Effects of glucagon-like peptide 1 and oxyntomodulin on neuronal activity of ghrelin-sensitive neurons in the hypothalamic arcuate nucleus

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Riediger T, Eisele N, Scheel C, Lutz TA. Effects of glucagon-like peptide 1 and oxyntomodulin on neuronal activity of ghrelin-sensitive neurons in the hypothalamic arcuate nucleus. Am J Physiol Regul Integr Comp Physiol 298: R1061–R1067, 2010. First published February 10, 2010; doi:10.1152/ajpregu.00438.2009.—Glucagon-like peptide 1 (GLP-1) and oxyntomodulin (OXM) are structurally related gastrointestinal hormones that are secreted in response to food intake. They reduce food intake and body weight and exert partly overlapping actions on glucose homeostasis and gastrointestinal function. The hypothalamic arcuate (ARC) nucleus is among the central structures expressing a high density of GLP-1 receptors (GLP-1R), which are known to be activated by both peptides. It was the aim of our electrophysiological studies to characterize the effects of GLP-1 and OXM on functionally defined ghrelin-sensitive ARC neurons. GLP-1R is densely expressed in hypothalamic areas, including the arcuate (ARC) and paraventricular nuclei and the area postrema/nucleus of the solitary tract region of the brain stem (3, 21, 34). As shown by in vivo imaging techniques in mice and humans, the GLP-1R-expressing brain areas are responsive to peripherally applied GLP-1 and OXM (2, 23).

In addition to their function as peripherally secreted hormones, GLP-1 and OXM are also expressed in the brain (5, 19). While the detailed distribution of OXM remains to be elucidated, the neuroanatomy of GLP-1-ergic pathways is well established. Enteroreceptive GLP-1-expressing neurons reside in the nucleus of the solitary tract and project to GLP-1R-expressing hypothalamic brain areas, including the ARC (19).

The GLP-1R seems to mediate the anorectic effect of OXM, because the GLP-1R antagonist exendin(9–39) blocks the OXM-induced suppression of food intake (4, 8, 9). Furthermore, GLP-1R-deficient mice do not show an anorectic response to OXM (4). The GLP-1R is densely expressed in hypothalamic areas, including the arcuate (ARC) and paraventricular nuclei and the area postrema/nucleus of the solitary tract region of the brain stem (3, 21, 34). As shown by in vivo imaging techniques in mice and humans, the GLP-1R-expressing brain areas are responsive to peripherally applied GLP-1 and OXM (2, 23).

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In the present study, we conducted extracellular single-cell recordings to characterize the effects of GLP-1 and OXM on neuronal activity in the ARC of rats. Similar to our previous studies (25, 26), we used the well-investigated orexigenic hormone ghrelin as a functional reference stimulus. In the ARC of rats, ghrelin or agonists of the ghrelin receptor [growth hormone secretagogue receptor (GHS-R)] induce two types of effects on neuronal activity: a direct postsynaptic excitatory effect that predominates in the medial ARC, and an indirect presynaptically mediated inhibition, which is more frequent in the lateral subdivision (14, 29, 35, 36). Based on c-Fos/in situ hybridization studies and Ca2+ signaling/GHS-R expression studies, the majority of the ghrelin-excited target cells carrying the GHS-R express neuropeptide Y (NPY); to a much lower degree, some ghrelin-excited cells express somatostatin, growth hormone releasing hormone (GHRH), tyrosine hydroxylase, and pro-opiomelanocortin (POMC) (1, 17, 38).

In addition to the characterization of OXM- and GLP-1-mediated effects on ghrelin-responsive ARC neurons, we used the receptor antagonist exendin(9–39) to pharmacologically

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assess the involvement of the GLP-1R. Finally, we investigated the neuronal effects of OXM and GLP-1 under synaptic blockade to dissociate direct postsynaptic actions from indirect presynaptic actions. 

MATERIALS AND METHODS

**Animals.** Adult male Wistar rats, housed in a temperature-controlled room (22°C), were used. They had free access to water and standard rodent chow and were kept under a 12:12-h light-dark cycle. All animal procedures were approved by the Veterinary Office of the Canton Zurich.

**Extracellular recordings.** The experimental procedures for the extracellular recordings were as previously described (25, 27, 35). Rats were decapitated, and their brains were quickly removed and superfused with ice-cold artificial cerebrospinal fluid (aCSF) of the following composition (in mM): 124 NaCl, 5 KCl, 1.2 NaH2PO4, 1.3 MgSO4, 1.2 CaCl2, 26 NaHCO3, 10 glucose, equilibrated with 95% O2 and 5% CO2, pH 7.4, 290 mosmol/kg. Coronal whole brain slices (700 µm thick) were cut at the mid-rostro-caudal level of the ARC using a vibratome (Leica VT1000S, Leica Microsystems). A rectangular (2 x 2 mm) piece of tissue containing the ARC was dissected by hand and transferred to a temperature-controlled (37.0°C) recording chamber constantly superfused with prewarmed aCSF at a rate of 1.6 ml/min.

Extracellular recordings of ARC neurons were obtained using glass-coated platinum-iridium electrodes. The recording area was restricted to the ARC region, according to the neuroanatomical brain map of Paxinos and Watson (24). The responsiveness to rat ghrelin, GLP-1(1-36)amide, and OXM (Bachem) was tested by switching to a solution containing the investigated peptides at concentrations of 10^{-7} M to 2 x 10^{-7} M (OXM), 10^{-7} M (GLP-1), and 10^{-7} M to 10^{-8} M (ghrelin). An OXM concentration of 10^{-7} M had been used under experimentally similar conditions, inducing the release of α-melanocyte stimulating hormone from hypothalamic explants (9). To test the involvement of GLP-1Rs, the GLP-1R antagonist exendin(9-39) was coapplied with GLP-1 and OXM in 10-fold molar excess relative to the concentration of these hormones.

To dissociate presynaptic and postsynaptic effects, the effects of GLP-1 and OXM on neuronal activity were investigated under synaptic isolation (16) using aCSF containing low Ca^{2+} (0.3 mM) and high Mg^{2+} (9 mM) concentrations.

**Data analysis of electrophysiological responses.** From the continuously recorded rate meter counts, the average discharge rate of each neuron was evaluated for 60 s before the stimulus. This value (spontaneous discharge rate) was used to normalize changes in firing rate, expressed as percent change of the spontaneous discharge rate. If the averaged change of discharge rate during the response was larger than ±20% and ±0.5 Hz, the neuron was considered sensitive to the applied substance. In addition to the mean change of the discharge rate during the entire responses, the peak values of the responses were calculated on a basis of a 30-s interval during which the firing rate was maximal or minimal, respectively. Finally, the duration between the application of the drugs and the onset of the effects (latency) and the duration of the entire responses were determined. The parameters of the electrophysiological responses were expressed as means ± SE. Statistics. For statistical comparisons of the electrophysiological response parameters, Student’s t-test was used. Paired t-tests were used for the statistical analysis of the antagonist studies. The proportions of responses were compared by χ^2 test. In all cases, P < 0.05 was considered significant. Results are presented as means ± SE.

**RESULTS**

For each of the two hormones, OXM and GLP-1, 89 spontaneously active ARC neurons were tested for their cosensitivities to ghrelin. In 42 recordings, the cosensitivity to OXM and GLP-1 was tested. The mean amplitude of the extracellularly recorded action potentials was 174 μV, allowing a proper discrimination from background noise (signal-to-noise ratio of ~16). There was no indication that repeated stimulations caused desensitizations. Furthermore, previous experiments under the same conditions indicated that control superfusions of aCSF without peptides do not cause unspecific changes in firing rate over time.

**Effects of OXM and GLP-1 on ghrelin-sensitive ARC neurons.** The effects of ghrelin on neuronal activity were consistent with previous electrophysiological studies (27) and are, therefore, not described in detail. Briefly, ~62% of the neuronal responses were excitatory, while a lower proportion of cells was inhibited by ghrelin (17%). Although we did not repeat the analysis of site specificity (27), excitatory responses were more frequent in the medial part of the ARC compared with the lateral subdivision. Conversely, inhibitory responses were more frequent in the lateral part.

OXM and GLP-1 exerted excitatory and inhibitory effects on the discharge rate of ARC neurons. Both hormones showed similar characteristics with respect to the types of responses and also concerning the cosensitivity of the investigated neurons to ghrelin. Among the ghrelin-excited neurons that showed cosensitivity to OXM and GLP-1, most cells showed excitatory (concordant) responses to these hormones, while lower proportions of cells were inhibited (Fig. 1, left). In contrast, the majority of the ghrelin-inhibited neurons responded with excitatory (discordant) responses to OXM or GLP-1; no (OXM) or only few (GLP-1) inhibitory (concordant) effects were observed (Fig. 1, middle). The number of OXM- and GLP-1-responsive neurons was generally lower in ghrelin-insensitive ARC neurons, in which both excitatory and inhibitory effects of OXM and GLP-1 occurred (Fig. 1, right).

Within each of the three types of cells (ghrelin excited; ghrelin inhibited, ghrelin insensitive), the proportions of OXM or GLP-1 sensitivities were not significantly different (χ^2 test). However, the proportions of OXM/GLP-1 responses opposite to the action of ghrelin were significantly higher in ghrelin-inhibited than in ghrelin-excited cells (χ^2 test, P < 0.0001 for OXM, P = 0.009 for GLP-1). Representative recordings of OXM and GLP-1 responses in ghrelin-excited and ghrelin-inhibited ARC neurons are shown in Figs. 2 and 3, respectively.

**Effect parameters and cosensitivity of ARC neurons to OXM and GLP-1.** The mean effect parameters of all responses induced by superfusion of OXM (10^{-7} M and 2 x 10^{-7} M) and GLP-1 (10^{-7} M) are summarized in Table 1. The mean spontaneous firing rates of the analyzed neurons were statistically not different for the different stimuli. There were no statistical differences in the effect parameters resulting from the two different OXM concentrations. Similarly, stimulation-induced changes in firing rates were statistically not different between both OXM concentrations compared with the GLP-1 stimulation. However, GLP-1 appeared to have generally shorter response latencies than OXM. This was statistically significant for the inhibitory responses to GLP-1 compared with the inhibitory responses to OXM (10^{-7} M). Similarly, the excitatory response latency of GLP-1 was significantly shorter than that of OXM (2 x 10^{-7} M). Furthermore, GLP-1 had a significantly longer excitatory effect duration than OXM (2 x 10^{-7} M).
Among the 42 neurons that were tested for their cosensitivity to OXM and GLP-1, the most frequent types of concordant responses were excitatory actions of GLP-1 and OXM or insensitivity to both hormones (Fig. 4). Across all 42 tested neurons, 64% showed concordant responses, while antagonistic effects occurred in only 8%.

**Effect of the GLP-1R antagonist exendin(9–39) on OXM- and GLP-1-induced responses.** To investigate the involvement of the GLP-1R in the mediation of the OXM- and GLP-1-induced effects, exendin(9–39) was coapplied with these peptides in 10-fold molar excess after OXM and GLP-1 sensitivity had been confirmed by a reference stimulus without the antagonist. Exendin(9–39) effectively blocked the GLP-1- and OXM-mediated responses. None of the seven GLP-1-excited and six GLP-1-inhibited neurons that were tested responded to GLP-1 in the presence of exendin(9–39). Likewise, the excitatory effect of OXM was blocked by exendin(9–39) in all five neurons that were tested (Fig. 5). The same was true for two OXM-inhibited cells that were tested with exendin(9–39) (data not shown). As exemplified by the recording shown in Fig. 6, the responsiveness to OXM recovered after washout of the antagonist.

**DISCUSSION**

We demonstrated similar effects of OXM and GLP-1 on the electrical activity of neurons in the ARC. Both hormones exerted excitatory and inhibitory effects, presumably in functionally different subsets of neurons. The response profiles indicate that ARC neurons displayed a high degree of cosensitivity to OXM and GLP-1. The effects of GLP-1 and OXM were effectively blocked by the GLP-1R antagonist exendin(9–39), pointing to a mediation via the GLP-1R.

**Functional considerations.** The ARC contains functionally and phenotypically distinct populations of neurons involved in...
the control of food intake, energy homeostasis, and neuroendocrine and autonomic mechanisms. We used ghrelin as a reference stimulus, because its actions on neuronal activity in the ARC and the phenotype of GHS-R-expressing neurons have been characterized in previous studies. In relation to ghrelin sensitivity, our studies revealed three main findings. First, the number of OXM- or GLP-1-responsive neurons seems to be higher in ghrelin-sensitive than in ghrelin-insensitive ARC neurons, irrespective of the types of responses. Second, among the ghrelin-inhibited neurons, OXM and GLP-1 primarily induced excitatory actions, i.e., opposite to the effect of ghrelin. Third, a considerable fraction of ghrelin-excited ARC neurons was concordantly stimulated by OXM and GLP-1; to a lesser degree, inhibitory effects were also observed in this population of cells.

Based on the fact that the excitatory effects of ghrelin in the ARC are direct postsynaptic (GHS-R-dependent) effects (27), the neurochemical profile of the ghrelin-excited cells can be inferred from the neuropeptide/neurotransmitter content of GHS-R expressing ARC neurons. The vast majority of neurons carrying the GHS-R have been identified as NPY neurons, but the GHS-R is also found in 8% of POMC cells, in 30% of somatostatin neurons, and in ~20% of GHRH neurons (38). It has to be taken into consideration that the two latter cell types are less abundant in the ARC than NPY neurons (38), so that the absolute number of GHS-R expressing ARC neurons can be estimated to be 7.5 times higher than the number of GHS-R-positive somatostatin neurons.

The phenotyping studies by Willesen et al. (38) are, in principle, consistent with c-Fos/insitu hybridizations double-labeling studies (10), showing that ARC neurons activated by the GHS-R agonist growth hormone releasing peptide-6 predominantly express NPY (51%), and, with lower frequencies, GHRH (23%), tyrosine hydroxylase (11%), POMC (11%), or somatostatin (4%). Similarly, the majority of ARC neurons activated by ghrelin have been shown to be NPY-ergic in mice (37).

It is conceivable that the OXM/GLP-1-responsive and ghrelin-excited neurons form a subpopulation of GHS-R-expressing neurons in the ARC. However, GLP-1Rs seem to be scarce in NPY neurons (30). This might further suggest that ghrelin-excited neurons expressing NPY largely overlap with the fraction of OXM/GLP-1-insensitive cells observed in our studies. Notably, the proportion of these cells (56 and 52%) in our study corresponds well with the 51% of NPY-ergic ARC neurons activated by GHS-R stimulation in vivo (10). Based on these considerations, the actions of OXM and GLP-1 in the ghrelin-excited neurons seem to be largely restricted to neurons with neuroendocrine or yet unidentified function. It has been proposed that phenotypically unidentified ARC neurons might be implicated in GLP-1R-mediated effects on glucose homeostasis (30). Further studies are required to shed more light on the phenotypical and functional nature of these cells.

In contrast to the effects of OXM and GLP-1 in ghrelin-excited neurons, the effects of these hormones in ghrelin-inhibited neurons seem to be less complex. The majority of these cells were excited by GLP-1 and OXM. Both in rats and in mice, the inhibitory actions of ghrelin or ghrelin receptor agonists are indirect effects that are presynaptically mediated (27, 36). In mice, inhibitory effects of ghrelin have been shown to occur in POMC neurons, while excitatory effects are restricted to NPY and non-POMC neurons (7), which is consistent with the low incidence of GHS-R expression in POMC neurons (38). Although similar electrophysiological studies

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**Table 1. Average effect parameters of the electrophysiological responses induced by OXM and GLP-1**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>OXM Excitation, $10^{-7}$ M</th>
<th>OXM Inhibition, $10^{-7}$ M</th>
<th>OXM Excitation, $2 \times 10^{-7}$ M</th>
<th>OXM Inhibition, $2 \times 10^{-7}$ M</th>
<th>GLP-1 Excitation, $10^{-7}$ M</th>
<th>GLP-1 Inhibition, $10^{-7}$ M</th>
</tr>
</thead>
<tbody>
<tr>
<td>$n$</td>
<td>20</td>
<td>3</td>
<td>15</td>
<td>5</td>
<td>16</td>
<td>16</td>
</tr>
<tr>
<td>Mean spontaneous activity, Hz</td>
<td>$4.1 \pm 0.5$</td>
<td>$3.9 \pm 1.5$</td>
<td>$3.7 \pm 0.4$</td>
<td>$4.8 \pm 0.8$</td>
<td>$3.8 \pm 0.3$</td>
<td>$3.5 \pm 0.4$</td>
</tr>
<tr>
<td>Mean latency, s</td>
<td>$201 \pm 33$</td>
<td>$298 \pm 136$</td>
<td>$282 \pm 38$</td>
<td>$215 \pm 75$</td>
<td>$167 \pm 23^*$</td>
<td>$99 \pm 22^*$</td>
</tr>
<tr>
<td>Mean response, %</td>
<td>$+51 \pm 10$</td>
<td>$-34 \pm 7$</td>
<td>$+43 \pm 5$</td>
<td>$-39 \pm 6$</td>
<td>$+44 \pm 6$</td>
<td>$-43 \pm 5$</td>
</tr>
<tr>
<td>Mean response, Hz</td>
<td>$+1.6 \pm 0.2$</td>
<td>$-1.1 \pm 0.3$</td>
<td>$+1.5 \pm 0.2$</td>
<td>$-1.7 \pm 0.1$</td>
<td>$+1.6 \pm 0.2$</td>
<td>$-1.5 \pm 0.2$</td>
</tr>
<tr>
<td>Mean peak response, Hz</td>
<td>$+2.6 \pm 0.3$</td>
<td>$-2.5 \pm 1$</td>
<td>$+2.2 \pm 0.3$</td>
<td>$-2.4 \pm 0.1$</td>
<td>$+2.7 \pm 0.3$</td>
<td>$-2.3 \pm 0.3$</td>
</tr>
<tr>
<td>Mean response duration, s</td>
<td>$1,017 \pm 91$</td>
<td>$1,133 \pm 343$</td>
<td>$786 \pm 90$</td>
<td>$881 \pm 108$</td>
<td>$1,082 \pm 82^*$</td>
<td>$661 \pm 79$</td>
</tr>
</tbody>
</table>

Values are means $\pm$ SE; $n$, no. of neurons. OXM, oxytomodulin; GLP-1, glucagon-like peptide 1. *Significant difference vs. OXM inhibition ($10^{-7}$ M); †significant difference vs. OXM excitation ($2 \times 10^{-7}$ M); $P < 0.05$, $t$-test.

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**Fig. 4. Cosensitivities of ARC neurons to GLP-1 and OXM.** The majority of neurons showed concordant responses to these hormones. $n$, No. of neurons.
with neurochemically identified neurons have not yet been conducted in rats, the high abundance (68%) of the GLP-1R in POMC neurons of rats suggests that these neurons also potentially belong to the major target cells for OXM and GLP-1. It, therefore, appears plausible that the ghrelin-inhibited and OXM/GLP-1-excited neurons in our study largely overlap with the POMC-expressing population of ARC neurons.

α-Melanocyte-stimulating hormone released from POMC neurons are well known to inhibit feeding (33). Therefore, excitatory effects induced by GLP-1 and OXM on ghrelin-inhibited cells observed in our experiments were the type of response that is, in principle, consistent with an anorectic mechanism, although the involvement of the ARC in the anorectic action of these hormones has so far only been demonstrated for OXM, but not for GLP-1 (9, 30).

GLP-1R mediated actions of GLP-1 and OXM. We did not detect considerable differences in the efficacy of GLP-1 and OXM on neuronal ARC activity when applied at equimolar concentrations. Notably, both hormones exerted potent effects at a concentration that is 10-fold lower than the lowest effective GLP-1 concentration found to excite hypocretin neurons in previous electrophysiological dose-response studies (1). Furthermore, the effects of GLP-1 and OXM were completely blocked by the same concentration of exendin(9–39). Against the background of well-established differences in binding activities of GLP-1 and OXM to the GLP-1R, it may appear surprising that GLP-1 and OXM were equally effective in our experiments. The only parameters reflecting a stronger action of GLP-1 vs. OXM were the somewhat shorter response latencies and longer effect duration of GLP-1. The affinity of GLP-1 and OXM for the GLP-1R were reported to differ by about two orders of magnitude, with published IC50 values of 0.16 vs. 8.2 nM (GLP-1 vs. OXM) in one study (8) and 0.34 vs. 33.1 nM in another study (11). The GLP-1 and OXM concentration used in the current work produced submaximal GLP-1 displacement in these competition binding studies. Therefore, we cannot exclude diverging efficacies for these peptides at lower concentrations.

For most investigated hormones, the threshold concentrations determined in electrophysiological studies (including the present work) are supraphysiological (1–100 nM), despite evidence for an in vivo action of the endogenous hormone. There are various possible reasons for this, including synergistic actions of hormones or metabolic cues in vivo, and the comparatively short duration of stimulation in vitro. Furthermore, it is important to consider that brain intrinsic ligands released from axonal terminals can reach much higher local concentrations, which may be a relevant mechanism for GLP-1, because it has been detected in brain stem projections innervating the ARC (19).

Perspectives and significance. We conclude that GLP-1 and OXM exert direct effects on the activity of ARC neurons via GLP-1R-mediated action. Our data point to heterogeneous (excitatory and inhibitory) actions of OXM and GLP-1 on ghrelin-excited neuroendocrine neurons or as-yet neurochemically unidentified neurons that have been proposed to be involved in glucose homeostasis. There are several other physiological functions that might be influenced by GLP-1/OXM-ergic actions in the ARC. For instance, GLP-1 and OXM have been implicated in neuroendocrine responses to acute stress and in the suppression of ghrelin secretion, respectively.
OXM and GLP-1 induced predominantly excitatory effects in ghrelin-inhibited ARC neurons, which are thought to form a subpopulation of POMC neurons. The latter effects are compatible with ARC-dependent anorectic actions mediated by GLP-1R stimulation.

While our present studies do not point to fundamentally different actions of OXM and GLP-1 with respect to their effects on neuronal activity and receptor pharmacology, the complexity of responses suggests multiple physiological functions that might be affected by these hormones via the ARC. Whether the effects of OXM and GLP-1 on ghrelin-sensitive ARC neurons are the correlate of further functions, in addition to the presumed effects on food intake and glucose homeostasis, remains to be investigated.

GRANTS

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DISCLOSURES

The authors declare no competing interests.

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