Neuroscience Letters 436 (2008) 181–184

Contents lists available at ScienceDirect

Neuroscience Letters

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

Reinvestigation of the effect of orexin A on catecholamine release from adrenal chromaffin cells

Xiao-wei Chen[a,b,d], Wei Huang[a,b], Jun-an Yan[a,c], Hong-xiao Fan[b], Ning Guo[a], Jing Lü[a], Yun Xiu[a], Jing-li Gu[a], Claire Xi Zhang[a], Huai-zhen Ruan[b], Zhi-an Hu[b], Zheng-ping Yu[d], Zhuan Zhou[a,*]

[a] Institute of Molecular Medicine, Peking University, Beijing 100871, China
[b] School of Basic Medicine, Third Military Medical University, Chongqing 400038, China
[c] Urological Research Institute of PLA, Southwest Hospital, Third Military Medical University, Chongqing 400038, China
[d] Department of Occupational Health, Third Military Medical University, Chongqing 400038, China

ARTICLE INFO

Article history:
Received 12 October 2007
Received in revised form 26 February 2008
Accepted 7 March 2008

Keywords:
Orexin
Adrenal medulla
Catecholamine
Amperometry

ABSTRACT

Orexins have been shown to be implicated in the regulation of adrenal medulla functions. However, there are still inconsistent investigations on the effects of orexins on catecholamine release from chromaffin cells in varying species. In the present study, using the carbon-fiber amperometry, we investigated whether orexin A would stimulate catecholamine release from rat and mouse adrenal chromffin cells. Puff application of orexin A dose-dependently induced amperometric currents in the cultured rat chromaffin cells, which was completely blocked by the selective OX1R antagonist SB-334867 or by the removal of extracellular calcium. Likewise, in the mouse adrenal medulla slices, orexin A also induced catecholamine release mainly through the activation of OX1R. These results gain insight into our understanding of the pharmacological relevance of orexin system in modulating neuroendocrine functions.

Hypothalamic orexins are a pair of excitatory peptides (orexin A and B) involved in the regulation of appetite, neuroendocrine and autonomic functions, and arousal [10,19,22]. They act on two G-protein-coupled receptors, orexin receptor-1 and -2 (OX1R and OX2R), which are distributed not only in the central nervous system but also in peripheral tissues [20,23,25]. Several lines of evidence have recently shown that orexins may affect adrenal medullary functions [7]. The expression of orexin receptors has been detected in the adrenal medulla of rats, human, cattle, and pigs [8,12,14,16]. Furthermore, various effects of orexins on the synthesis and release of catecholamine have repeatedly been reported in adrenal medulla and in cells derived from adrenals. Orexins stimulate catecholamine synthesis in bovine adrenal medullary cells [8] and evoke catecholamine secretion from porcine chromaffin cells [16] and from human pheochromocytomas [13]. In contrast, orexins suppress dopamine synthesis and secretion in rat pheochromocytoma cells [17]. In human and bovine adrenomedullary cells, orexins do not apparently affect catecholamine release [8,14]. Therefore, these conflicting reports prompted us to further investigate the effects of orexins on catecholamine release from adrenals of other species including rats and mice.

Comparing with the biochemical assay, high performance liquid chromatography used in the aforementioned studies [8,13,14,16,17], amperometry provides high temporal and spatial resolution in directly detecting catecholamine release from chromaffin cells [6,26,28]. In the present study, using this real-time measurement, we determined whether orexin A would affect catecholamine release from either cultured rat chromaffin cells or mouse adrenal medullary slices.

Cultured rat adrenal chromaffin cells were prepared as described previously [18]. All experimental procedures were approved by the Third Military Medical University Animal Care Committee or by the Peking University Committee on Ethics in the Care and Use of Laboratory Animals. Adrenal medulla slices were prepared according to a previous report [15] with a minor modification. In brief, adrenal glands were removed from 6 to 10-week-old DBA/2J mice and were immediately immersed in ice-cold, low calcium bicarbonate-buffered saline (BBS) solution containing (in mM): 125 NaCl, 2.5 KCl, 0.1 $CaCl_2$, 5 $MgCl_2$, 1.25 NaH_2PO_4, 26 $NaHCO_3$, 10 glucose, pH 7.4 when gassed with 95%O_2/5%CO_2. After that, single gland was glued with cyanoacrylate to the stage of a vibratome chamber and covered with the same cold, O_2-saturated BBS solution. Slices (100–200 μm) were cut parallel to the larger base of the gland, with razor blade on a vibratome (Vibratome 1000, St. Louis, MO, USA). They were then incubated for 30 min at room temperature (24–26 °C) in normal BBS solution containing (in mM) 125 NaCl, 2.5 KCl, 2 $CaCl_2$, 1 $MgCl_2$, 1.25 NaH_2PO_4, 26 $NaHCO_3$, 10 glucose gassed with 95%O_2/5%CO_2. Slices could

* Corresponding author: Institute of Molecular Medicine, Peking University, Beijing 100871, China. Tel.: +86 10 6275 3212; fax: +86 10 6275 3212.
E-mail address: zzhou@pku.edu.cn (Z. Zhou).

0304-3940/$ – see front matter © 2008 Elsevier Ireland Ltd. All rights reserved.
doi:10.1016/j.neulet.2008.03.014

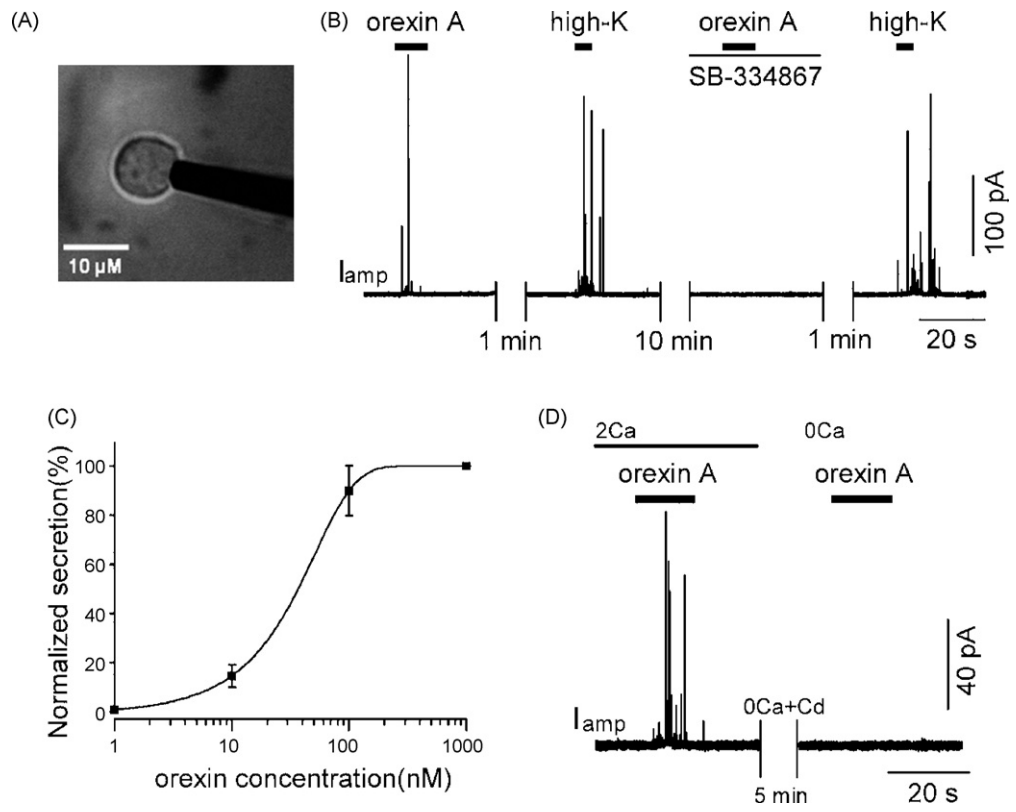

Fig. 1. Orexin A induces catecholamine release from cultured rat adrenal chromaffin cells. (A) Infrared DIC image showing a micrograph of a micro-carbon fiber electrode (black bar) attached to a chromaffin cell. (B) Puff application of 100 nM orexin A evoked amperometric current traces (I_{amp}). Pretreatment of the selective OX1R antagonist, SB-334867 (1 μM), the orexin A-induced response was almost completely blocked ($n = 7$, $p < 0.001$). High-K solution was repeatedly applied as a positive control. (C) The normalized responses induced by orexin A are plotted against various concentrations (1 nM, 10 nM, 100 nM, and 1000 nM). These data are fitted to a sigmoid curve ($n = 4$). (D) Orexin-induced secretion was dependent on extracellular Ca^{2+}. Application of Ca^{2+}-free ACSF containing 1 mM EGTA and 200 μM $CdCl_2$ for at least 5 min completely blocked secretion induced by 100 nM orexin A ($n = 3$, $p < 0.01$).

be used for up to 8 h after cutting. For amperometric measurements, slices were transferred to a recording chamber attached to the stage of an upright microscope equipped with an infrared sensitive CCD camera and continuously superfused with normal BBS solution at room temperature. We performed amperometry by using an EPC9/2 amplifier and Pulse software (HEKA Elektronik, Lambrecht/Pfalz, Germany) as described elsewhere [5,15,29]. Five-micrometer carbon fiber electrodes (CFE) (Dagan, Minneapolis, MN, USA) were used to measure catecholamine release from cultured chromaffin cells or adrenal slices. The amperometric current (I_{amp}) was measured at a holding potential of 780 mV. Amperometric signals were low-pass filtered at 0.3 kHz and digitized at 1 kHz. The CFE surface was positioned in contact with the membrane of a clean cell. And the close proximity of the electrode surface to the cell surface was confirmed by a slight deformation in the outline of the cell. Eighty millimolars high-K^+ solution, all secretagogues used in our experiments, were applied using a perfusion system with a fast exchange time (PCR-2B, INBIO, Wuhan, China).

Orexin A binds to both OX1R and OX2R with a high affinity, whereas orexin B binds only to OX2R with a similar high affinity [21]. Thus we used only orexin A in this study. To characterize the effect of orexin A on catecholamine release from chromaffin cells, we first performed electrochemical recordings by using CFEs on the surfaces of cultured rat adrenal chromaffin cells (Fig. 1A). Puff application of 100 nM orexin A significantly resulted in a burst of amperometric spikes representing the fusion of exocytotic vesicles [6,26,28]. To our knowledge, these fast amperometric spikes presumably reflect vesicles released close to the electrode [9]. In the presence of the selective OX1R antagonist SB-334867 (1 μM), orexin A (100 nM) failed to produce any amperometric signals. As a positive control, high-K (80 mM) solution was repeatedly applied to chromaffin cells, evoking reliable amperometric responses (Fig. 1B). Quantitative analysis of normalized integral of amperometric signals in response to orexin A concentrations ranging from 1 to 1000 nM showed these effects to be concentration dependent (Fig. 1C). In addition, orexin has been shown to increase $[Ca^{2+}]_i$ through extracellular Ca^{2+} influx or intracellular Ca^{2+} release in different cells [11,24,27], prompting us to investigate which pathway would be responsible for the orexin-induced catecholamine release from chromaffin cells. As shown in Fig. 1D, when we replaced the extracellular solution with Ca^{2+}-free solution containing 1 mM EGTA and 200 μM Cd^{2+} for at least 5 min, orexin A-induced secretion was totally blocked, confirming that extracellular Ca^{2+} influx plays a major role. Therefore, these results clearly demonstrate that orexin A triggers catecholamine release from cultured rat adrenal chromaffin cells, which is dependent on the activation of OX1R and the extracellular Ca^{2+} influx.

We next investigated whether orexin A would evoke catecholamine release from mouse adrenal chromaffin cells. Since tissue slice provide a more physiological condition as compared to cultured cells, we used mouse adrenal slices in this section [4,15]. As shown in Fig. 2A, an electrode attached to a relative clear cell in adrenal slice. Puff application of orexin A (100 nM) significantly produced a slow signal accompanied by several amperometric spikes. Comparing with the fast spikes, the slow signals arise from the vesicles slightly further away [2,9]. In addition, the evoked amperometric currents by orexin A was dramatically inhibited by pretreatment with the selective OX1R antagonist SB-334867 (1 μM). However, SB-334867 itself had no effect on the cate-

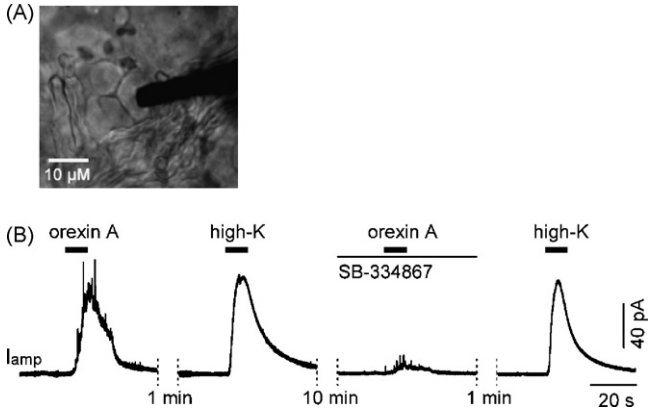

Fig. 2. Orexin A evokes catecholamine release from mouse chromaffin cells in adrenal slices. (A) Representative image showing a carbon fiber electrode (black bar) attached to a chromaffin cell in a mouse adrenal slice. (B) Application of 100 nM orexin A markedly induced amperometric signals. In the presence of SB-334867 (1 μM), the orexin A-induced response was significantly inhibited ($n = 6$, $p < 0.001$). High-K solution was used as a positive control.

cholamine release. As a positive control, high-K solution repeatedly induced significant secretory signals (Fig. 2B). These results demonstrate that orexin A stimulates catecholamine secretion from mouse adrenal chromaffin cells, which is mainly mediated by OX1R.

In recent years evidence has accumulated that orexins affect peripheral organs, such as adrenal gland [7]. However, conflicting findings have been reported about the effects of orexins on catecholamine secretion from human, bovine and porcine adrenal medulla and from human and rat pheochromocytomas [8,13,14,16,17]. In the present study, we further found that orexin A (100 nM) stimulated catecholamine release from both the cultured rat chromaffin cells and the mouse adrenal slices by using electrochemical amperometry, which provides direct evidence to show orexins modulating adrenal medulla function in rats and mice. These results are consistent with the observations obtained from porcine chromaffin cells [16] and human pheochromocytomas [13]. In contrast, our results argue against the findings that orexins reduce catecholamine secretion in rat pheochromocytoma cells [17] and do not play a role in human and bovine adrenomedullary cells [8,14]. Actually, several possible explanations for these inconsistent observations should be considered. Firstly, we obtained the results by using two different approaches, namely high performance liquid chromatography used elsewhere [8,13,14,16,17] and carbon-fiber amperometry in our study. Compared with the biochemical assay, amperometry is a powerful tool with high temporal and spatial resolution [6,26,28] and thus detect orexin-induced catecholamine release from chromaffin cells immediately. Secondly, species differences in the effects of orexin on catecholamine release may exist in adrenal medulla. Hence, more studies in future should be carried out using amperometry in other species.

OX1R and OX2R expression has been detected in rat adrenal medulla [12]. However, there are still conflicting reports regarding the relative expression of orexin receptors. Mazzocchi et al. reported high expression of OX1R but very low expression of OX2R in human adrenal medulla [14]. Kawada et al. observed robust expression of OX1R in bovine adrenal medulla [8]. Contrarily, some reports described only OX2R expression in human pheochromocytomas [13] and rat adrenal medulla [17]. In parallel, the receptor subtype involved in the effect of orexins on adrenal medulla also remained controversial. OX1R mediates the stimulation of catecholamine synthesis by orexin A in bovine adrenals [8], while OX2R contributes to catecholamine release in human pheochromocytomas [13]. In the present study, treatment with the selective OX1R antagonist SB-334867 almost completely blocked orexin-induced secretion in cultured rat chromaffin cells and significantly inhibited the secretory response in mouse adrenal medulla slices. These results provide strong evidence that activation of OX1R plays a major role in the effect of orexin A on catecholamine release in rat and mouse adrenals. On the other hand, catecholamine release has been widely shown to be Ca^{2+}-dependent in the adrenal chromaffin cells [3,30]. Either extracellular Ca^{2+} influx or intracellular Ca^{2+} release contributes to the orexin-mobilized $[Ca^{2+}]_i$ in varying cells [11,24,27]. The present results demonstrate that orexin A-induced secretion is mainly dependent on extracellular Ca^{2+} in chromaffin cells.

Finally, this study showed that orexin A ranging from 1 to 1000 nM dose-dependently induced catecholamine release from adrenal chromaffin cells. However, the dose in this range is relatively high as compared to the plasma levels of orexin (about 2 pM) in healthy humans [1]. Therefore, the present study provides evidence showing the pharmacological effect of orexin in regulating adrenal function, while the physiological significance of serum orexin A remains unclear.

References

[1] Z. Arihara, K. Takahashi, O. Murakami, K. Totsune, M. Sone, F. Satoh, S. Ito, T. Mouri, Immunoreactive orexin-A in human plasma, Peptides 22 (2001) 139–142.
[2] G. Arroyo, J. Fuentealba, N. Sevane-Fernandez, M. Aldea, A.G. Garcia, A. Albillos, Amperometric study of the kinetics of exocytosis in mouse adrenal slice chromaffin cells: physiological and methodological insights, J. Neurophysiol. 96 (2006) 1196–1202.
[3] G.J. Augustine, E. Neher, Calcium requirements for secretion in bovine chromaffin cells, J. Physiol. 450 (1992) 247–271.
[4] J.G. Barbara, J.C. Poncer, R.A. McKinney, K. Takeda, An adrenal slice preparation for the study of chromaffin cells and their cholinergic innervation, J. Neurosci. Methods 80 (1998) 181–189.
[5] X.K. Chen, L.C. Wang, Y. Zhou, Q. Cai, M. Prakriya, K.L. Duan, Z.H. Sheng, C. Lingle, Z. Zhou, Activation of GPCRs modulates quantal size in chromaffin cells through G(betagamma) and PKC, Nat. Neurosci. 8 (2005) 1160–1168.
[6] R.H. Chow, L. von Ruden, E. Neher, Delay in vesicle fusion revealed by electrochemical monitoring of single secretory events in adrenal chromaffin cells, Nature 356 (1992) 60–63.
[7] O. Johren, N. Bruggemann, P. Dominiak, Orexins (hypocretins) and adrenal function, Horm. Metab. Res. 36 (2004) 370–375.
[8] Y. Kawada, S. Ueno, K. Asayama, M. Tsutsui, K. Utsunomiya, Y. Toyohira, N. Morisada, K. Tanaka, A. Shirahata, N. Yanagihara, Stimulation of catecholamine synthesis by orexin-A in bovine adrenal medullary cells through orexin receptor 1, Biochem. Pharmacol. 66 (2003) 141–147.
[9] D.S. Koh, B. Hille, Modulation by neurotransmitters of catecholamine secretion from sympathetic ganglion neurons detected by amperometry, Proc. Natl. Acad. Sci. U.S.A. 94 (1997) 1506–1511.
[10] J.P. Kukkonen, T. Holmqvist, S. Ammoun, K.E. Akerman, Functions of the orexinergic/hypocretinergic system, Am. J. Physiol. 283 (2002) C1567–C1591.
[11] K.P. Larsson, H.M. Peltonen, G. Bart, L.M. Louhivuori, A. Penttonen, M. Antikainen, J.P. Kukkonen, K.E. Akerman, Orexin-A-induced Ca2+ entry: evidence for involvement of trpc channels and protein kinase C regulation, J. Biol. Chem. 280 (2005) 1771–1781.
[12] M. Lopez, R. Senaris, R. Gallego, T. Garcia-Caballero, F. Lago, L. Seoane, F. Casanueva, C. Dieguez, Orexin receptors are expressed in the adrenal medulla of the rat, Endocrinology 140 (1999) 5991–5994.
[13] G. Mazzocchi, L.K. Malendowicz, F. Aragona, P. Rebuffat, L. Gottardo, G.G. Nussdorfer, Human pheochromocytomas express orexin receptor type 2 gene and display an in vitro secretory response to orexins A and B, J. Clin. Endocrinol. Metab. 86 (2001) 4818–4821.
[14] G. Mazzocchi, L.K. Malendowicz, L. Gottardo, F. Aragona, G.G. Nussdorfer, Orexin A stimulates cortisol secretion from human adrenocortical cells through activation of the adenylate cyclase-dependent signaling cascade, J. Clin. Endocrinol. Metab. 86 (2001) 778–782.
[15] T. Moser, E. Neher, Rapid exocytosis in single chromaffin cells recorded from mouse adrenal slices, J. Neurosci. 17 (1997) 2314–2323.
[16] T. Nanmoku, K. Isobe, T. Sakurai, A. Yamanaka, K. Takekoshi, Y. Kawakami, K. Goto, T. Nakai, Effects of orexin on cultured porcine adrenal medullary and cortex cells, Regul. Pept. 104 (2002) 125–130.
[17] T. Nanmoku, K. Isobe, T. Sakurai, A. Yamanaka, K. Takekoshi, Y. Kawakami, K. Ishii, K. Goto, T. Nakai, Orexins suppress catecholamine synthesis and secretion in cultured PC12 cells, Biochem. Biophys. Res. Commun. 274 (2000) 310–315.
[18] A. Neely, C.J. Lingle, Effects of muscarine on single rat adrenal chromaffin cells, J. Physiol. 453 (1992) 133–166.
[20] T. Sakurai, Orexins and orexin receptors: implication in feeding behavior, Regul. Pept. 85 (1999) 25–30.

[19] T. Sakurai, The neural circuit of orexin (hypocretin): maintaining sleep and wakefulness, Nat. Rev. Neurosci. 8 (2007) 171–181.
[21] T. Sakurai, A. Amemiya, M. Ishii, I. Matsuzaki, R.M. Chemelli, H. Tanaka, S.C. Williams, J.A. Richarson, G.P. Kozlowski, S. Wilson, J.R. Arch, R.E. Buckingham, A.C. Haynes, S.A. Carr, R.S. Annan, D.E. McNulty, W.S. Liu, J.A. Terrett, N.A. Elshourbagy, D.J. Bergsma, M. Yanagisawa, Orexins and orexin receptors: a family of hypothalamic neuropeptides and G protein-coupled receptors that regulate feeding behavior, Cell 92 (1998) 573–585.
[22] J.G. Sutcliffe, L. de Lecea, The hypocretins: setting the arousal threshold, Nat. Rev. Neurosci. 3 (2002) 339–349.
[23] P. Trivedi, H. Yu, D.J. MacNeil, L.H. Van der Ploeg, X.M. Guan, Distribution of orexin receptor mRNA in the rat brain, FEBS Lett. 438 (1998) 71–75.
[24] 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, J. Neurosci. 18 (1998) 7962–7971.
[25] T. Voisin, P. Rouet-Benzineb, N. Reuter, M. Laburthe, Orexins and their receptors: structural aspects and role in peripheral tissues, Cell Mol. Life Sci. 60 (2003) 72–87.
[26] R.M. Wightman, J.A. Jankowski, R.T. Kennedy, K.T. Kawagoe, T.J. Schroeder, D.J. Leszczyszyn, J.A. Near, E.J. Diliberto Jr., O.H. Viveros, Temporally resolved catecholamine spikes correspond to single vesicle release from individual chromaffin cells, Proc. Natl. Acad. Sci. U.S.A. 88 (1991) 10754–10758.
[27] J.X. Xia, X.W. Chen, S.Y. Cheng, Z.A. Hu, Mechanisms of orexin A-evoked changes of intracellular calcium in primary cultured cortical neurons, Neuroreport 16 (2005) 783–786.
[28] Z. Zhou, S. Misler, Action potential-induced quantal secretion of catecholamines from rat adrenal chromaffin cells, J. Biol. Chem. 270 (1995) 3498–3505.
[29] Z. Zhou, S. Misler, R.H. Chow, Rapid fluctuations in transmitter release from single vesicles in bovine adrenal chromaffin cells, Biophys. J. 70 (1996) 1543–1552.
[30] R.S. Zucker, Calcium and transmitter release, J. Physiol. Paris 87 (1993) 25–36.