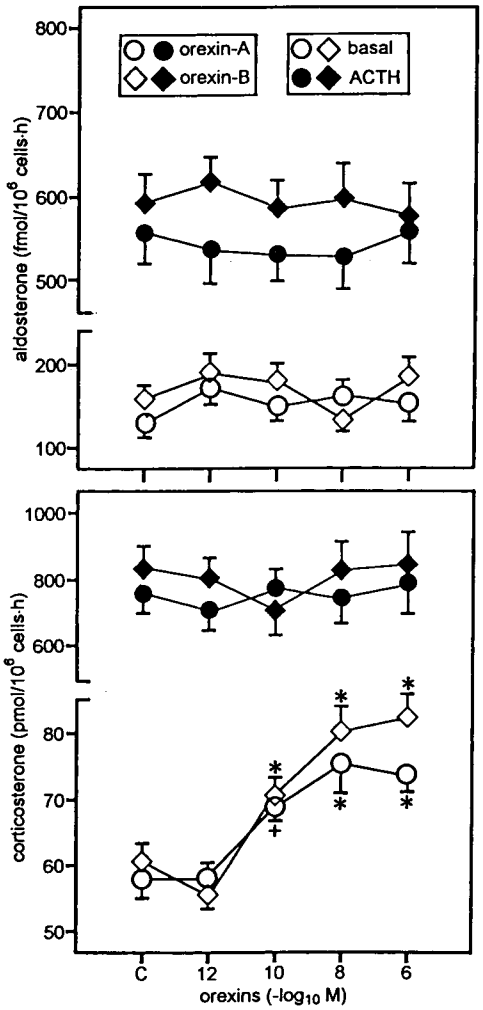

Fig. 1. Effects of orexins on basal and ACTH (10^{-9} M)-stimulated aldosterone and corticosterone secretion of dispersed rat ZG and ZF/R cells, respectively. Data are means ± SEM ($n = 6$). †$P < 0.05$ and *$P < 0.01$ from the respective control value (C).

The data were averaged per experimental group and SEM was calculated. Their statistical comparison was done by ANOVA, followed by the Multiple Range Test of Duncan.

3. Results

Orexins did not affect either basal or ACTH (10^{-9} M)-stimulated aldosterone secretion of dispersed capsular cells or ACTH-stimulated corticosterone production by inner cells. In contrast, both orexin-A and orexin-B enhanced basal corticosterone release by inner cells, minimal and maximal effective concentration being 10^{-10} and 10^{-8} M, respectively (Fig. 1). CIP (10^{-6} M) abolished the corticosterone secretago-

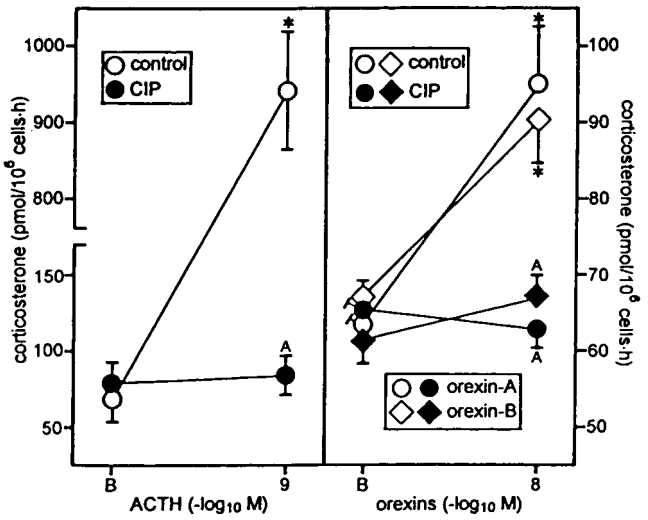

Fig. 2. Effect of CIP (10^{-6} M) on ACTH (10^{-9} M)- and orexin (10^{-8} M)-stimulated corticosterone secretion of dispersed rat ZF/R cells. Data are means ± SEM ($n = 6$). $^{*}P < 0.01$ from the respective basal value (B); $^{A}P < 0.01$ from the respective control value.

gue effect of 10^{-9} M ACTH, but did not affect that of 10^{-8} M orexins (Fig. 2). Orexins (10^{-8} M) increased cyclic-AMP release by dispersed ZF/R cells, and H-89 (10^{-5} M) annulled corticosterone response to both 10^{-8} M orexins (Fig. 3) and 10^{-9} M ACTH (data not shown). Both orexin-A and orexin-B evoked a marked dose-dependent rise in corticosterone plasma concentration (Fig. 4), without significantly affecting the blood levels of aldosterone (data not shown).

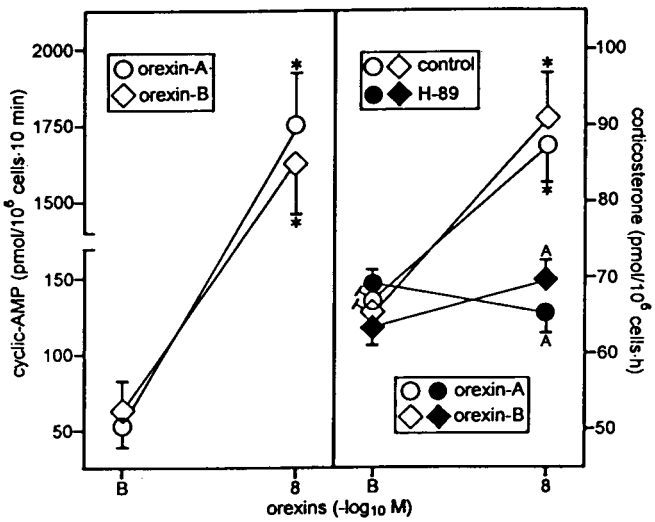

Fig. 3. Effect of orexins (10^{-8} M) on cyclic-AMP release by dispersed rat ZF/R cells (left panel), and of H-89 (10^{-5} M) on orexin (10^{-8} M)-simulated corticosterone secretion of dispersed inner adrenocortical cells. Data are means ± SEM ($n = 6$). $^{*}P < 0.01$ from the respective baseline value; $^{A}P < 0.01$ from the respective control value.

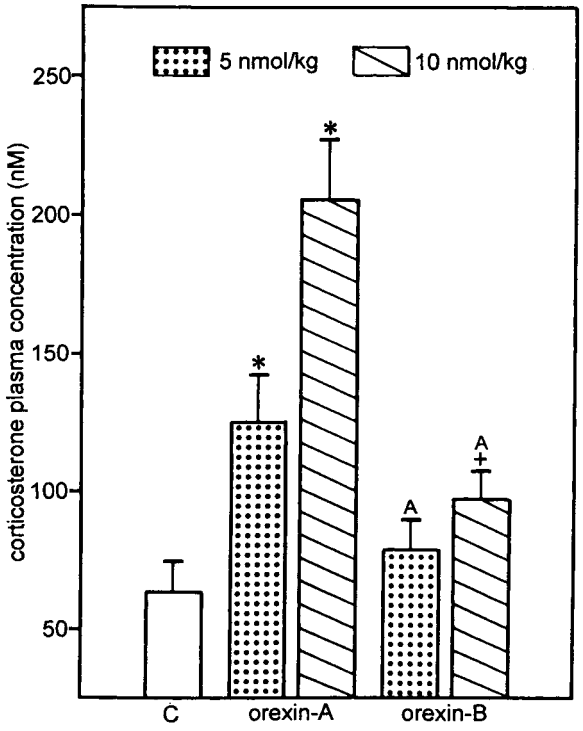

Fig. 4. Effect of orexins on plasma corticosterone concentration in rats. Data are means ± SEM ($n = 6$). $^{\dagger}P < 0.05$ and $^{*}P < 0.01$ from control value (C); $^{A}P < 0.01$ from the respective orexin-A value.

4. Discussion

The present findings provide the first demonstration that orexin-A and orexin-B stimulate basal glucocorticoid secretion of dispersed rat adrenocortical cells. The ACTH-receptor antagonist CIP [13], at a concentration which is able to annul ACTH secretagogue action, does not affect glucocorticoid response to orexins, thereby ruling out the possibility that orexins may bind and activate ACTH receptors in a nonspecific manner.

The lack of the effect of orexins on ACTH-stimulated corticosterone secretion, makes it likely that these neuropeptides activate the same post-receptorial events involved in the intracellular transduction of the ACTH secretagogue signal. It is current knowledge that the main mechanism whereby ACTH stimulates glucocorticoid secretion involves the activation of the adenylate cyclase/protein kinase A (PKA)-dependent pathway (see refs. [14,15] for review). Our results clearly indicate that orexins act on rat ZF/R cells through the same signaling mechanism. In fact, both orexins markedly raise cyclic-AMP release by inner adrenocortical cells, and the selective PKA inhibitor H-89 [16], at a concentration abolishing ACTH secretagogue action, is able to block corticosterone response to both orexins. H-89 per se does not alter basal corticosterone secretion, thereby excluding the possibility

that its effect may be due to a nonspecific toxic lesion of the steroidogenic machinery.

Our in vivo findings validate in vitro ones. Moreover, they show that the corticosterone secretagogue effect of orexin-A was much more intense than that of orexin-B. Since OX_1-R selectively binds orexin-A and OX_2-R is a nonselective receptor for both orexins [1], our results could suggest that rat inner adrenocortical cells are mainly provided with the OX_1-R subtype.

In conclusion, the present investigation indicates that orexins, like NPY and leptin (see Introduction), are able to affect glucocorticoid secretion in the rat, thereby stressing that close interrelationships may occur between adrenal function, food intake and adipose tissue metabolism.

References

[1] T. Sakurai, A. Amemiya, M. Ishii, I. Matsuzaki, R.M. Chemelli, H. Tanaka, S.C. Williams, J.A. Richardson, G.P. Kozlowski, S. Wilson, J.R.S. Arch, R.E. Buckingham, A.C. Haynes, S.A. Carr, R.S. Annan, D.E. McNulty, W.S. Liu, J.A. Terret, N.A. Elshourbagy, D.J. Bergsma, M. Yanagisava, Orexins and orexin receptors: a family of hypothalamic neuropeptides and G protein-coupled receptors that regulate feeding behavior, Cell 92 (1998) 573–585.

[2] A.N. van den Pol, X.B. Gao, K. Obrietan, T.S. Kilduff, A.B. Belousov, Presynaptic and postsynaptic actions and modulation of neuroendocrine neurons by a new hypothalamic peptide, hypocretin-orexin, Journal of Neuroscience 18 (1998) 7962–7971.

[3] E. Spinedi, R.C. Gaillard, A regulatory loop between the hypothalamo-pituitary-adrenal (HPA) axis and circulating leptin: a physiological role of ACTH, Endocrinology 139 (1998) 4016–4020.

[4] G. Wolf, Orexins: a newly discovered family of hypothalamic regulators of food intake, Nutrition Reviews 56 (1998) 172–189.

[5] F. Rohner-Jeanrenaud, I. Cusin, A. Sainsbury, K.E. Zakrzewska, B. Jeanrenaud, The loop system between neuropeptide Y and leptin in normal and obese rodents, Hormone and Metabolic Research 28 (1996) 642–648.

[6] G.G. Nussdorfer, Paracrine control of adrenal cortical function by medullary chromaffin cells, Pharmacological Reviews 48 (1996) 495–530.

[7] S.R. Bornstein, K. Uhlmann, A. Haidan, M. Ehrhart-Bornstein, W.A. Scherbaum, Evidence for a novel peripheral action of leptin as a metabolic signal to adrenal gland. Leptin inhibits cortisol release directly, Diabetes 46 (1997) 1235–1238.

[8] L.K. Malendowicz, G.G. Nussdorfer, A. Markowska, Effects of recombinant murine leptin on steroid secretion of dispersed rat adrenocortical cells, Journal of Steroid Biochemistry and Molecular Biology 63 (1997) 123–125.

[9] G.G. Nussdorfer, G. Gottardo, Neuropeptide-Y family of peptides in the autocrine-regulation of adrenocortical function, Hormone and Metabolic Research 30 (1998) 368–373.

[10] J.P. Hinson, S. Kapas, C.D. Orford, G.P. Vinson, Vasoactive intestinal peptide stimulation of aldosterone secretion by the rat adrenal cortex may be mediated by the local release of catecholamines, Journal of Endocrinology 133 (1992) 253–258.

[11] G. Neri, L.K. Malendowicz, P.G. Andreis, G.G. Nussdorfer, Tyrotropin-releasing hormone inhibits glucocorticoid secretion of rat adrenal cortex: in vivo and in vitro studies, Endocrinology 133 (1993) 511–514.

[12] L.K. Malendowicz, G.G. Nussdorfer, A. Markowska, K.W. Nowak, L. Torlinski, Effect of neuromedin-N on the pituitary-adrenocortical axis of dexamethasone-suppressed rats, Neuropeptides 24 (1993) 1–4.

[13] C.H. Li, D. Chung, D. Yamashiro, C.Y. Lee, Isolation, characterization and synthesis of a corticotropin-inhibiting peptide from human pituitary glands, Proceedings of the National Academy of Sciences of the USA 75 (1978) 4306–4309.

[14] W.L. Miller, Molecular biology of steroid hormone synthesis, Endocrine Reviews 9 (1988) 295–318.

[15] A. Ganguly, J.S. Davis, Role of calcium and other mediators in aldosterone secretion from adrenal glomerulosa cells, Pharmacological Reviews 46 (1994) 417–447.

[16] T. Chijiwa, A. Mishima, M. Hagiwara, M. Sano, K. Hayashi, T. Inoue, K. Naito, T. Toshioka, H. Hidaka, 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-isoquinolinesulfonamide (H-89), of PC12D pheochromocytoma cells, Journal of Biological Chemistry 265 (1990) 5267–5272.