



Orexins control intestinal glucose transport by distinct neuronal, endocrine and direct epithelial pathways

| | |
|-------------------------------|--|
| Journal: | <i>Diabetes</i> |
| Manuscript ID: | DB-07-0614.R2 |
| Manuscript Category: | Original Article |
| Date Submitted by the Author: | 05-Jul-2007 |
| Complete List of Authors: | Ducroc, Robert; INSERM Unit 773, Centre de Recherche Biomédicale Bichat-Beaujon Voisin, Thierry; INSERM Unit 773, Centre de Recherche Biomédicale Bichat-Beaujon El Firar, Aadil; INSERM Unit 773, Centre de Recherche Biomédicale Bichat-Beaujon Laburthe, Marc; INSERM Unit 773, Centre de Recherche Biomédicale Bichat-Beaujon |
| Key Words: | Sodium Glucose Cotransporter, CCK receptor, Orexin receptor, Ussing chamber, CCK receptor |
| | |



**Orexins control intestinal glucose transport
by distinct neuronal, endocrine and direct epithelial pathways**

Robert DUCROC, Thierry VOISIN, Aadil El FIRAR, Marc LABURTHE

Institut National de la Santé et de la Recherche Médicale, U 773
Centre de Recherche Biomédicale Bichat-Beaujon, CRB3, Paris F-75018 ;
Université Denis Diderot, UMR S 773, Paris 75018, France

Running Head

Orexins regulate intestinal glucose absorption

Corresponding Author

R. Ducroc, Inserm, U773, Centre de Recherche Biomédicale Bichat-Beaujon, 16 rue Henri Huchard, BP16, 75870 Paris cedex 18, France. Phone : 33-1 4485-6133; Fax : 33-1 4228-8765; E-mail : ducroc@bichat.inserm.fr

Key words

Ussing chamber; CCK receptor; tetrodotoxin; SGLT-1; orexin receptor

Abstract

Objective : Orexins are neuropeptides involved in energy homeostasis. We investigated the effect of orexin A (OxA) and OxB on intestinal glucose transport in the rat. Research Design and Methods : Injection of orexins led to a decrease in the blood glucose level in OGTT. Effects of orexins on glucose entry were analysed in Ussing chamber using the Na⁺-dependent increase in short-circuit current to quantify jejunal glucose transport. Results & Conclusions : The rapid and marked increase in I_{sc} induced by luminal glucose was inhibited by 10 nmol/l OxA or OxB (53 and 59% respectively). Response' curves to OxA and OxB were not significantly different with IC₅₀ at 0.9 and 0.4 nmol/l, respectively. On the one hand, OxA-induced inhibition of I_{sc} was reduced by the neuronal blocker TTX, and by a CCK2R antagonist, indicating involvement of neuronal and endocrine CCK-releasing cells. The OX1R antagonist SB334867 had no effect on OxA-induced inhibition, which is likely to occur via a neuronal and/or endocrine OX2R. On the other hand, SB334867 induced a significant right shift of the concentration-effect curve for OxB. This OxB-preferring OX1R pathway was not sensitive to TTX or to CCKR antagonists, suggesting that OxB may act directly on enterocytic OX1R. These distinct effects of OxA and OxB are consistent with the expression of OX1R and OX2R mRNA in the epithelial and non-epithelial tissues, respectively. Our data delineate a new function for orexins as inhibitors of intestinal glucose absorption and provide a new basis for orexin-induced short-term control of energy homeostasis.

Orexins/hypocretins are peptides discovered by orphan receptor technologies (1) or subtractive cDNA cloning (2). The two orexins, A and B, are encoded by a single gene and are derived from a common prepro-orexin that is processed into the 33-amino acid orexin-A (OxA) and the 28-amino acid OxB. These peptides share 46% amino acid identity in rat (3). Orexins are neuropeptides present in the hypothalamic neurons that project throughout the central nervous system (CNS) to nuclei involved in the control of feeding, sleep-wakefulness, neuroendocrine homeostasis and autonomic regulation (1; 3). Two orexin receptor subtypes, OX₁R and OX₂R (1; 2), are serpentine G protein-coupled receptors that bind both orexins. As shown in rat, OX₂R binds OxA and OxB with equal affinity whereas OX₁R has a preference for OxA (1; 3). Both receptors appear to be coupled to calcium mobilisation (1; 3). As with other peptides found in the hypothalamic area (ie. PYY, NPY, leptin, ghrelin, galanin) belonging to the so-called brain-gut axis, expression of orexins has also been reported in enteric neurons and endocrine cells of the digestive tract (4; 5). OxA was demonstrated to trigger cholecystokinin (CCK) release in STC-1 cells, an intestinal neuroendocrine cell model that expresses OX₁R and OX₂R (6). These studies, together with those characterising the orexin receptors in the enteric nervous system, pancreas and intestinal mucosa cells (4; 5; 7), suggest that orexins can exert direct control on gut functions.

The hypothalamus has been the focus of considerable attention regarding its role in the regulation of energy homeostasis, but the gut is now also considered as a major

player in the regulation of food intake. To achieve energy homeostasis, the CNS integrates signals coming from the peripheral organs that provide information about the level of energy fluxes and stores through neuronal pathways. Primary input is mediated by signal molecules conveyed in the intestinal lumen. Glucose, the major form of absorbed carbohydrate, is also an important signal molecule. Glucose levels in the bloodstream are critically evaluated by integrated hypothalamic circuits of neurons and neurochemicals that regulate appetite, energy expenditure and metabolism. Orexin neurons have been recently identified to play a major role in this regulation (8-10). Orexin glucosensing neurons can modulate their intrinsic electrical activity according to the ambient fluctuations in the levels of nutrients and appetite-regulating hormones, and are believed to translate directly rises and falls in body energy levels into different states of consciousness (10).

Following a meal, exogenous glucose is rapidly transported from the lumen of the small intestine into the blood stream and tissues. The first step of this process is the intracellular accumulation of glucose in enterocytes by the sodium-dependent glucose transporter 1, SGLT1. Intracellular glucose is then released into the interstitial space and blood *via* the GLUT2 glucose transporter located in the basolateral membrane. The activity of SGLT1 is highly regulated by hormones and intestinal peptides (11-15) indicating that control of intestinal glucose entry is crucial in the maintenance of energy homeostasis. Indeed, expression of SGLT1 is dramatically increased in diabetic humans (16). Although orexins and their receptors

have been found in the gastrointestinal tract, their role in the regulation of intestinal glucose transport has not yet been studied.

The present study was conducted to determine whether orexins A and B modulate intestinal glucose transport. We demonstrate a new physiological role for both OxA and OxB in the inhibitory control of intestinal glucose absorption and we describe distinct cellular pathways for their action that allow OX₁R and OX₂R to be distinguished.

For Peer Review Only

Research Design and Methods

Animals. Male Wistar rats weighing 240–280 g (Centre Elevage Janvier, Le Genest-St-Isle, France) were caged under standard laboratory conditions with tap water and regular food provided *ad libitum*, in a 12-h/12-h light/dark cycle at a temperature of 21–23°C. The animals were treated in accordance with European Community guidelines concerning the care and use of laboratory animals.

Oral glucose tolerance test. Oral glucose tolerance test (OGTT) was performed on conscious rats, following a 18 h fasting. Blood samples from fasted animals were first taken from the tail vein by 10:00 o'clock in the morning. OxA or OxB (55 µg/kg) diluted in NaCl 0.9% were administered by i.p. route five min before the OGTT. Controls received vehicle only. Rats in all groups were fed a 30% D-glucose solution (1 g/kg body weight) and blood samples taken by tail bleeds at 15, 30, 60 and 120 min after glucose administration. Glucose determination in blood was run immediately using an Accu-Chek Go (Roche Diagnostics, Meylan, France). Area under the curves were calculated according to the trapeze method and expressed in arbitrary units.

Tissue preparation and short-circuit measurement. Rats were fasted 16 h with water *ad libitum*. Animals were killed by i.p. pentobarbital overdose and the proximal jejunum was dissected out and rinsed in cold saline solution. The mesenteric border was carefully stripped off and the intestine was opened along the mesenteric border.

Four adjacent proximal samples were mounted in Ussing chambers as described (11). The tissues were bathed on each side with carbogen-gassed Krebs-Ringer bicarbonate (KRB) solution having the following composition (in mmol/l): NaCl 115.4, KCl 5, MgCl₂ 1.2, NaH₂PO₄ 0.6, NaHCO₃ 25, CaCl₂ 1.2 and glucose 10. In the solution bathing the mucosal side of the tissue, glucose was replaced with mannitol. Mannitol was kept in the bathing solution during glucose challenge. Both solutions were gassed with 95% O₂-5% CO₂ and kept at constant temperature of 37°C (pH at 7.4).

Electrogenic ion transport was monitored continuously as short-circuit current (I_{sc}) using an automated voltage clamp apparatus (DVC 1000, WPI, Aston, England) linked through a MacLab 8 to a MacIntosh computer. Orexins were added in the serosal bath 2 min before luminal glucose challenge. Results were expressed as the difference (ΔI_{sc}) between the peak I_{sc} after glucose challenge (maximum measured after 3 min) and the basal I_{sc} (measured just before the addition of glucose). Response' curves to orexins were studied non-cumulatively. ΔI_{sc} response to carbachol (100 μ mol/l) was used at the end of the experiment as a control.

Epithelial cell isolation and RT-PCR analysis

Epithelial and non epithelial cellular fractions of rat jejunum were obtained from everted jejunum shaken in a dispersing solution containing EDTA as described in detail previously (17; 18). Non-epithelial tissues were obtained after complete removal of epithelial cells.

Total RNA was extracted from cultured cell lines (CHO/ OX₁R or CHO/ OX₂R) or from intestinal cells using Trizol® reagent (Invitrogen, Cergy-Pontoise, France). All RNA preparations were treated with RNase free-DNAse (Promega, Charbonnières, France) for 60 min at 37°C. Five µg of RNA were reverse transcribed using oligo(dT) primers. Twenty-five percent of the cDNA mixture were amplified using human OX₁R sense primer (5'-CCTGTGCCTCCAGACTATGA-3') and OX₁R antisense primer (5'-ACACTGCTGACATTCCATGA-3') or OX₂R sense primer (5'TAGTTCCTCAGCTGCCTATC-3') and OX₂R anti-sense primer (5'CGTCCTCATGTGGTGGTTCT-3') or GADPH sense primer (5'TGAAGGTCGGAGTCAACGGATTGGT-3') and GADPH anti-sense primer (5'CATGTGGGCCATGAGGTCACACAC-3'). Each of the 35 cycles of amplification consisted of 94°C for 1 min, 62°C for 1 min and 72°C for 1 min. Amplicons were separated by electrophoresis in 1% agarose gels, stained with ethidium bromide and viewed under UV illumination.

Chemicals. Orexins A and B were purchased from R&D Systems (R&D Systems Europe Ltd, Abingdon, United Kingdom). Tetrodotoxin (TTX) was purchased from Alomone labs (Alomone Labs Ltd, Jerusalem, Israel). SB334867, an OX₁R specific antagonist, was purchased from Tocris Bioscience, (Bristol UK). Antagonists and TTX were added in serosal bath 10 min before orexins. Ala¹¹,D-Leu¹⁵OxB, an OX₂R specific agonist, was purchased from Calbiochem (La Jolla, CA). All other chemical reagents were purchased from Sigma (St. Louis, MO, USA).

Statistical Analysis. All results were expressed as means \pm SEM with n = number of tissues. One-way ANOVA with Tukey-Kramer multiple comparison post test was performed using GraphPad Prism version 3.0 for Windows (Graphpad software Inc., San Diego, CA). The level of significance was set at $P < 0.05$.

For Peer Review Only

Results

Oral glucose tolerance test. To evaluate the impact of peripheral OxA and OxB on glucose homeostasis, we performed oral glucose tolerance tests (OGTT) on conscious rats after i.p. administration of OxA or OxB (55 µg/kg). As depicted in Fig. 1, blood glucose concentration following glucose feeding was significantly reduced in rats receiving i.p. injection of OxA. Blood glucose level at 15 min was not significantly different from those in controls but glycemia was markedly decreased at 30 (P<0.008) and 60 (P<0.012) min. With OxB, blood glucose level was also decreased but it reached statistical significance for 15 min only (Fig. 1). Areas under the curve (AUC) were significantly decreased for both OxA and OxB as compared to control (Fig. 1, inset). Altogether, these data indicate that peripherally administered OxA and OxB can reduce intestinal absorption of glucose in a glucose tolerance test. When glucose is given luminally, the OGTT is an index of intestinal glucose entry through the glucose transporter SGLT-1 (19). Therefore, we further examined *in vitro* the effect of orexins on active intestinal absorption of glucose.

Intestinal glucose transport *in vitro* is inhibited by OxA and OxB. Luminal addition of 10 mmol/l glucose to the jejunal preparation (control) induced a rapid and marked increase in I_{sc} (*vs* basal condition before glucose challenge; Fig. 2A) which plateaued at 3 min : $\Delta I_{sc} = 27.0 \pm 2.3 \mu A/cm^2$, n=20. Serosal addition of OxA or OxB (10 nmol/l), two minutes before glucose challenge, significantly decreased glucose-induced I_{sc},

with ΔI_{sc} values at plateau of $11.6 \pm 3.9 \mu A/cm^2$ (n= 5) and $13.3 \pm 2.5 \mu A/cm^2$ (n= 8), for OxA and B respectively (57 and 50.7% inhibition respectively). We then studied dose-effect of OxA and OxB. As shown in Figure 2B, maximal effects of OxA and OxB were observed at 100 nmol/l peptide concentration and represented a ~70% inhibition of glucose-induced I_{sc} . The IC_{50} values for OxA and OxB were 0.9 and 0.4 nmol/l, respectively. To analyse the pathways involved in this inhibition, we examined the effect of the OX_1R antagonist SB334867. The response to OxA was not significantly modified by SB334867 (Fig. 3A). In sharp contrast, the OX_1R antagonist markedly shifted to the right the dose-response curve to OxB (Fig. 3B). This clearly indicates that the effect of OxB involves an OX_1 receptor whereas the effect of OxA is mostly independent of the OX_1 receptor. In the absence of available OX_2R antagonist, we could not further explore the orexin receptor mediating the effect of OxA.

Analysis of the pathway involved in the inhibitory action of OxB on glucose-induced I_{sc} . Recent morphological data showed that OX_1R immunoreactivity is present in the gut in neuronal cell bodies and mucosal epithelial cells, including enteroendocrine cells and enterocytes (5). We first examined the effect of the neuroblocker tetrodotoxin (TTX, 5 μM) on OxB-mediated inhibition of glucose-induced I_{sc} . As shown in Fig 4A, TTX had no effect on OxB-induced inhibition, indicating that neuronal cells do not play a significant role in this effect. We then examined the possible relay of endocrine cells in the action of OxB. We focused our attention to endocrine I cells for three reasons: i) CCK-producing I cells are present in

the vicinity of epithelial cells in duodenum and proximal small intestine (20); ii) endocrine I cells release CCK in response to different stimuli, including orexins (6); iii) CCK is an inhibitor of SGLT1 and subsequently an inhibitor of glucose absorption (12). In order to explore the possible involvement of endocrine I cells and CCK in the response to OxB, we tested the effects of a mixture of CCK1R and CCK2R antagonists in our Ussing chamber model. As shown in Fig 4B, CCKR antagonists did not modify the effect of OxB on glucose-induced *Isc*. These data clearly indicate that OxB does not target enteric neurons or endocrine I cells for inhibiting glucose absorption. Therefore, our hypothesis is that OxB inhibits glucose absorption through direct interaction with OX₁R on enterocytes.

*Analysis of the pathway involved in the inhibitory action of OxA on glucose-induced *Isc*.* We first examined the effect of the neuronal blocker TTX on OxA action. As shown in Fig. 4C, dose-effect curve for OxA was markedly shifted to the right in the presence of TTX. However, for high concentrations of OxA, TTX only partially blocked the effect of OxA, suggesting the existence of a TTX-insensitive component in OxA action.

The TTX-resistant effect of OxA could be due to the activation of the OX₁R identified in enterocytes (see above). We therefore ran additional experiments in which the effect of 100 nmol/l OxA in the presence of TTX was determined with or without the OX₁R antagonist SB334867. Our data clearly indicated that the OX₁R antagonist completely blocked the TTX-insensitive component in the action of OxA : control 24.3

$\pm 1.7 \mu\text{A}/\text{cm}^2$ (n= 4); OxA 100 nmol/L alone : $8.0 \pm 0.8 \mu\text{A}/\text{cm}^2$ (n= 4); OxA 100 nmol/L plus TTX: $17.0 \pm 4.1 \mu\text{A}/\text{cm}^2$ (n= 3); OxA 100 nmol/L plus TTX plus SB334867: $28.3 \pm 4.4 \mu\text{A}/\text{cm}^2$ (n= 3).

The TTX-sensitive component of OxA plays a major role in its inhibitory effect on glucose absorption. The neurochemistry of neurons involved in the action of OxA remains unclear since no information is currently available regarding the expression of orexin receptors in mucosa while OX_1R is reported in myenteric plexus . However, the fact that OxA was shown previously to increase CCK release (6), prompted us to investigate the role of CCK receptor antagonists on OxA action. As shown in Fig 4D, the inhibition of glucose transport induced by OxA was reduced by a mixture of both CCK1R and CCK2R antagonists. To gain further insight into the subtype of CCK receptor involved in this pathway, we set out to determine the sensitivity of a single concentration of 10 nmol/l OxA, which is close to IC_{50} , in presence of either antagonists. As shown in Fig 5, the inhibition of glucose transport induced by 10 nmol/l OxA was completely abolished by the CCK2R antagonist YM022 (1 nmol/l), but not by the CCK1R antagonist L-364718. These data indicate that the OxA effect on intestinal glucose absorption involves a CCK2R. Since this major component of OxA action is not mediated by the OX_1R (see Fig. 3A), it is likely that it involves the other orexin receptor, i.e. OX_2R . This is in agreement with our finding that a specific OX_2R agonist triggers an inhibition of glucose absorption (Fig. 6). In line with the effect of TTX on OxA-induced inhibition, the effect of the OX_2R agonist was sensitive to TTX. Also in accordance with the involvement of CCK2R in this pathway,

inhibition induced by OX₂R agonist was found sensitive to CCK2R antagonist YM022 (Fig.6).

Analysis of OXRs mRNA distribution in jejunal mucosa

Finally, RT-PCR analysis of OxRs in rat jejunal mucosa showed the expression of OX₁R in epithelial cells (Fig 7; lane 5) but not in non-epithelial fraction (Fig 7; lane 7), in line with our functional results. In contrast, OX₂R was found essentially in non-epithelial fraction (Fig 7; lane 7), a localization in good agreement with the expression of OX₂R in neurons. A small amplicon was also found in the epithelial fraction, possibly corresponding to the presence of OX₂R in enteroendocrine cells (Fig 7; lane 5).

Discussion

Our results demonstrate that OxA and OxB acutely inhibit the active absorption of luminal glucose mediated by SGLT-1. This delineates a new function for orexins in the short-term control of enterocyte glucose absorption and provides an original insight into the role of orexins as a link between peripheral energy balance and the CNS (21; 22). As glucose is both an energy-rich molecule and a signal molecule, the initial arrival of glucose in the intestinal lumen and its absorption into enterocytes through SGLT-1 is a major event in the energy homeostasis process. The nature of the peripheral neural circuitry through which homeostatic pathways may be integrated into the regulation of energy balance, appetite, locomotor control and body weight is not yet fully understood. Many recent studies have shown that orexin neurons are sensitive to changes in nutritional status. In normal mice, orexin expression negatively correlates with changes in blood glucose (23). Fasting or insulin-induced hypoglycemia upregulates rat prepro-orexin (1) and orexin mRNA (24) and stimulates c-Fos expression in orexin neurons (25), indicating that changes in circulating glucose concentration can activate orexin neurons. OxA secretion from the endocrine pancreas is also stimulated by low glucose levels (7). Our finding that orexin A and B can induce a short-term modulation of the incoming flux of glucose gives a new insight into the involvement of orexins in glucose regulation.

We found that both orexins reduced significantly blood glucose when administered by i.p. route. In previous studies, it was found that OxA, when given I.V., had no

effect on glycemia (5) or increased it (7). Whether difference in the dose delivered and/or in the route of administration (i.p. vs. I.V.) is concerned remains to be determined.

Uptake of alimentary glucose by SGLT1 is a fundamental process that is regulated by several hormones and peptides. These peptides can lead to a decrease (i.e. leptin (11; 26), CCK (12) or an increase (i.e. adenosine (27), EGF (13), GLP-2 (14), GIP (28) or insulin (15)) in glucose transport. The activity of SGLT-1 and the resulting glycaemia occur in the context of a balance between the activating and inhibitory hormones, peptides and neuropeptides that modulate the activity of the glucose transporter according to food availability. The relevance of the effect of endogenous orexins on glycemia should be considered in this physiological context. This should explain why orexin knockout mice exhibit normal blood glucose (29).

Another important finding is the distinct mode of action of orexins A and B on enterocyte function. Such marked differences in the mechanism of action of orexin peptides have not been reported previously. Indeed, either OxA or OxB were found to be active *in vivo* and *in vitro* with IC₅₀ values in the low nanomolar range and significant activities still being observed in the pM range. In this context, we were able to dissect the pathways involved in orexins actions leading to propose a novel scheme to illustrate how endogenous OxA and OxB inhibits SGLT1-mediated glucose absorption across enterocytes (Figure 8). We found that OxA was sensitive to TTX (indicating the involvement of neuronal cells) and also sensitive to the CCK2R antagonist (indicating the involvement of endocrine I cells). These results may be

explained by several potential mechanisms. First, neurosecretion of OxA could activate OX₂R present on as yet uncharacterized neuronal cell types which, in turn, release an unknown neurotransmitter to activate neuroendocrine I cells to secrete CCK (Figure 8; pathway 1). The nature of the neuronal cells involved in this pathway is unknown and requires further investigation. Second, OxA could directly activate endocrine I cells via OX₂R (Figure 8; pathway 2). This is consistent with a previous report on neuroendocrine STC-1 cells, which express OX₂R and release CCK in response to orexin A (6). Third, OxA could also directly activate efferent CCK-releasing neuronal cells via OX₂R (Figure 8; pathway 3). Such efferent CCK cells have been described in submucosal networks of enteric neurons (30). In our scheme, these pathways are not mutually exclusive. In all cases, CCK released by endocrine I cells is certainly a major mediator of OxA action in the inhibition of SGLT-1 activity. However, the fact that the action of OxA is not completely inhibited by the CCKR antagonist cocktail (Fig. 4C) suggests the possible involvement of minor mediators in this action, the nature of which remains to be established. Here, we further demonstrate that this CCK-mediated effect of OxA involved a CCK₂R subtype. This finding is in agreement with previous data showing that inhibition by leptin of glucose absorption in rat jejunum is mediated by CCK (11).

Conversely, our data indicate that OxB produced and secreted by neuronal cells in the mucosa could directly activate the OX₁R on enterocytes (Figure 8; pathway 4). Indeed, we found that OxB-induced inhibition was not sensitive to TTX, indicating the absence of a neuronal intermediate between the local release of neuropeptide and

its action on epithelial cells. As STC-1 cells have been shown to express both types of orexin receptors, it is conceivable that these cells may also be responsive to OxB. The absence of significant modifications in the effect of OxB in the presence of CCKR antagonists (Figure 4B) indicates that this effect does not involve CCK-releasing cells but rather suggests that it is a direct effect on enterocytes. The inhibitory effect of OxB appears to be mediated by OX₁R, as indicated by the sensitivity of the OxB effect to the OX₁R antagonist SB334867 (Fig. 3B). Analysis of OxR transcripts in the epithelial and non-epithelial compartments of mucosa gave results that were consistent with this global scheme. Indeed, OX₁R was found only in the epithelial fraction that corresponds to enterocytes. In contrast, OX₂R expression was found mainly in the non-epithelial fraction of the mucosa containing neuronal cells, with also a small signal in epithelial fraction that may be ascribed to the presence of endocrine cells within the jejunal epithelium. Another analysis of OXR distribution in the gut by RT-PCR showed the expression of OX₁R mRNA in submucosal and myenteric plexus of the guinea-pig duodenum and rat ileum (31). Whether this discrepancy is related to difference in species or organ distribution remains to be determined.

The nature of the peripheral neural circuitry through which signals from the homeostatic pathways may be integrated into the regulation of energy balance, appetite, locomotor control and body weight is not yet fully understood. Orexin neurons have been recently demonstrated to sense blood glucose levels in both central and peripheral areas (10). Our finding that orexins have a peripheral effect on

glucose entry and its consequences on glucose level *in vivo* suggests a possible link between intestinal entry of glucose and orexin glucosensing neurons in the submucosal plexus that may respond to and integrate signals involved in the regulation of energy homeostasis, resulting in negative feedback and adjustment of energy levels. In line with their recently demonstrated role in glucose sensing in the CNS, orexins appear to be important players in the physiological regulation of peripheral glucose levels. Orexins have been shown to be involved in the central regulation of wakefulness and locomotor activity that support food seeking. There are increasing data to support the concept that reduced food availability has a global stimulatory effect on reward perception (32). By decreasing neuronal input from adiposity signals, energy restriction increases responses to rewarding stimuli. A model has been proposed in which peripheral signals reflecting negative energy balance, such as reduced plasma glucose, induce fasting-related arousal by triggering increased activity of orexin neurons (23). We propose that orexins released by neurons and endocrine cells in the gut, and acting via distinct pathways, are connected to glucose transporter activity to allow sensing and control of incoming levels of glucose (as a part of a glucose “sensing” cell network that includes the pancreatic cells (7) and duodenal SGLT-3 sensing (33)). Modulation of glucose levels through this postulated loop, in close relationship with other hormones and neuropeptides (*i.e.* leptin, CCK, ghrelin), could in turn result in an increase in neuronal activity (32) and in production/secretion of peripheral orexin (7).

In conclusion, our data provide a rational basis for orexin-induced short-term regulation of intestinal glucose transport. Together with the recent findings that orexin neurons are specialised cells for sensing blood glucose in the hypothalamus (10), our results indicate that orexins can also modulate peripheral glucose disposal. In addition, they suggest that orexins are also involved in a regulatory loop between enteric glucose entry and central evaluation of peripheral available energy levels, enlarging the group of gut peptides involved in energy homeostasis (34). Further studies are needed to evaluate this possible role of orexins in pathologies associated with anomalies in energy homeostasis including obesity and diabetes.

Acknowledgements

We would like to thank Emma Pilling for re-reading of the manuscript and Jean-Pierre Laigneau for drawing Figures. A.E.F. is supported by Ministère de l'Éducation et de la Recherche (an MRT fellowship). This study was presented in part at the 21st meeting of European Intestinal Transport Group held in Oberwiesenthal, Germany on 3-6 march, 2007.

Legends to Figures

Figure 1. Oral glucose tolerance test (1 g/kg) in rats. Rats were injected i.p. with saline (control, ○), or with 55 µg/kg OxA (●) or OxB (■) five min before they were challenged by oral administration of a 30% D-glucose solution. Results are presented as mean ± SEM. n= 6-10. *P<0.05. Area under the curve (insert) is expressed in arbitrary units (AU).

Figure 2. Effect of OxA and OxB on glucose-induced short-circuit current (Isc). A. Typical recording of Isc (in µA/cm²) across rat jejunum mounted in Ussing chamber. OxA (or OxB, not shown) diluted in saline was added serosally two minutes before luminal glucose challenge (10 mmol/l); maximal increase in Isc measured at plateau was taken as an index of SGLT-1 activity. B. Non cumulative dose-response curves to OxA (○) or OxB (□) in reducing glucose-induced Isc. number of tissues studied : 5-7.

Figure 3. Effect of OX₁R-antagonist SB334867 on inhibition of glucose-induced Isc triggered by OxA or OxB. (A) Non cumulative dose-effect curves for OxA alone (○) or in presence of 5 µmoles/l SB334867 (●) (B) Non cumulative dose-effect curves for OxB alone (□) or in presence of 5 µmoles/l SB334867 (■). No significant effect of the OX₁R antagonist was observed for OxA. By contrast, the antagonist markedly

inhibited the inhibitory response induced by OxB at all concentrations. n = 5-7; *, P<0.05.

Figure 4. Effect of tetrodotoxin (A, C) and CCK receptor antagonists (B, D) on OxB- or OxA-induced inhibition of glucose transport. A: Non-cumulative dose-effect curves to OxB alone (□) or in presence (■) of tetrodotoxin (TTX, 5 μmoles/l). (B) Dose-effect curves to OxB alone (□) or in presence (▣) of CCK2R antagonist YM022 (1 nmoles/l) plus CCK1R antagonist L-364,718 (1 nmoles/l). (C) Non-cumulative dose-effect curves to OxA alone (○) or in presence (●) of TTX; *, P<0.05. (D) Dose-effect curves to OxA alone (○) or in presence (●) of CCK2R antagonist. Number of tissues studied = 5-8

Figure 5. Effect of CCK receptor antagonists on OxA-induced inhibition of glucose transport. Inhibitory effect of OxA (10 nmoles/l) was studied in presence of CCK₁ receptor antagonist, L-364,718 (1 nmoles/l) or CCK₂ receptor antagonist, YM022 (1 nmoles/l). n=4 - 5 different tissues. YM022 alone had no effect. *, P<0.05.

Figure 6. Effect of the OX₂R agonist Ala¹¹,D-Leu¹⁵OxB on inhibition of glucose transport. Inhibitory effect of OX₂R agonist (10 nmoles/l) was studied alone or in presence of TTX (5 μmoles/l) or in the presence of CCK2 receptor antagonist, YM022 (1 nmoles/l). n= 5-7; *, P<0.05 vs control.

Figure 7. RT-PCR analysis of OxR expression in epithelial and non-epithelial fractions of rat jejunal mucosa. OX₁R (top panel), OX₂R (middle panel) amplicon from CHO/OX₁R cells and CHO/OX₂R (positive controls) are shown in lane 1 and 3, respectively. Expression of OX₁R transcripts was found only in epithelial cells fractions (lane 5), whereas expression of OX₂R transcripts was found mainly in non-epithelial cells (lane 7) with a small positive signal in epithelial cell fraction (lane 5) which may be ascribed to the presence of enteroendocrine cells in the epithelial fraction. Bottom panel : expression of GAPDH transcripts. Lane 2, 4, 6, 8 : negative controls of RT. Lane 9 : negative control of PCR.

Figure 8. Schematic drawing of inhibitory pathways involved in OxA and OxB inhibition of glucose absorption by enterocyte. SB334867 : specific antagonist of OX₁R; TTX : neuronal blocker tetrodotoxin; YM022 : CCK2 receptor antagonist.

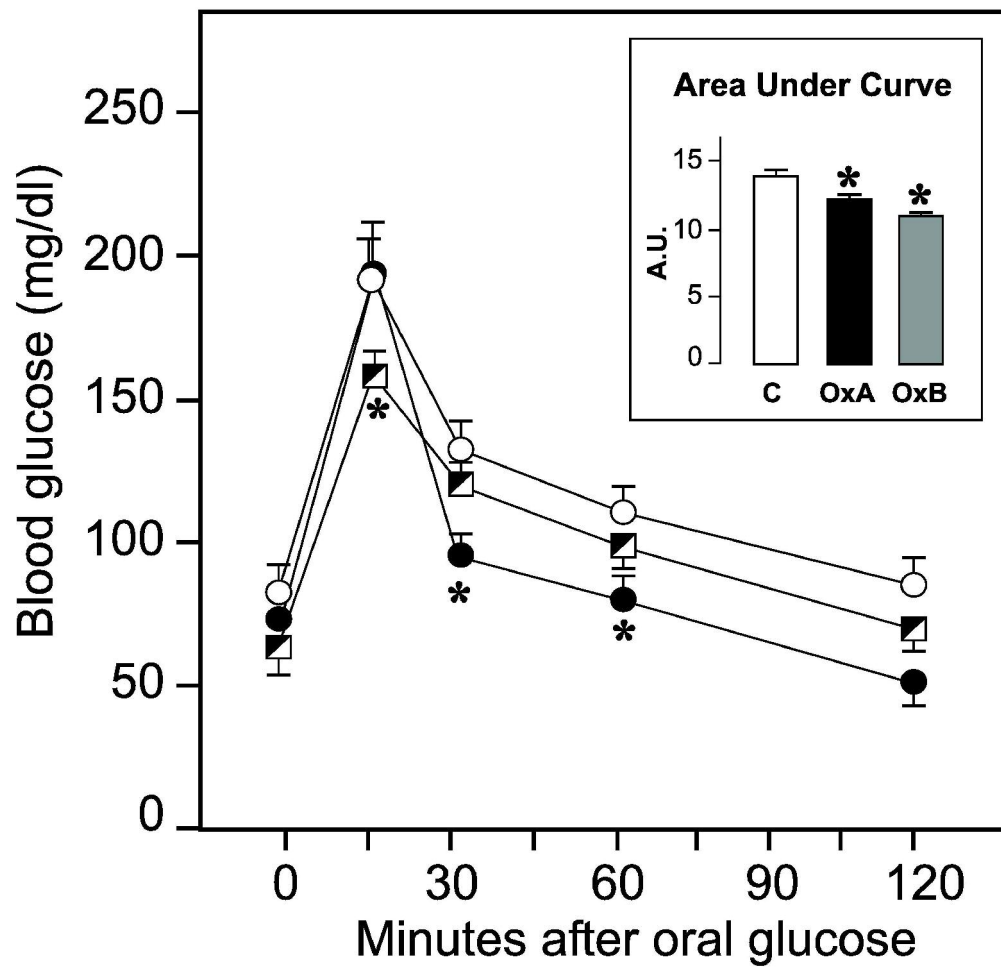
References

1. Sakurai T, Amemiya A, Ishii M, Matsuzaki I, Chemelli RM, Tanaka H, Williams SC, Richardson JA, Kozlowski GP, Wilson S, Arch JR, Buckingham RE, Haynes AC, Carr SA, Annan RS, McNulty DE, Liu WS, Terrett JA, Elshourbagy NA, Bergsma DJ, Yanagisawa M: Orexins and orexin receptors: a family of hypothalamic neuropeptides and G protein-coupled receptors that regulate feeding behavior. *Cell* 92:573-585, 1998
2. de Lecea L, Kilduff TS, Peyron C, Gao X, Foye PE, Danielson PE, Fukuhara C, Battenberg EL, Gautvik VT, Bartlett FS, 2nd, Frankel WN, van den Pol AN, Bloom FE, Gautvik KM, Sutcliffe JG: The hypocretins: hypothalamus-specific peptides with neuroexcitatory activity. *Proc Natl Acad Sci U S A* 95:322-327, 1998
3. Voisin T, Rouet-Benzineb P, Reuter N, Laburthe M: Orexins and their receptors: structural aspects and role in peripheral tissues. *Cell Mol Life Sci* 60:72-87, 2003
4. Kirchgessner AL, Liu M: Orexin synthesis and response in the gut. *Neuron* 24:941-951, 1999
5. Ehrstrom M, Gustafsson T, Finn A, Kirchgessner A, Gryback P, Jacobsson H, Hellstrom PM, Naslund E: Inhibitory effect of exogenous orexin a on gastric emptying, plasma leptin, and the distribution of orexin and orexin receptors in the gut and pancreas in man. *J Clin Endocrinol Metab* 90:2370-2377, 2005
6. Larsson KP, Akerman KE, Magga J, Uotila S, Kukkonen JP, Nasman J, Herzig KH: The STC-1 cells express functional orexin-A receptors coupled to CCK release. *Biochem Biophys Res Commun* 309:209-216, 2003
7. Ouedraogo R, Naslund E, Kirchgessner AL: Glucose regulates the release of orexin-a from the endocrine pancreas. *Diabetes* 52:111-117, 2003

8. Burdakov D, Luckman SM, Verkhatsky A: Glucose-sensing neurons of the hypothalamus. *Philos Trans R Soc Lond B Biol Sci* 360:2227-2235, 2005
9. Burdakov D, Gerasimenko O, Verkhatsky A: Physiological changes in glucose differentially modulate the excitability of hypothalamic melanin-concentrating hormone and orexin neurons in situ. *J Neurosci* 25:2429-2433, 2005
10. Burdakov D, Jensen LT, Alexopoulos H, Williams RH, Fearon IM, O'Kelly I, Gerasimenko O, Fugger L, Verkhatsky A: Tandem-pore K⁺ channels mediate inhibition of orexin neurons by glucose. *Neuron* 50:711-722, 2006
11. Ducroc R, Guilmeau S, Akasbi K, Devaud H, Buyse M, Bado A: Luminal leptin induces rapid inhibition of active intestinal absorption of glucose mediated by sodium-glucose cotransporter 1. *Diabetes* 54:348-354, 2005
12. Hirsh AJ, Cheeseman CI: Cholecystokinin decreases intestinal hexose absorption by a parallel reduction in SGLT1 abundance in the brush-border membrane. *J Biol Chem* 273:14545-14549, 1998
13. Chung BM, Wong JK, Hardin JA, Gall DG: Role of actin in EGF-induced alterations in enterocyte SGLT1 expression. *Am J Physiol* 276:G463-469, 1999
14. Cheeseman C: Upregulation of SGLT-1 transport activity in rat jejunum induced by GLP-2 infusion in vivo. *Am J Physiol* 273:R1965-1971, 1997
15. Stumpel F, Kucera T, Gardemann A, Jungermann K: Acute increase by portal insulin in intestinal glucose absorption via hepatoenteral nerves in the rat. *Gastroenterology* 110:1863-1869, 1996
16. Dyer J, Wood IS, Palejwala A, Ellis A, Shirazi-Beechey SP: Expression of monosaccharide transporters in intestine of diabetic humans. *Am J Physiol* 282:G241-248, 2002

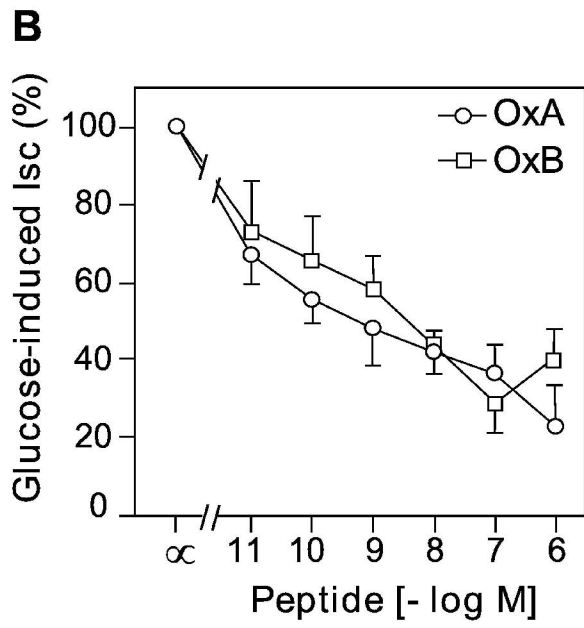
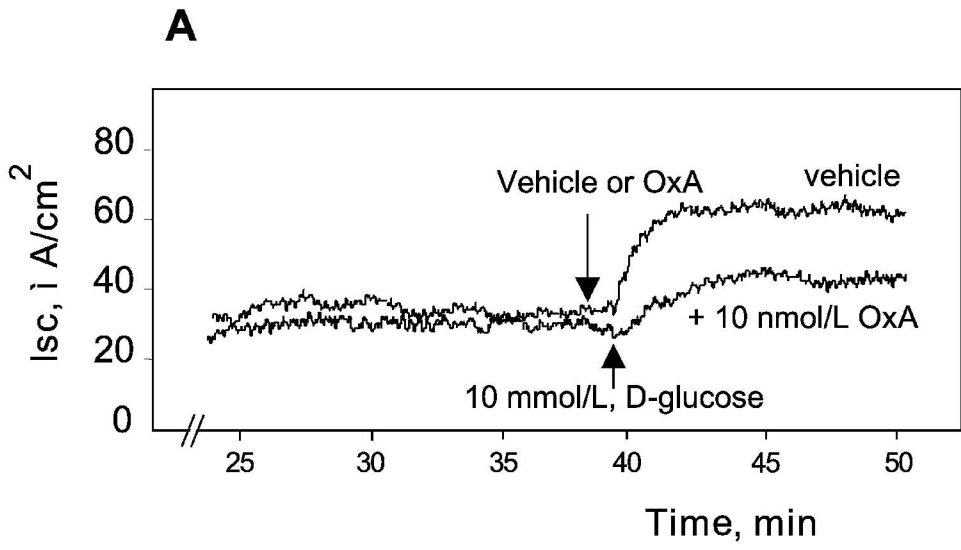
17. Goumain M, Voisin T, Lorinet AM, Laburthe M: Identification and distribution of mRNA encoding the Y1, Y2, Y4, and Y5 receptors for peptides of the PP-fold family in the rat intestine and colon. *Biochem Biophys Res Commun* 247:52-56, 1998
18. Laburthe M, Rouyer-Fessard C, Gammeltoft S: Receptors for insulin-like growth factors I and II in rat gastrointestinal epithelium. *Am J Physiol* 254:G457-462, 1988
19. Stumpel F, Burcelin R, Jungermann K, Thorens B: Normal kinetics of intestinal glucose absorption in the absence of GLUT2: evidence for a transport pathway requiring glucose phosphorylation and transfer into the endoplasmic reticulum. *Proc Natl Acad Sci U S A* 98:11330-11335, 2001
20. Ritter RC, Brenner LA, Tamura CS: Endogenous CCK and the peripheral neural substrates of intestinal satiety. *Ann N Y Acad Sci* 713:255-267, 1994
21. Sakurai T: Roles of orexins and orexin receptors in central regulation of feeding behavior and energy homeostasis. *CNS Neurol Disord Drug Targets* 5:313-325, 2006
22. Sakurai T: Orexin: a link between energy homeostasis and adaptive behaviour. *Curr Opin Clin Nutr Metab Care* 6:353-360, 2003
23. Yamanaka A, Beuckmann CT, Willie JT, Hara J, Tsujino N, Mieda M, Tominaga M, Yagami K, Sugiyama F, Goto K, Yanagisawa M, Sakurai T: Hypothalamic orexin neurons regulate arousal according to energy balance in mice. *Neuron* 38:701-713, 2003
24. Griffond B, Risold PY, Jacquemard C, Colard C, Fellmann D: Insulin-induced hypoglycemia increases preprohypocretin (orexin) mRNA in the rat lateral hypothalamic area. *Neurosci Lett* 262:77-80, 1999
25. Moriguchi T, Sakurai T, Nambu T, Yanagisawa M, Goto K: Neurons containing orexin in the lateral hypothalamic area of the adult rat brain are activated by insulin-induced acute hypoglycemia. *Neurosci Lett* 264:101-104, 1999

26. Lostao MP, Urdaneta E, Martinez-Anso E, Barber A, Martinez JA: Presence of leptin receptors in rat small intestine and leptin effect on sugar absorption. *FEBS Lett* 423:302-306, 1998
27. Kimura Y, Turner JR, Braasch DA, Buddington RK: Luminal adenosine and AMP rapidly increase glucose transport by intact small intestine. *Am J Physiol Gastrointest Liver Physiol* 289:G1007-1014, 2005
28. Cheeseman CI, Tsang R: The effect of GIP and glucagon-like peptides on intestinal basolateral membrane hexose transport. *Am J Physiol* 271:G477-482, 1996
29. Chemelli RM, Willie JT, Sinton CM, Elmquist JK, Scammell T, Lee C, Richardson JA, Williams SC, Xiong Y, Kisanuki Y, Fitch TE, Nakazato M, Hammer RE, Saper CB, Yanagisawa M: Narcolepsy in orexin knockout mice: molecular genetics of sleep regulation. *Cell* 98:437-451, 1999
30. Larsson LI, Rehfeld JF: Localization and molecular heterogeneity of cholecystokinin in the central and peripheral nervous system. *Brain Res* 165:201-218, 1979
31. Kirchgessner AL: Orexins in the brain-gut axis. *Endocr Rev* 23:1-15, 2002
32. Morton GJ, Cummings DE, Baskin DG, Barsh GS, Schwartz MW: Central nervous system control of food intake and body weight. *Nature* 443:289-295, 2006
33. Freeman SL, Bohan D, Darcel N, Raybould HE: Luminal glucose sensing in the rat intestine has characteristics of a sodium-glucose cotransporter. *Am J Physiol* 291:G439-445, 2006
34. Murphy KG, Bloom SR: Gut hormones and the regulation of energy homeostasis. *Nature* 444:854-859, 2006

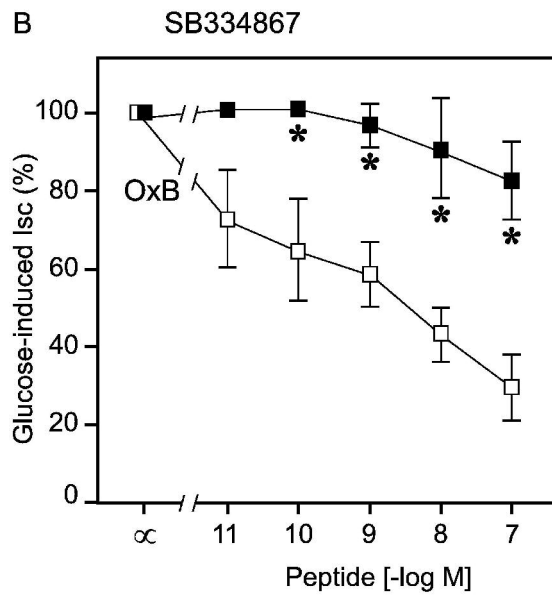
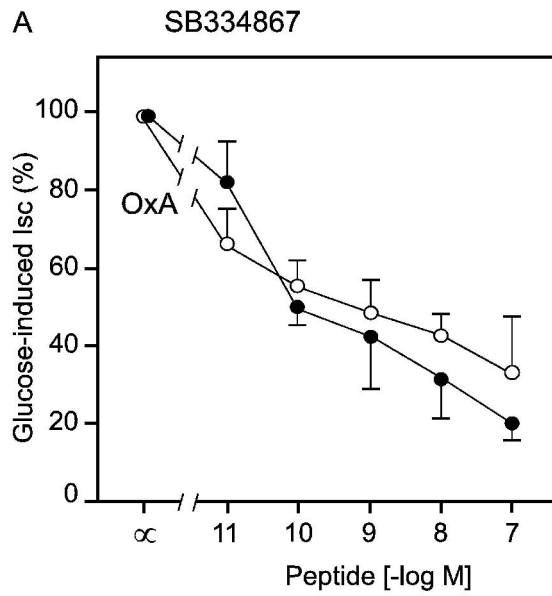


129x124mm (600 x 600 DPI)

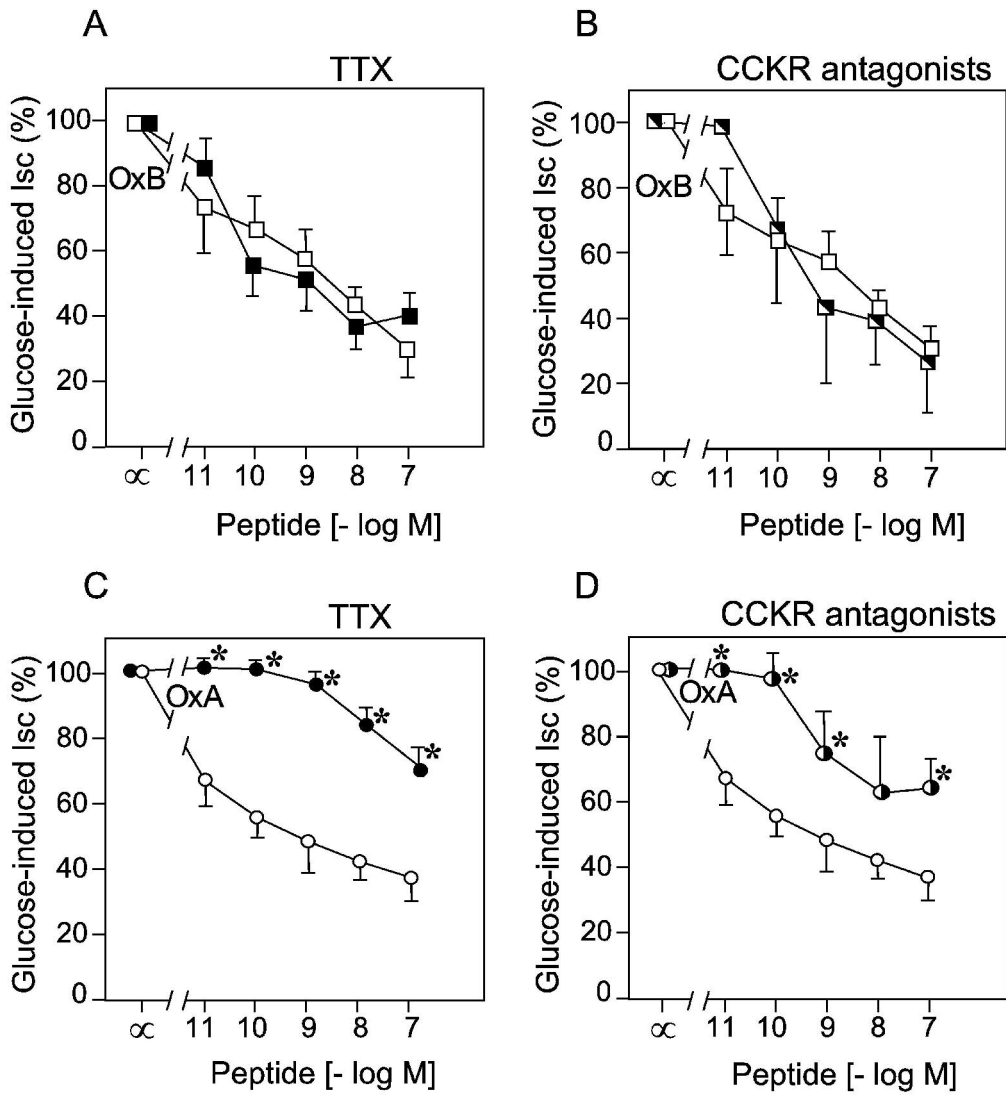
Only



138x173mm (600 x 600 DPI)

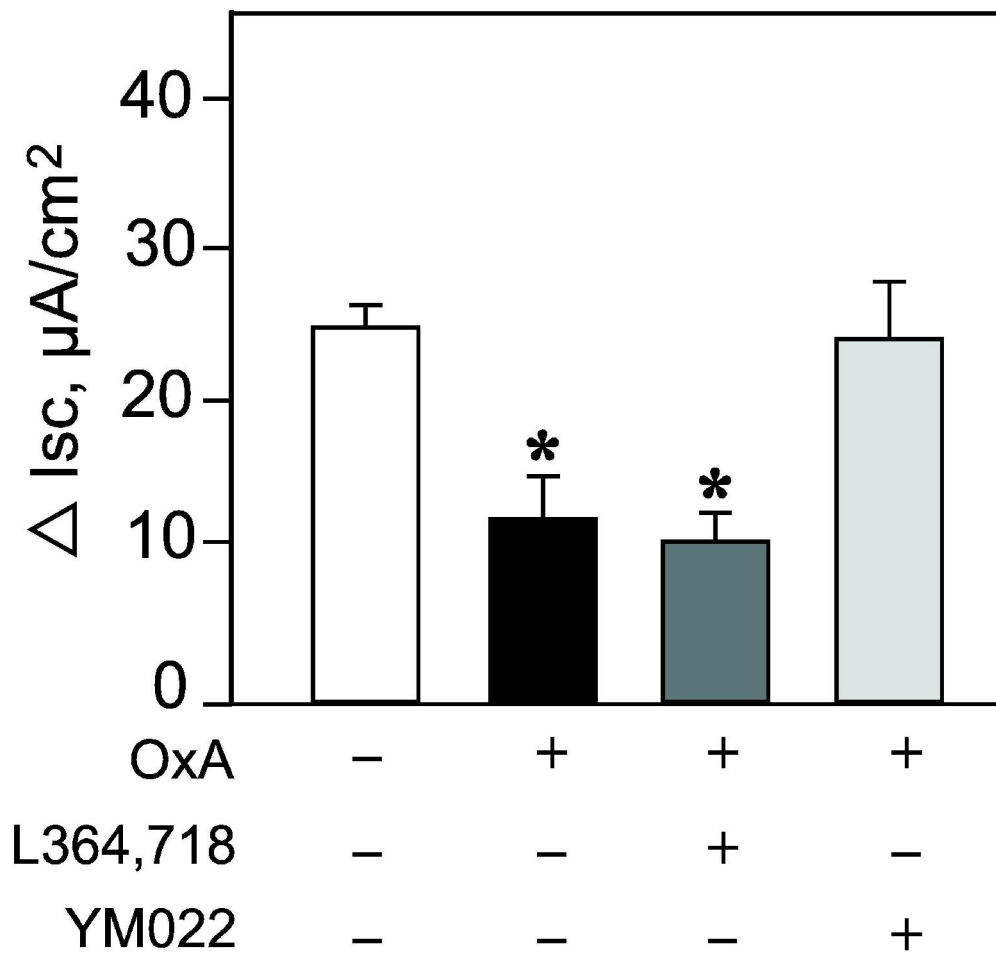


93x202mm (600 x 600 DPI)



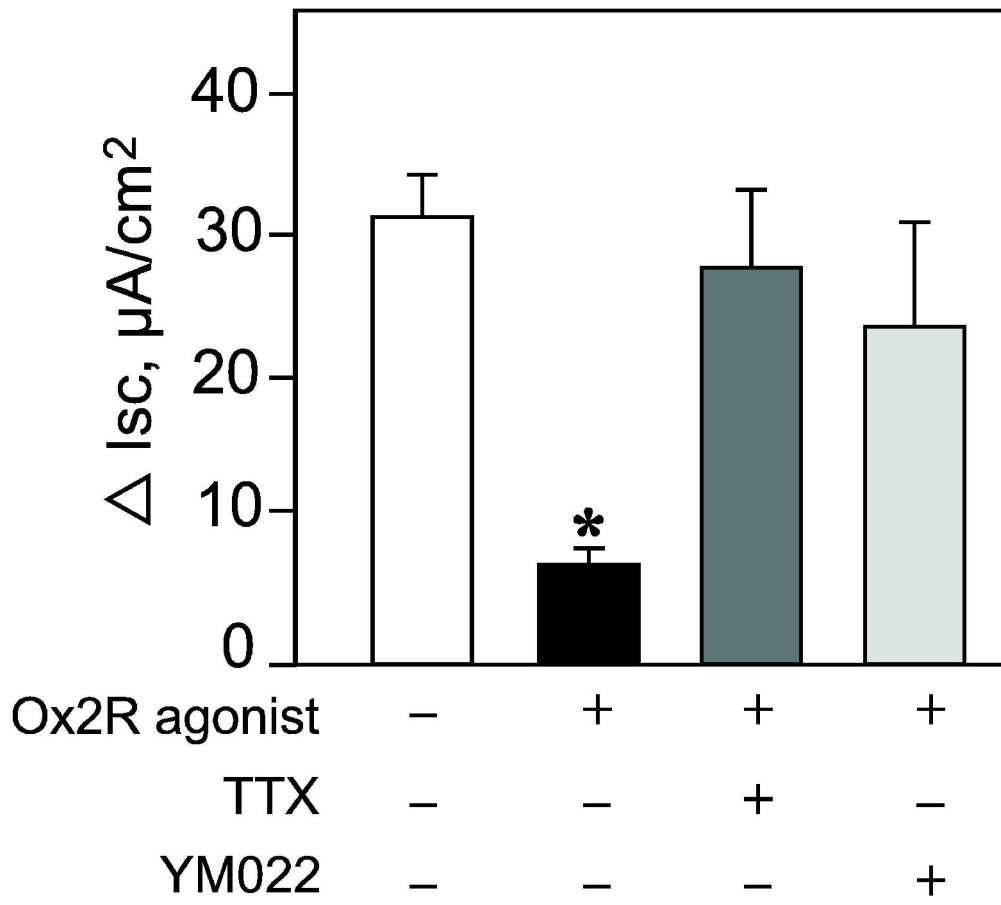
152x165mm (600 x 600 DPI)





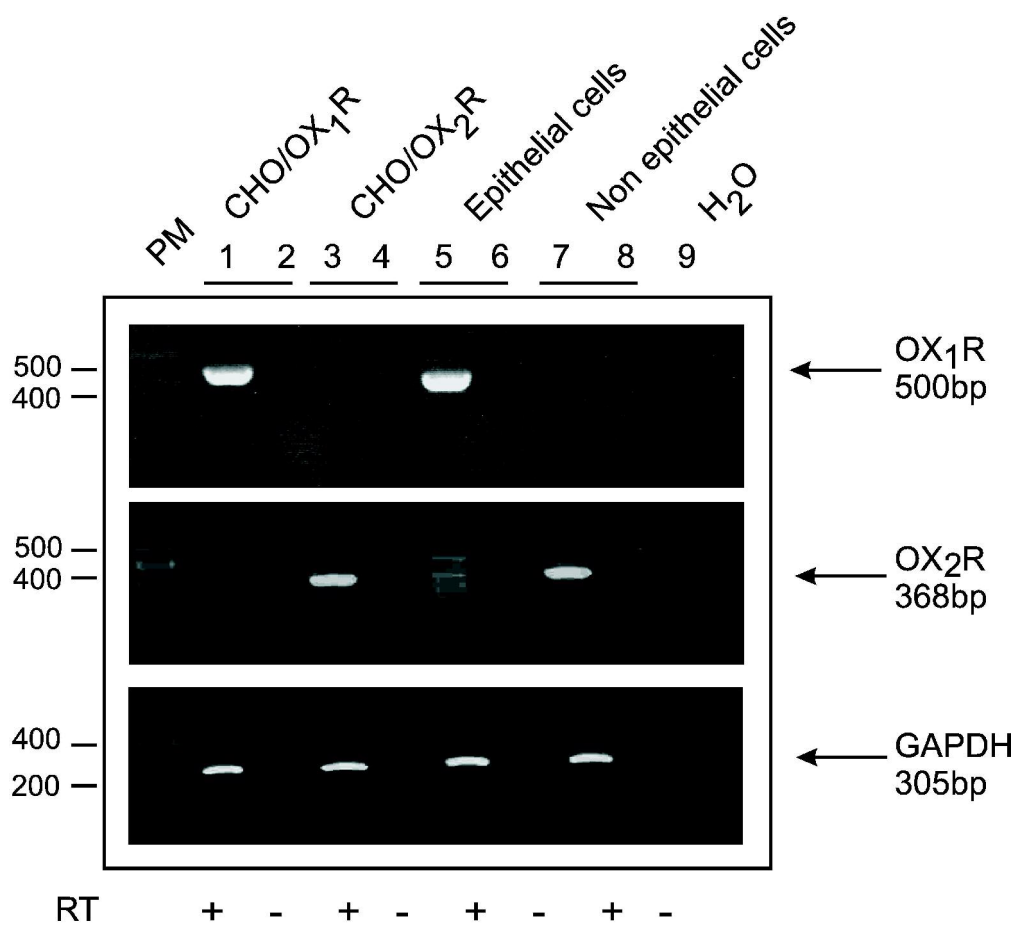
117x111mm (600 x 600 DPI)

Only



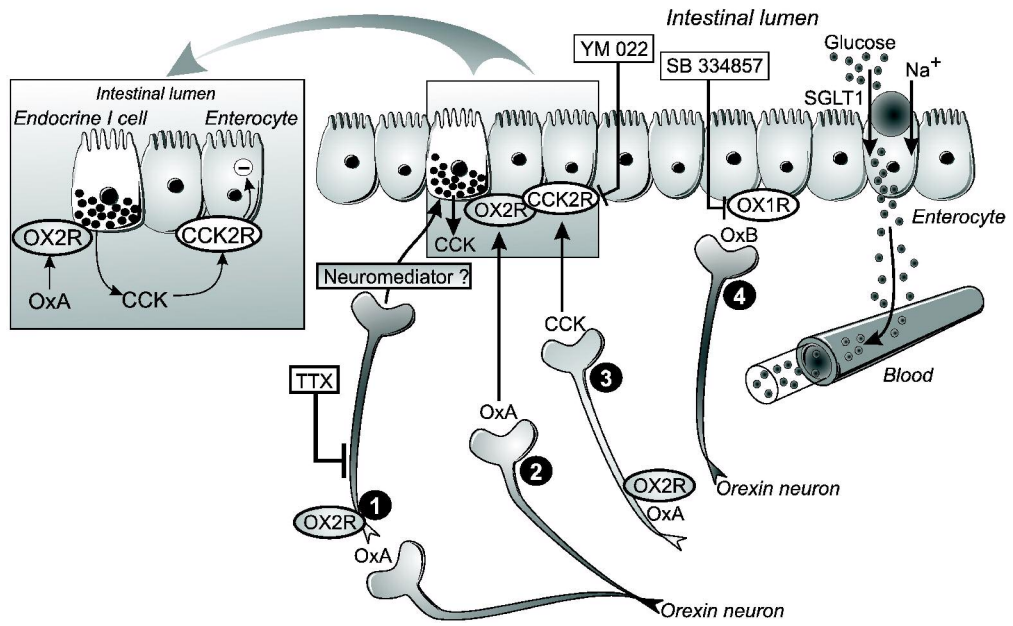
124x111mm (600 x 600 DPI)

Only



168x153mm (600 x 600 DPI)

Only



189x120mm (600 x 600 DPI)

Review Only