

**Oxyntomodulin increases intrinsic heart rate in mice independent of the glucagon-like peptide-1 receptor.**

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**Keywords:** GLP-1R, Glucagon receptor, Core body temperature, Exendin-4, Insulin, food intake, Mean arterial pressure, rats, Activity

**Running title:** OXM and heart rate

## Abstract

Oxyntomodulin (OXM), a postprandially released intestinal hormone, inhibits food intake via the glucagon-like peptide-1 receptor (GLP-1R). While OXM may have clinical value in treating obesity, the cardiovascular effects of OXM are not well understood. Using telemetry to measure heart rate (HR), body temperature ( $T_b$ ) and activity in conscious and freely moving mice, we tested 1) whether OXM affects HR, and 2) whether this effect is mediated by the GLP-1R. We found that peripherally administered OXM significantly increased HR in wildtype mice, raising HR by over 200 bpm to a maximum of  $728 \pm 11$  bpm. To determine the extent to which the sympathetic nervous system (SNS) mediates the tachycardia of OXM, this hormone was delivered to mice deficient in dopamine  $\beta$  hydroxylase (*Dbh*  $-/-$  mice), littermate controls (*Dbh*  $+/-$  mice), and autonomically-blocked C57Bl mice. OXM increased HR equally in all groups ( $192 \pm 13$ ,  $197 \pm 21$ ,  $216 \pm 11$  bpm, respectively), indicating that OXM elevated intrinsic HR. Intrinsic HR was also vigorously elevated by OXM in *Glp-1R*  $-/-$  mice ( $200 \pm 28$  bpm). In addition, peripherally-administered OXM inhibited food intake and activity levels in wildtype mice, and lowered  $T_b$  in autonomically blocked mice. None of these effects were observed in *Glp-1R*  $-/-$  mice. These data suggest multiple modes of action of OXM: 1) it directly elevates murine intrinsic HR through a GLP-1R independent mechanism, perhaps via the glucagon receptor or an unidentified OXM receptor, and 2) it lowers food intake, activity, and  $T_b$  in a GLP-1R dependent fashion.

## Introduction

OXM is a 37 amino acid peptide, containing the entire 29 amino acid sequence of glucagon, followed by an 8 amino acid carboxyterminal extension (17). OXM was first characterized in 1982 (7), and is so named after its ability to inhibit gastric acid secretion in gastric oxyntic glands (6, 24, 31, 32). Belonging to a family of hormones known as proglucagon derived peptides (PGDPs), OXM originates from the proglucagon gene, which is expressed in the L-cells of the small intestine, the pancreas and the central nervous system (CNS) (47). The proglucagon gene product is posttranslationally cleaved into different PGDPs depending on tissue type. While glucagon is the primary cleavage product of proglucagon in the pancreas, OXM and GLP-1 are the major cleavage products in the brain and gut (22).

OXM inhibits food intake and causes weight loss when administered peripherally in humans, sparking interest in the use of OXM as a potential therapy for obesity (11, 17, 45, 46). Further, central administration of OXM into rodents can also inhibit food intake (2, 13, 14). OXM has a weak affinity for both the glucagon receptor (GCGR) (2, 6) and the GLP-1R (13). The GLP-1R is necessary for the anorectic effects of OXM (2) as well as the anorectic effects of other GLP-1R agonists, namely GLP-1 and Exendin-4 (Ex-4) (2, 13, 14). The GLP-1 receptor is widely expressed, being found centrally in the hypothalamus (34, 42) and peripherally in the pancreas, heart, lung, brain, kidney and gastrointestinal tract (8, 44). Although no specific OXM receptor has been identified, a separate OXM receptor may exist, as the pattern of neuronal activation observed after administration of peripheral OXM differs from the pattern seen after peripheral administration of GLP-1 (14).

While little is known about the cardiovascular effects of OXM, hormones similar to OXM, namely GLP-1 and Ex-4, can regulate the cardiovascular system in two distinct ways. First, central administration of GLP-1 and Ex-4 elevate HR and blood pressure through activation of preganglionic sympathetic neurons (3, 28, 48). Second, GLP-1 can have a direct impact on the heart, elevating HR independent of catecholamines (4). Indeed, GLP-1 elevates intracellular cAMP in target cells that express the GLP-1R, including rat heart cells (43). Further, a protein similar to OXM isolated from eel gut was found to increase beating rate and atrium contractile force in a dose dependent manner when incubated with isolated eel heart, an effect that was not inhibited by the  $\beta 1$  adrenergic receptor antagonist betaxonal (41).

In the absence of any nervous or hormonal influences, the murine heart will beat at approximately 510 beats/min (23). This intrinsic HR is modified by the activity of parasympathetic and/or sympathetic nerves at the sino-atrial node, which lower or raise HR, respectively. In addition to the tachycardia mediated via activation of the sympathetic nervous system (SNS) in behaviors such as stress or escape (36), peripheral hormones such as insulin increase SNS outflow and elevate HR (26). Importantly, OXM administration has been shown to stimulate insulin secretion (25). In addition to autonomic control of HR, certain hormones, such as thyroid hormone and prostanoids, can influence intrinsic HR directly (18, 30, 37). We hypothesized that peripherally administered OXM would increase HR in mice in a GLP-1R dependent manner by increasing intrinsic HR. To test whether OXM influences HR directly in a GLP-1R dependent manner, we used radiotelemetry to measure the HR,  $T_b$ , and activity responses to OXM in A) wildtype mice with chemically-induced type I diabetes, B) mice lacking

the sympathetic neurotransmitters norepinephrine and epinephrine due to deficiency in the enzyme dopamine  $\beta$  hydroxylase (*Dbh*  $-/-$  mice) (39), C) mice deficient in the GLP-1R (*Glp-1R*  $-/-$  mice) (33), and D) mice treated with pharmacological agents to block autonomic signaling at the heart.

## Materials and Methods

### Animals

Female C57Bl mice weighing 22-24 g (3 months old) were obtained from Harlan and used in OXM-dose response experiments, as well as the streptozotocin (STZ)-induced diabetes experiment. Female *Dbh*<sup>-/-</sup> mice and *Dbh*<sup>+/-</sup> controls (3-4 months old) were shipped from Emory University. *Glp-1R*<sup>-/-</sup> mice, approximately 6 months old, were shipped from University of Toronto to Williams College for physiological assessment. Age-matched female C57Bl mice (from Harlan) served as controls for *Glp-1R*<sup>-/-</sup> mice. Female Sprague-Dawley rats were obtained from Harlan. Upon receipt, all of the animals were housed at 28-30 °C and maintained on a 12 hr light/dark cycle. Studies were approved by each of the local IACUCs.

### Reagents

Porcine OXM and Ex-4 were obtained from California Peptide Research Inc. (Napa, CA). Metoprolol, atropine, dobutamine, glucagon, and STZ were obtained from Sigma (St. Louis).

### Implantation of EKG and blood pressure telemeters

Mice were anesthetized initially with 5% isoflurane in an oxygen stream, and maintained on 2-3% isoflurane. Mice were kept on a heating pad (38 °C) throughout implantation of the EKG telemeter (ETAF20; Data Sciences International) in the abdominal cavity and subcutaneous placement of the EKG leads. Some C57Bl mice (n=6) were implanted with a blood pressure telemeter (PAC20; Data Sciences International) as described previously (35). Implantation of the blood pressure telemeter (PAC40; Data Sciences International) into the abdominal aorta of rats was performed as

described previously (35). Mice and rats were maintained on a heating pad for 48 hours following the surgery, and then housed individually at 28-30 °C for one week to allow time for recovery.

#### Cardiovascular, temperature, and activity data collection

Data from mouse EKG and rat blood pressure telemeters were recorded at 500 Hz. HR, body temperature ( $T_b$ ), and activity levels were collected every minute for 5 seconds for the hour preceding and two hours following each injection of peptide or vehicle.

Activity levels were measured as described previously (35, 36) using software from Data Sciences International, which calculates activity from the change in signal strength coming from the telemeter as the animal moves about the cage. Ambient temperature was maintained at 28-30 °C for the entire duration of the experiments.

#### Experimental protocols

For analysis of the effect of OXM and Ex-4 on HR,  $T_b$ , and activity, animals were injected IP with either vehicle (saline) or peptide (dissolved in saline) at the onset of the light cycle. Food was freely available to the mice at all times, unless otherwise noted. Animals were injected alternately such that half of the animals received saline and half received peptide on any given day. If mice were injected with peptide on day one, they were injected with saline on day two (and vice versa) to allow for paired comparisons between effects of peptide and saline. Unless stated otherwise, mice were injected with either 15 µg OXM, 2.5 µg Ex-4, or saline. Rats were injected with 100 µg OXM, 7.5 µg Ex-4, or saline. These dosages were chosen based on previous reports of the effects of these hormones on feeding and blood pressure (2, 4, 14). The doses chosen for the dose-response glucagon were based on the effects of OXM. Type 1 diabetes mellitus was

induced in C57Bl mice by a single IP injection of STZ (200 mg/kg body wt). The effects of OXM and saline were tested ten days after STZ treatment. An autonomic block was induced by IP injection of metoprolol (12 mg/kg) and atropine (5 mg/kg) twenty minutes before administration of OXM or saline. A SNS block was confirmed by the absence of a HR response to IP injection of dobutamine (1 mg/kg), a specific  $\beta_1$  adrenergic receptor agonist.

#### Blood samples and assays

Approximately 0.5 ml blood was drawn directly from the heart of anesthetized STZ-treated mice, which were then immediately sacrificed. Blood was centrifuged at 10,000g at 4°C for five minutes and plasma was stored at -80°C. Plasma glucose was measured with a glucose oxidase kit (Sigma) while insulin was measured with an ELISA kit with a detection limit of 0.2 ng/ml (LINCO research).

#### Feeding Experiment

For analysis of food intake, *Glp-1R*  $-/-$  mice and C57Bl mice were housed at 28-30 °C and fasted for 15 hours, consisting of the last 3 hours of the light cycle and the entire dark cycle. Food was given at the onset of the light cycle immediately after intraperitoneal (IP) administration of peptides or vehicle, and weighed at regular time intervals thereafter. Experiments were conducted in 3 separate trials, with at least 4 days between trials, such that every mouse was injected with OXM (22  $\mu$ g), Ex-4 (2.5  $\mu$ g) and saline in a randomized fashion. As a result, each animal acted as her own control, which allowed for paired comparisons between the effects of peptides and saline.

#### Statistics



All results are reported as means  $\pm$  SE. Paired t-tests were used to compare light and dark cycle averages of HR,  $T_b$ , and activity. Unpaired t-tests were used to compare baseline parameters between the two groups. Both sets of tests were corrected for multiple statistical analysis using a Bonferroni procedure. OXM effects on HR, blood pressure,  $T_b$ , and activity were statistically assessed using a repeated measures ANOVA, with a 2x2 design, followed by a post-hoc Bonferroni test for statistical significance. A repeated measures ANOVA, with a 3x2 design, was used to assess differences between saline, Ex-4, and OXM. This was also followed by a Bonferroni post-hoc test. Significance levels of  $P < 0.05$  were accepted.

## Results

### Peripheral OXM dose-dependently elevates HR in wildtype mice

HR increased after administration of vehicle, from baseline levels of  $473 \pm 11$  bpm to  $605 \pm 25$  bpm. OXM at all doses tested ( $1.5 \mu\text{g}$ ;  $n=11$ ;  $15 \mu\text{g}$ ;  $n=9$ ;  $45 \mu\text{g}$ ;  $n=4$ ) elevated HR above vehicle ( $p<0.05$ ) five minutes after administration of peptide or vehicle, reaching a maximum of  $728 \pm 11$  bpm (Figure 1). HR was significantly elevated above saline for 40 minutes after administration of  $1.5 \mu\text{g}$  OXM, for 60 minutes after administration of  $15 \mu\text{g}$  OXM and for 80 minutes after administration of  $45 \mu\text{g}$  OXM (Figure 1).

### OXM-induced tachycardia is independent of insulin

To test the hypothesis that the tachycardia induced by OXM was mediated through insulinotropic effects, the same C57Bl mice used in the dose response experiment ( $n=9$  at the  $15 \mu\text{g}$  dose) were treated with STZ to induce a diabetic state. STZ-treated mice had undetectable levels of plasma insulin in the fed state (data not shown) and plasma glucose levels of  $384 \pm 40$  mg/dL. Baseline HRs in both the dark and light cycles were lower in these diabetic mice than before treatment with STZ (Table 1 and Figure 2), as has been seen previously in rats (21). STZ-treated mice had a lower  $T_b$  and were less active than before treatment, but displayed circadian differences in each of these measurements (Table 1). Administration of OXM elevated HR by over 200 bpm in these diabetic mice to  $542 \pm 23$  bpm (Figure 2).

### OXM elevates intrinsic HR

To determine whether the effects of OXM were mediated via the SNS, the effect of OXM on HR was determined in *Dbh*<sup>-/-</sup> mice ( $n=6$ ), which lack the SNS transmitters norepinephrine and epinephrine (39), and their littermate controls (*Dbh* <sup>+/-</sup> mice,  $n=6$ ).

Baseline average HR,  $T_b$ , and activity obtained the day previous to the injection are shown in Table 1. As we have shown previously (36), *Dbh*<sup>-/-</sup> mice exhibit a much lower HR than littermate controls during both the dark and light cycles (Table 1). Also, we now show that these mice are cooler than littermate controls during the dark cycle, and do not display the normal circadian rhythm of HR, activity, or  $T_b$  (Table 1). Administration of vehicle to *Dbh*<sup>-/-</sup> mice had no effect on HR (Figure 3A), indicating the lack of a HR response to animal handling. However, administration of OXM increased HR in *Dbh*<sup>-/-</sup> mice by  $192 \pm 13$  bpm (Figure 3A). This increase is similar to the increase in HR observed in *Dbh*<sup>+/-</sup> mice after OXM administration ( $197 \pm 21$  bpm). To confirm that OXM works independently of the SNS in wildtype mice, we investigated the effects of OXM in mice in which both sympathetic and parasympathetic input to the heart was chemically blocked. A new set of 6-month old C57Bl mice (n=6) were treated with metoprolol, a  $\beta_1$  adrenergic receptor antagonist, and atropine, a muscarinic receptor antagonist, 20 minutes prior to peptide or vehicle administration. Blocking both inputs of the autonomic nervous system (ANS) to the heart resulted in an intrinsic HR of  $525 \pm 11$  bpm, similar to the intrinsic HR of mice measured by others (23). As shown in figure 3B, OXM increased intrinsic HR by  $181 \pm 9$  bpm. As a control, autonomically-blocked mice were injected either with dobutamine, a  $\beta_1$  receptor agonist, or vehicle. Neither of these affected HR in autonomically-blocked C57Bl mice, as expected (Figure 3B).

### **OXM induced tachycardia is not mediated through the GLP-1 receptor**

To determine whether OXM-induced tachycardia is mediated via the GLP-1 receptor, we measured the physiological responses to OXM in *Glp-1R*<sup>-/-</sup> mice (n=6) and their age-matched controls (C57Bl: n=6). These C57Bl mice are the same mice tested in the

previous section. Baseline physiological parameters of these two sets of mice are shown in Table 1. *Glp-1R*  $-/-$  mice exhibit equivalent resting HRs,  $T_b$ , and activity as compared to C57Bl mice (Table 1). These *Glp-1R*  $-/-$  mice also display normal circadian rhythms in all parameters measured (Table 1). Twenty minutes after administration of metoprolol and atropine, intrinsic HR was found to be  $501 \pm 45$  bpm in *Glp-1R*  $-/-$  mice.

Surprisingly, injection of OXM into *Glp-1R*  $-/-$  mice elevated intrinsic HR just as vigorously as seen in C57Bl mice ( $200 \pm 28$  bpm: Figure 4), indicating that the GLP-1 receptor does not mediate the tachycardia induced by OXM.

### **Glucagon has similar effects as OXM on HR in the mouse**

Previous studies have shown that glucagon has chronotropic effects independent of the sympathetic nervous system (10). To determine whether this effect could be observed in the conscious, free-roaming mouse, and to compare the HR effects of glucagon with those of OXM, mice were pretreated with metoprolol and atropine 20 minutes prior to intraperitoneal administration of glucagon or OXM at the following amounts: 15  $\mu$ g, 1.5  $\mu$ g, or 0.3  $\mu$ g. As seen with resting HR in unblocked mice (Figure 1), OXM induced a dose-dependent increase in intrinsic HR in these blocked mice (Figure 5), with no detectable response at the lowest dose used (0.3  $\mu$ g). Glucagon (15  $\mu$ g) also increased intrinsic HR in mice by over 200 beats/min in a similar manner to that of OXM (Figure 5). At the lower concentrations of 1.5  $\mu$ g and 0.3  $\mu$ g, glucagon elicited a higher intrinsic HR than OXM (Figure 5B and C).

### **OXM has no effect on blood pressure in mice or rats**

To determine whether OXM can influence blood pressure, a new set of 6-month old C57Bl mice (n=6) were implanted with blood pressure telemeters. While HR was

elevated in mice injected with OXM (Figure 6) as seen previously, mean blood pressure (Figure 6) was not different when injected with OXM or saline. The mean blood pressure and HR of Sprague-Dawley rats (n=5) was also measured with telemetry. Surprisingly, OXM had no effect on either HR or blood pressure of these rats (Figure 6).

#### **Exendin-4 effects are rat-specific**

Because others have shown that GLP-1 agonists influence HR in rats (4, 48), we wished to confirm this using Exendin-4, a potent GLP-1 agonist, in both mice and rats. In rats, the intrinsic HR of  $302 \pm 9$  bpm was elevated by  $55 \pm 15$  bpm after injection with Ex-4 (Figure 7). We found that the HR of mice did not respond to Ex-4 (Figure 7), further supporting the hypothesis that the GLP-1 receptor does not mediate the HR effects of OXM.

#### **OXM affects $T_b$ in autonomically-blocked mice**

To determine whether OXM affects core  $T_b$  in mice, we were able to use  $T_b$  data from our telemeters in the previously described experiments. OXM had no effect on  $T_b$  in mice with a normally functioning autonomic nervous system (Figure 8, top). However, OXM induced a significant drop in  $T_b$  of  $0.7 \pm 0.2$  °C in autonomically-blocked 6-month old C57Bl mice (Figure 8, bottom) and autonomically-blocked 3-month old C57Bl (data not shown). This hypothermic effect was also observed in *Dbh*  $-/-$  mice, with a drop in core  $T_b$  of  $1.1 \pm 0.3$  °C (Figure 8, bottom). Importantly, the fall in  $T_b$  in response to OXM administration was not observed in autonomically-blocked *Glp-1R*  $-/-$  mice (Figure 8, bottom).

#### **OXM induces inactivity in mice**

To determine whether OXM may affect general cage activity, we were able to use activity level data from our telemeters in the previously described experiments. Six month old C57Bl mice became significantly less active 12 to 24 minutes after peripheral OXM treatment, independent of whether they were autonomically-blocked (Figure 9A) or not (data not shown). Importantly, OXM-induced inactivity was not observed in *Glp-1R*<sup>-/-</sup> mice (Figure 9B), suggesting that this, like the drop in  $T_b$ , is mediated through the GLP-1 receptor. Ex-4 also induced inactivity in C57Bl mice, but not in *Glp-1R*<sup>-/-</sup> mice (Figure 9B).

### **Peripheral OXM inhibits food intake in mice**

Previous research has shown that IP administration of OXM does not impact cumulative food intake over a period of 2 hours (2). Given the transient but robust effects of OXM on HR,  $T_b$ , and activity, we wanted to reexamine whether food intake is suppressed by IP administration of OXM in mice over shorter time periods. *Glp-1R*<sup>-/-</sup> mice and their control C57Bl mice received 22 µg OXM or vehicle IP after a 15 hour fast. Food intake was significantly suppressed for 60 minutes after administration of OXM than after vehicle in wildtype mice, which was not observed in *Glp-1R*<sup>-/-</sup> mice (Figure 10). Ex-4 (2.5 µg) invoked a similar, but more potent suppression of food intake in C57Bl mice (Figure 10), as has been shown previously (2). This effect of Ex-4 was also not observed in *Glp-1R*<sup>-/-</sup> mice. At the lower dose of 15 µg OXM, food intake was significantly inhibited in C57Bl mice for 30 minutes (data not shown).

## Discussion

While the effects of OXM on food intake have been investigated extensively (2, 11, 14, 17, 45, 46), little is known about the effects of OXM on the cardiovascular system. In this study we showed that peripheral OXM vigorously increases intrinsic HR in mice independent of the GLP-1 receptor. In contrast, we found that peripheral OXM inhibits food intake and induces a drop in  $T_b$  and activity levels via GLP-1R dependent mechanisms. It should be noted that we are uncertain whether the primary dose used throughout these studies (15  $\mu$ g) results in physiological, sub-pharmacological, or pharmacological levels of circulating OXM as compared with post-prandial levels. The dose chosen is within the range reported by others using peripheral injections in animals studies (14). Further, a low dose of 1.5  $\mu$ g, which is similar to that chosen for weight-loss in human studies (45) evoked a cardiovascular response greater than that achieved with saline (Figure 1).

Because GLP-1 elevates HR in rats independent of the SNS (3), we hypothesized that OXM would have a similar tachycardic effect on HR in mice independent of the SNS. Since the HR effects of OXM are seen in wildtype mice pretreated with metoprolol and atropine as well as in *Dbh*<sup>-/-</sup> mice which lack catecholamines, we conclude that OXM can influence HR in the mouse independently of the ANS. Our experiments do not rule out the possibility that OXM can also influence HR by altering autonomic outflow in the mouse. In fact, OXM may elevate SNS output directly (3, 28, 48) and indirectly through elevation of circulating insulin (25), although the lack of a blood pressure response to OXM in both mice and rats argues against major changes in ANS activity. Further, we found the magnitude of the HR change in response to OXM was similar

(~200 bpm) in the presence or absence of autonomic influence. These data suggest that if OXM influences HR via the ANS, the contribution may be quite small.

For peripheral OXM to increase intrinsic HR by over 200 bpm (approximately 40%) in only a few minutes, OXM almost certainly binds receptors directly on the heart. The GLP-1R appears not to be that receptor, as the HR of *Glp-1R*<sup>-/-</sup> mice responded just as robustly as the HR of C57Bl mice to OXM. Given that mice exhibit tachycardia independent of the GLP-1R, and Ex-4 action is likely mediated through the GLP-1R (5), it is not surprising that the HR of mice tested herein did not respond to Ex-4 (Figure 7). These data support the notion that OXM functions independently of the GLP-1R at the heart, and that the GLP-1R plays little role in mediating any of the cardiovascular effects of gut hormones in the mouse. Three lines of evidence suggest that the glucagon receptor (GCGR) may mediate the HR effects of OXM. First, OXM contains the entire sequence of glucagon (17). Second, OXM has between 2-10% affinity for the GCGR as that of glucagon (2, 6). Third, we show here that at the highest dose tested (Figure 5A), the effects of glucagon on intrinsic HR in the mouse are nearly identical in both magnitude and duration as OXM on intrinsic HR. Importantly, at the lower doses tested (Figure 5B and C), glucagon had a much greater effect on  $I_{HR}$  than OXM, consistent with the decreased affinity of OXM for the GCGR. These data are a strong indication that OXM elevates  $I_{HR}$  through the GCGR. Contrary to this hypothesis, however, is the fact that rats express the GCGR in the heart (9, 20), but OXM has no effect on either HR or  $I_{HR}$  of the rat (Figures 6 and 7). Collectively, these data suggest that OXM either activates the GCGR in a species-specific manner or acts via an, as yet unidentified, OXM-specific



receptor; the existence of which has been alluded to by others (13, 14). Further experimentation is required to test this hypothesis.

While the HR of rats responds robustly to Ex-4 (Figure 7 and refs (5, 48)), the murine HR does not respond to Ex-4 (Figure 7). However, Ex-4 does evoke other physiological effects in the mouse include diminished feeding (2, 38) and reversal of hepatic steatosis (16). Similarly, while the HR of mice responds vigorously to OXM, the HR of rats does not respond to OXM (Figure 7) although OXM clearly has physiological effects in the rat, including inhibition of food intake (14, 15), inhibition of pancreatic secretion (1), and elevation of intestinal glucose uptake (12). The species-specific HR response to OXM extends to humans. Recently, Wynne et al. have shown that OXM does not influence HR or blood pressure in humans (45). Together, these data suggest that both Ex-4 and OXM can have both organ-specific and species-specific effects.

Previous studies have found that administration of a GLP-1R agonist causes a decrease in core  $T_b$  in rats (29). We extend these findings here to show a hypothermic effect of OXM in the mouse. Importantly, we only observed a lower  $T_b$  in mice with compromised SNS signaling (*Dbh*  $-/-$  mice or autonomic block of control mice). This suggests that the SNS may play an important role in maintaining  $T_b$  in the presence of elevated OXM. *Dbh*  $-/-$  mice certainly have an impaired ability to generate heat due to the lack of catecholamines (40), and it may be that the metoprolol used here to block  $\beta_1$  receptors on the heart also blocked  $\beta_3$  receptors on heat-generating organs, like brown adipose tissue. Hormones similar to OXM can activate the SNS (3, 28, 48); hence it may be that OXM activates the SNS which contributes to heat generation in the mouse.

This fall in  $T_b$  of a mouse in response to OXM likely results from depressed metabolism which may become manifest as a result of inactivity (Figure 9A) or the lack of food intake (Figure 10). Each is an important contributor to heat production in the rodent (19, 27). In support of this notion, *Glp-1R*  $-/-$  mice, which do not exhibit hypothermia in response to OXM, lack both the satiation and inactivity effects of OXM. However, a preliminary study suggests that the lack of food intake after OXM administration is not responsible for the drop in core  $T_b$  (GLS, unpublished observations). Hence, the hypothermia induced by OXM in SNS-compromised mice may only exhibit dependence on the GLP-1R because of the requirement for GLP-1R for inactivity. This remains to be tested.

In summary, OXM elevates HR in mice independent of the ANS and likely through actions mediated directly on cardiac cells. While it is clear that OXM increases intrinsic HR independent of the GLP-1R, we expect that with further investigation we will better elucidate the mechanisms through which OXM exerts a HR effect in the periphery and how this may integrate with other effects of OXM, both centrally and peripherally.

### **Acknowledgments:**

The authors would like to thank Molly Gutilla and Auyon Mukharji for their technical assistance for the experiments in this manuscript. This work was supported by a grant R15 HL081101-01 (SJS), grants from the CIHR and the Heart and Stroke Foundation of Canada (DJD), and in part by a Howard Hughes Medical Institute grant to Williams College.

Table 1 – Baseline physiological characteristics

Mouse Model	Heart rate (bpm)		T <sub>b</sub> (°C)		Activity (arbitrary units)	
	Dark cycle	Light cycle	Dark cycle	Light cycle	Dark cycle	Light cycle
C57Bl (3 month old)	453 ± 7 <sup>c</sup>	379 ± 7	37.8 ± 0.1 <sup>c</sup>	36.7 ± 0.1	16.9 ± 2.5 <sup>c</sup>	7.3 ± 0.9
STZ-treated C57Bl	403 ± 8 <sup>a,c</sup>	332 ± 13 <sup>a</sup>	37.1 ± 0.1 <sup>a,c</sup>	35.9 ± 0.3 <sup>a</sup>	7.6 ± 1.2 <sup>a,c</sup>	3.1 ± 0.4 <sup>a</sup>
<i>Dbh</i> +/-	545 ± 22 <sup>c</sup>	486 ± 9	36.9 ± 0.3 <sup>c</sup>	36.4 ± 0.3	5.0 ± 0.5 <sup>c</sup>	2.8 ± 0.3
<i>Dbh</i> -/-	404 ± 13 <sup>b</sup>	385 ± 2 <sup>b</sup>	36.2 ± 0.4 <sup>b</sup>	36.2 ± 0.1 <sup>b</sup>	5.7 ± 0.5	4.2 ± 0.6 <sup>b</sup>
C57Bl (6 month old)	495 ± 19 <sup>c</sup>	424 ± 12	36.8 ± 0.7 <sup>c</sup>	35.5 ± 0.8	11.9 ± 2.5 <sup>c</sup>	4.1 ± 0.8
<i>Glp-1R</i> -/-	484 ± 11 <sup>c</sup>	432 ± 9	37.7 ± 0.1 <sup>c</sup>	36.5 ± 0.1	13.9 ± 2.5 <sup>c</sup>	3.4 ± 0.3

a - C57Bl (3 month) significantly different from STZ-treatment within the same light/dark cycle

b - *Dbh* +/- mice significantly different from *Dbh* -/- mice within the same light/dark cycle

c - Light cycle significantly different from dark cycle within the same group

## Figures

Figure 1. Peripheral OXM increased HR in the mouse. 3-month old C57Bl mice were injected IP at the onset of the light cycle with saline or OXM at doses of 1.5  $\mu$ g, 15  $\mu$ g, 45  $\mu$ g.

\*  $P < 0.05$ : 1.5  $\mu$ g OXM vs. saline

\*\*  $P < 0.05$ : 15  $\mu$ g OXM vs. saline;

\*\*\*  $P < 0.05$ : 45  $\mu$ g OXM vs. saline

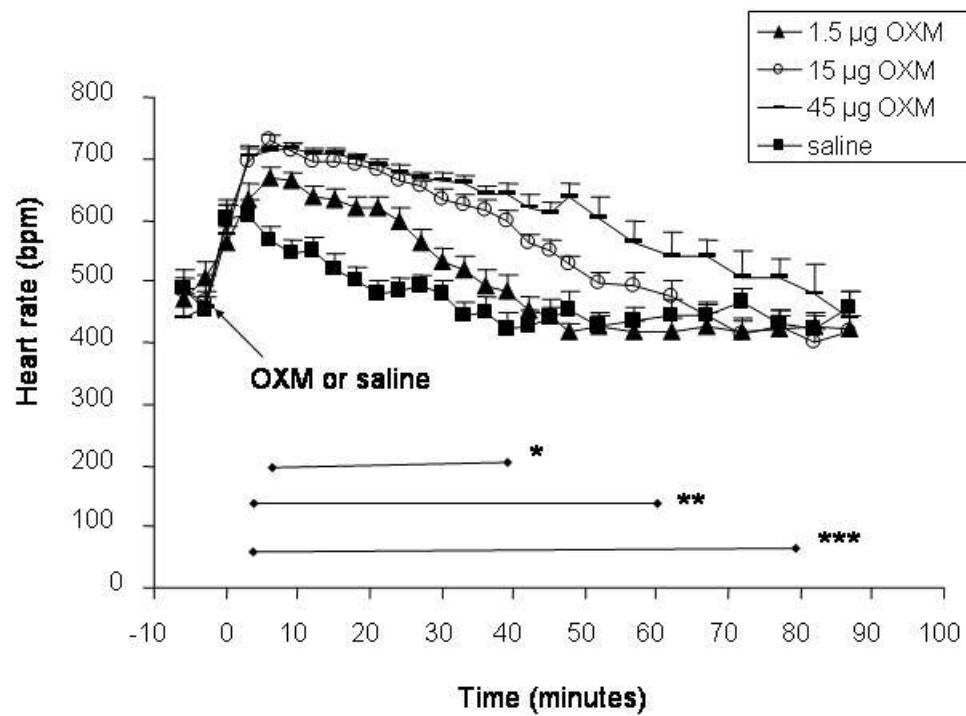


Figure 2. OXM increased HR independent of insulin. C57Bl mice were treated with STZ ten days previous to the OXM injection. Saline induced a minimal HR response in these STZ-treated mice, while administration of OXM increased HR by over 200 bpm.

\*  $P < 0.05$  vs. saline.

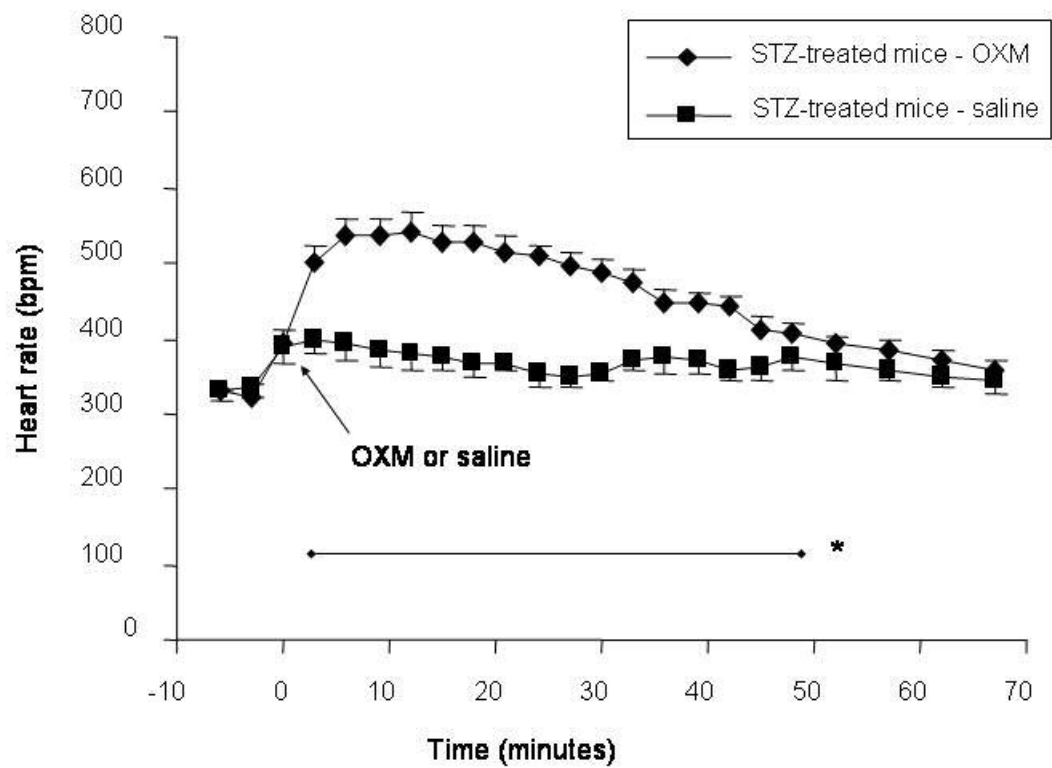


Figure 3. OXM increased intrinsic HR. A) *Dbh*<sup>-/-</sup> mice, lacking norepinephrine and epinephrine, were injected with either saline or OXM. While saline had no effect on HR, OXM increased HR by  $192 \pm 13$  bpm in these mice. \*  $P < 0.05$  vs. saline. B) Pretreatment of C57Bl mice with metoprolol and atropine blocks both inputs of the autonomic system to the heart and results in an intrinsic HR. While saline and dobutamine (a  $\beta 1$  adrenergic receptor agonist) did not influence HR in these autonomically-blocked mice, OXM increased intrinsic HR.

\*  $P < 0.05$  vs. saline.

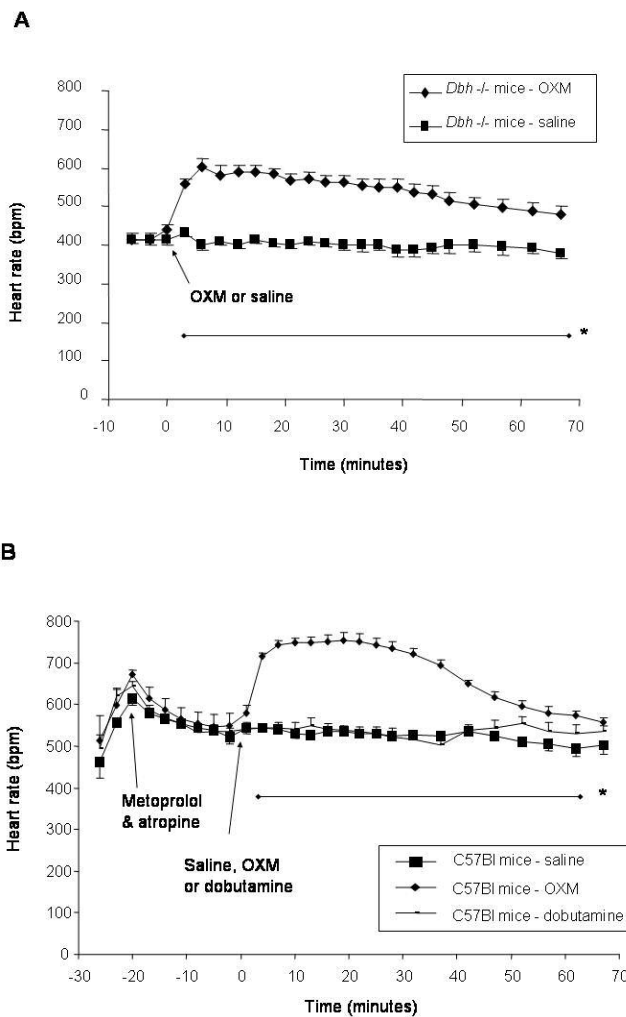


Figure 4. OXM mediates tachycardia independent of the GLP-1R. *Glp1R*  $-/-$  mice were treated with metoprolol and atropine to measure intrinsic HR. Saline induced a minimal response in intrinsic HR of *Glp1R*  $-/-$  mice, whereas OXM elevated intrinsic HR approximately 200 bpm. This indicates that OXM does not exert its HR effect via the GLP-1 receptor.

\*  $P < 0.05$  vs. saline.

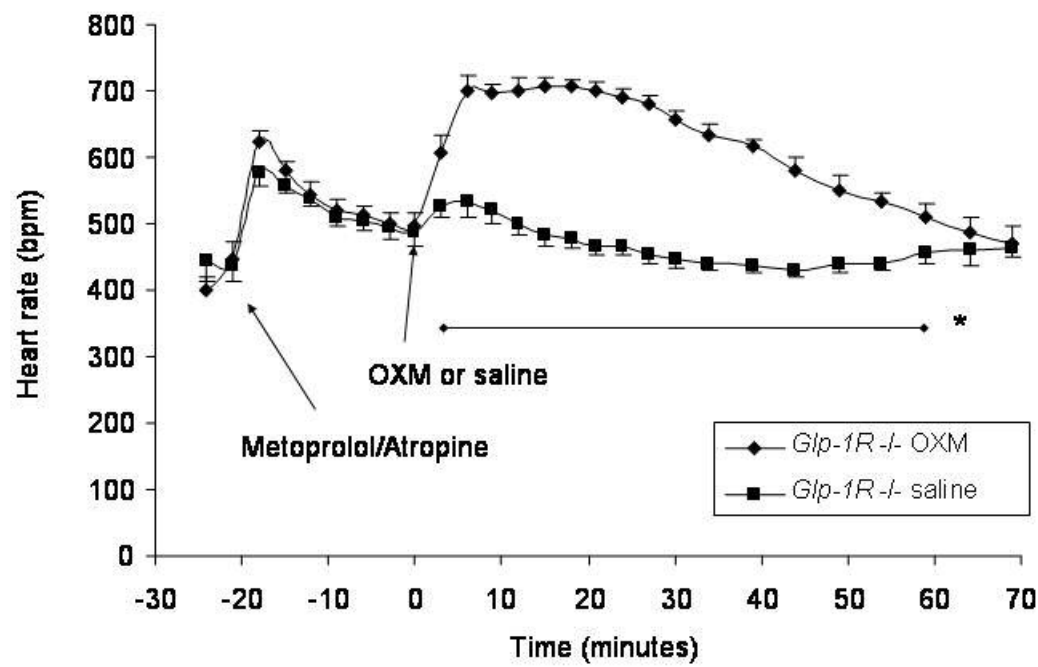
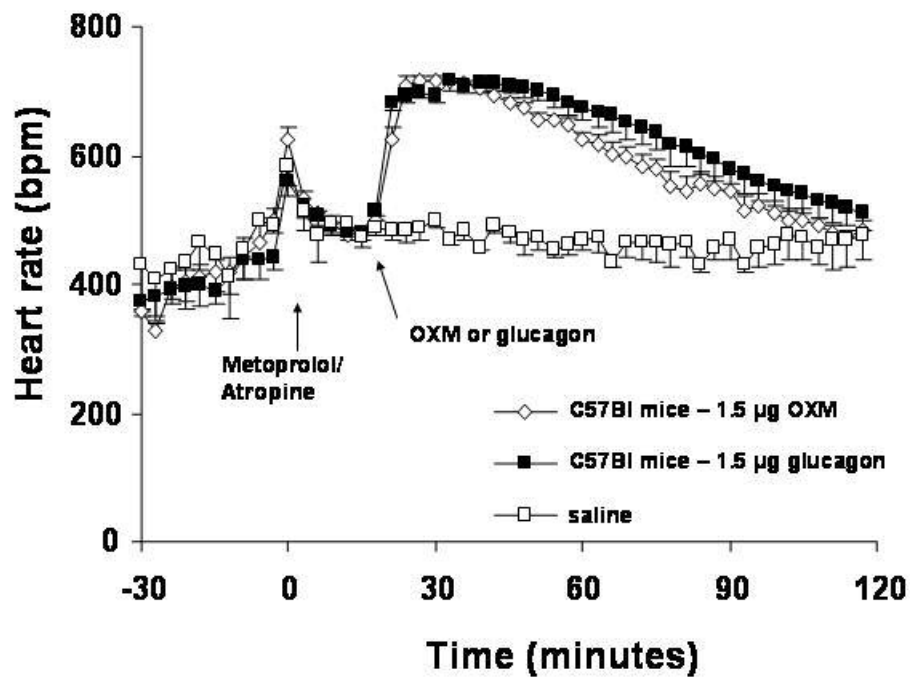


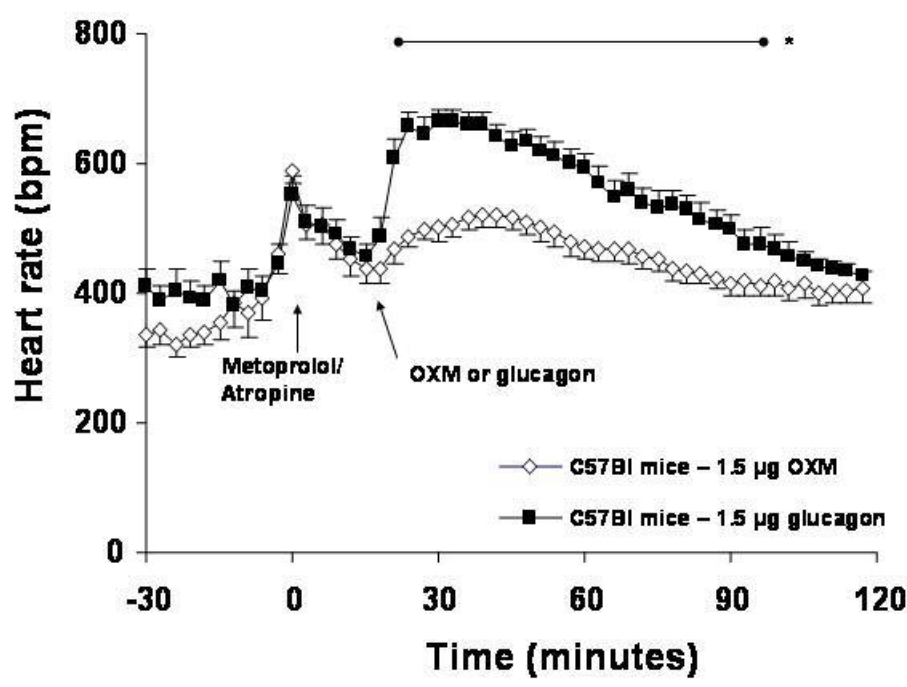
Figure 5 – Glucagon elevates intrinsic HR in mice at lower doses to a greater extent than OXM. C57Bl mice were pretreated with metoprolol and atropine to measure intrinsic HR. The mice were injected with either glucagon or OXM at 1 of 3 doses: 15  $\mu$ g (A), 1.5  $\mu$ g (B), and 0.3  $\mu$ g (C). \*  $P < 0.05$  vs. saline.

**A**





**B**



**C**

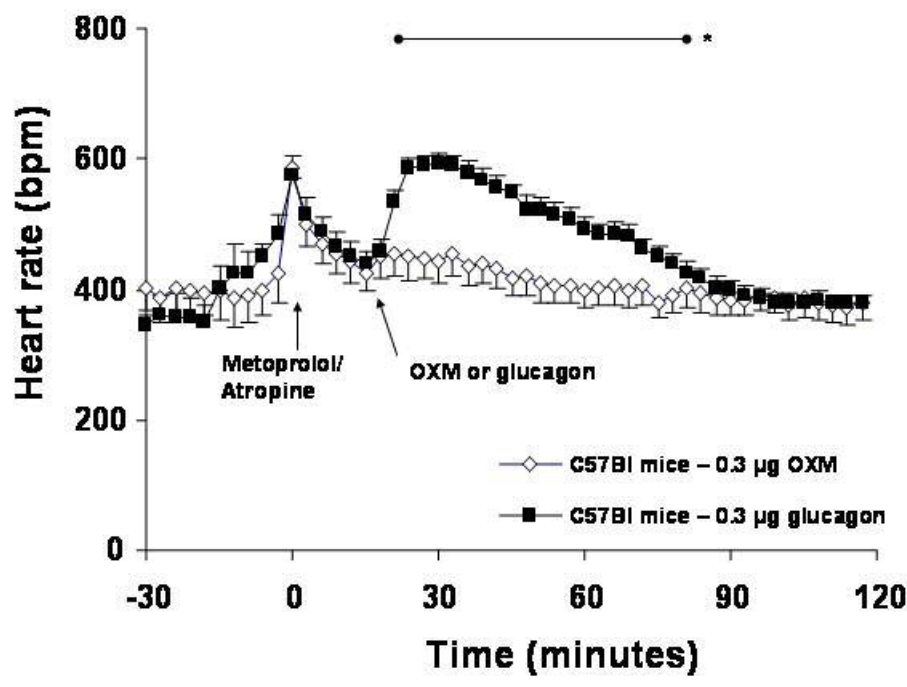


Figure 6 – OXM does not alter blood pressure in mice or rats. C57Bl mice and Sprague-Dawley rats were implanted with blood pressure telemeters to measure mean blood pressure and heart rate. OXM did not significantly alter blood pressure in either mice or rats. OXM elevated heart rate only in mice, but not rats.

\*  $P < 0.05$  vs. saline.

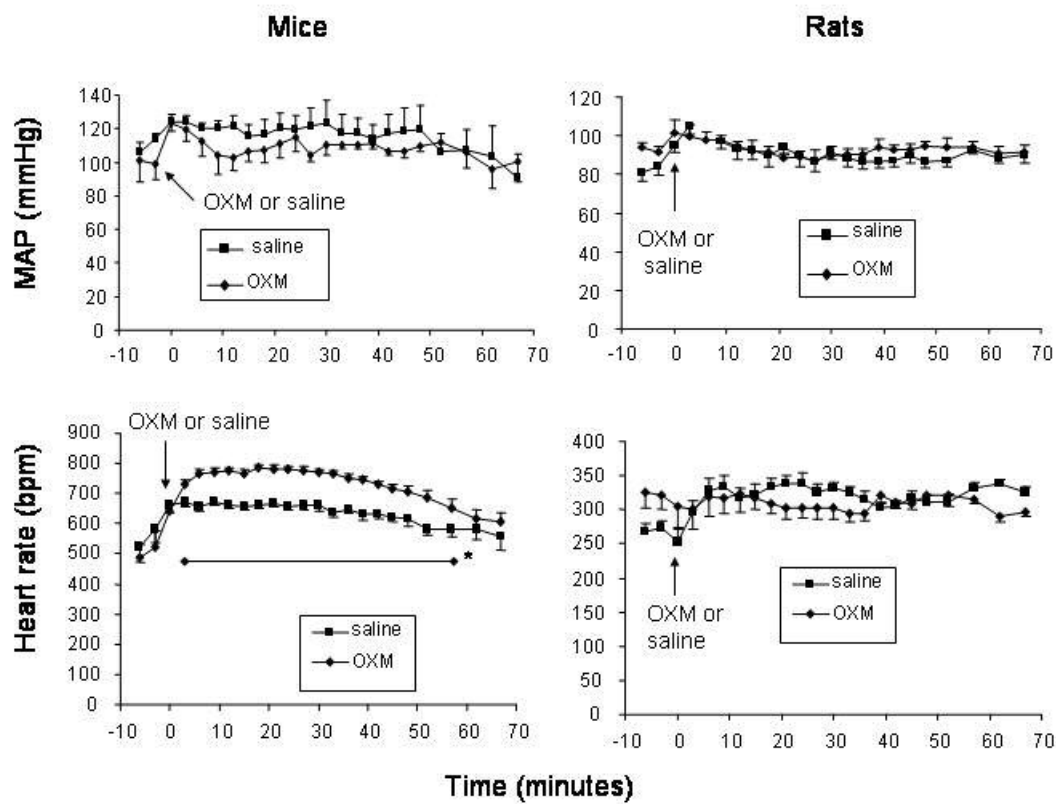


Figure 7. Exendin-4 only alters intrinsic HR in rats. Mice and rats were pretreated with metoprolol and atropine to measure intrinsic HR. After 20 minutes, animals were injected with saline, OXM, or Exendin-4. While OXM elevated intrinsic HR in mice as seen previously, it had no effect on HR in rats. Similarly, exendin-4 elevated intrinsic HR in rats, but had no effect on HR in mice.

\*  $P < 0.05$  OXM vs. saline

\*\*  $P < 0.05$  Ex-4 vs. saline

\*\*\*  $P < 0.05$  Ex-4 vs. OXM

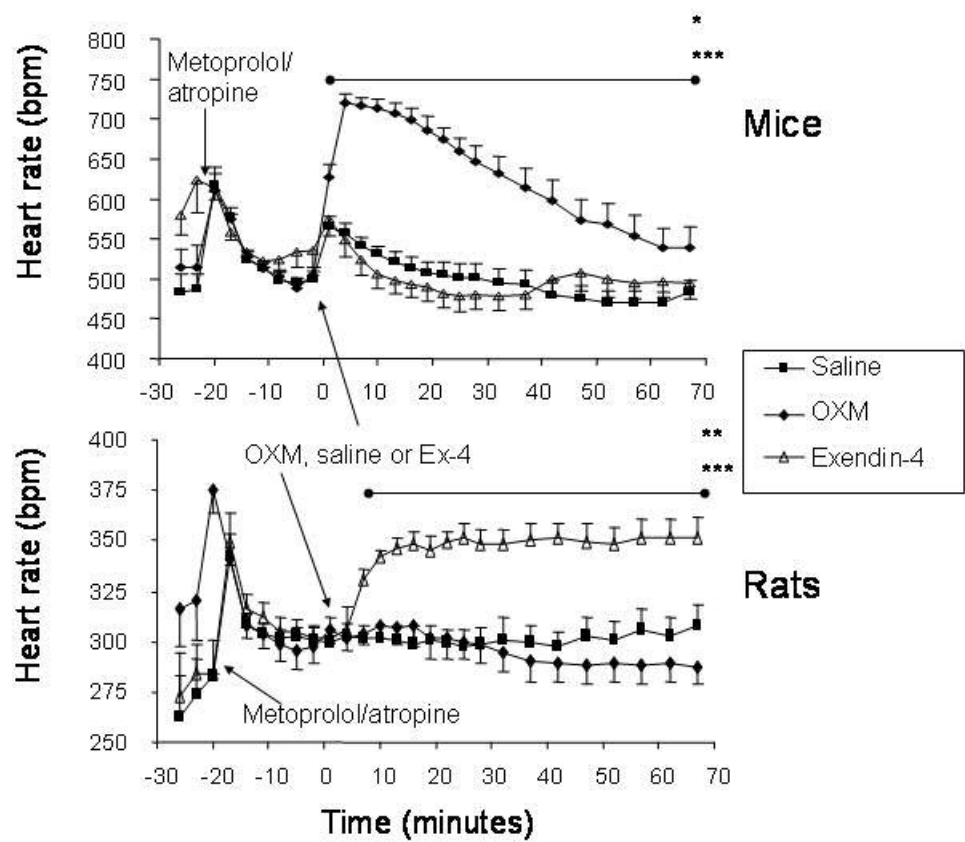


Figure 8 - OXM-induced hypothermia requires the GLP-1 receptor. *Top* – OXM or saline was administered to C57Bl mice, *Glp-1R*  $-/-$  mice, and *Dbh*  $+/-$  mice. Both saline and OXM induced a transient elevation in core  $T_b$ , followed by a fall in  $T_b$ . Core  $T_b$  after OXM or saline were not significantly different in these mice. *Bottom* – OXM or saline was administered to autonomically-blocked C57Bl mice, and autonomically-blocked *Glp-1R*  $-/-$  mice as well as to *Dbh*  $-/-$  mice, which are deficient in SNS neurotransmitters. OXM induced a significant hypothermia in ANS-blocked wildtype mice and *Dbh*  $-/-$  mice, but not in *Glp-1R*  $-/-$  mice, suggesting that OXM-induced hypothermia is mediated via the GLP1 receptor.

\*  $P < 0.05$  vs. saline.

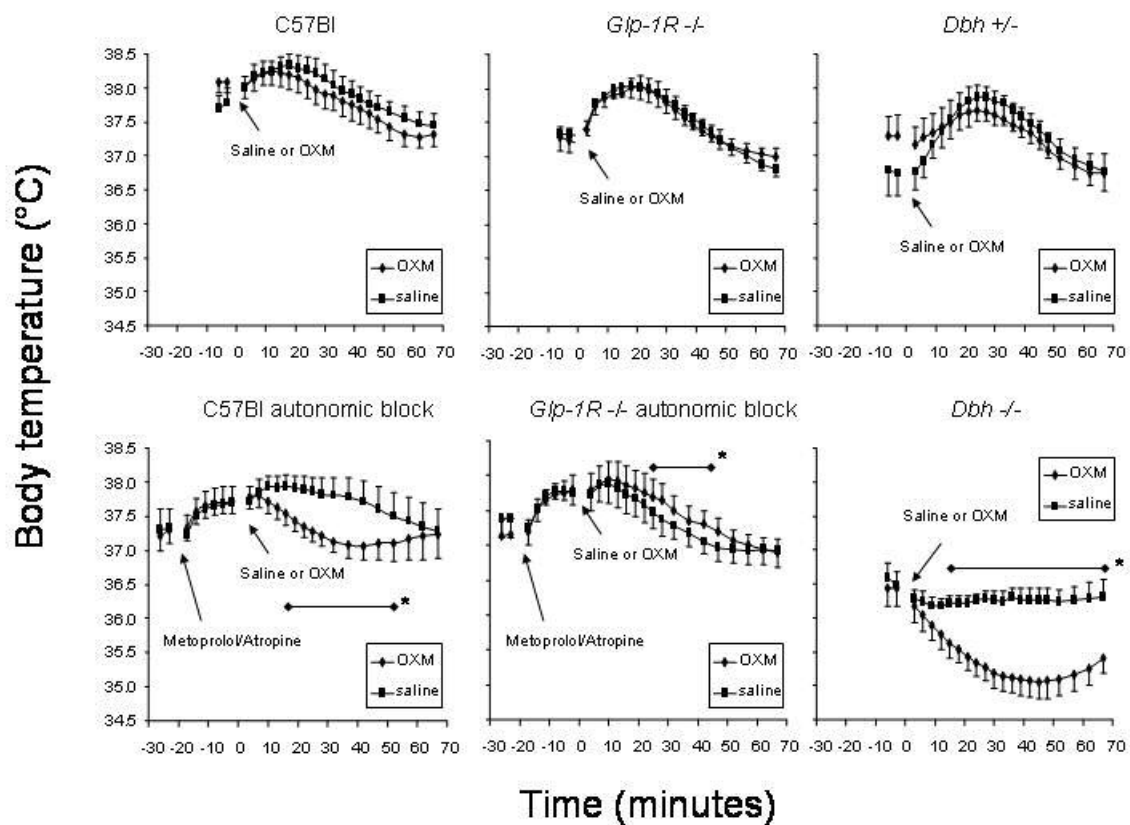


Figure 9 - Transient inactivity induced by OXM requires the GLP-1 receptor. A) General cage activity levels in C57Bl mice were quantified using Data Sciences Int. software. These mice demonstrated significantly lower activity levels in the first 12 to 24 minutes post OXM injection than after saline injection. \*  $P < 0.05$  vs. saline B) Average activity levels in C57Bl and *Glp-1R*  $-/-$  mice were quantified over this same timeframe after OXM or Ex-4 injection. Cage activity was significantly less after OXM administration as compared to saline in C57Bl mice but not in *Glp-1R*  $-/-$  mice, indicating that these peptides suppress activity via the GLP-1 receptor. \*  $P < 0.05$  vs. saline.

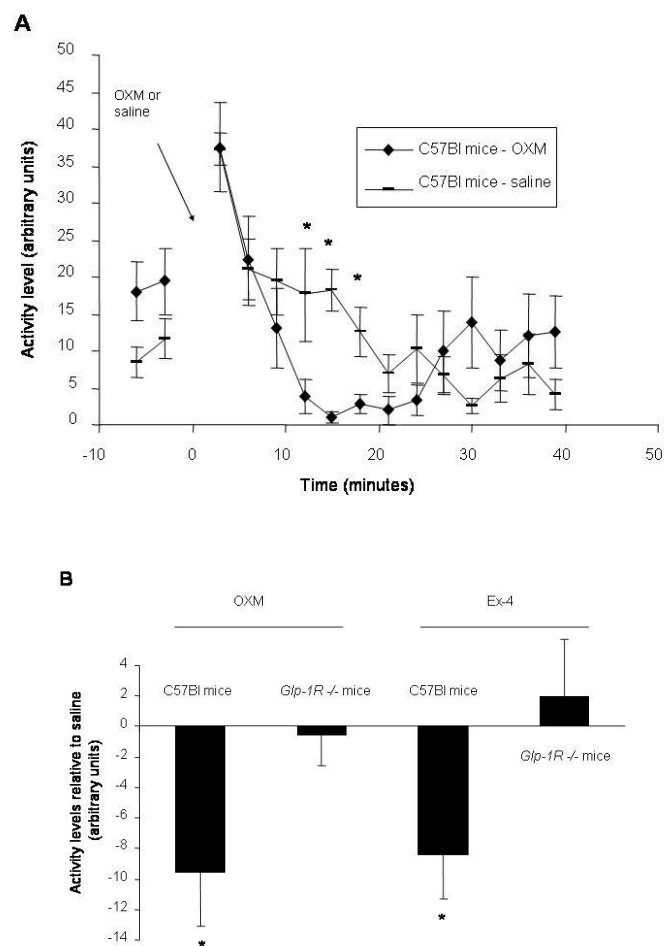
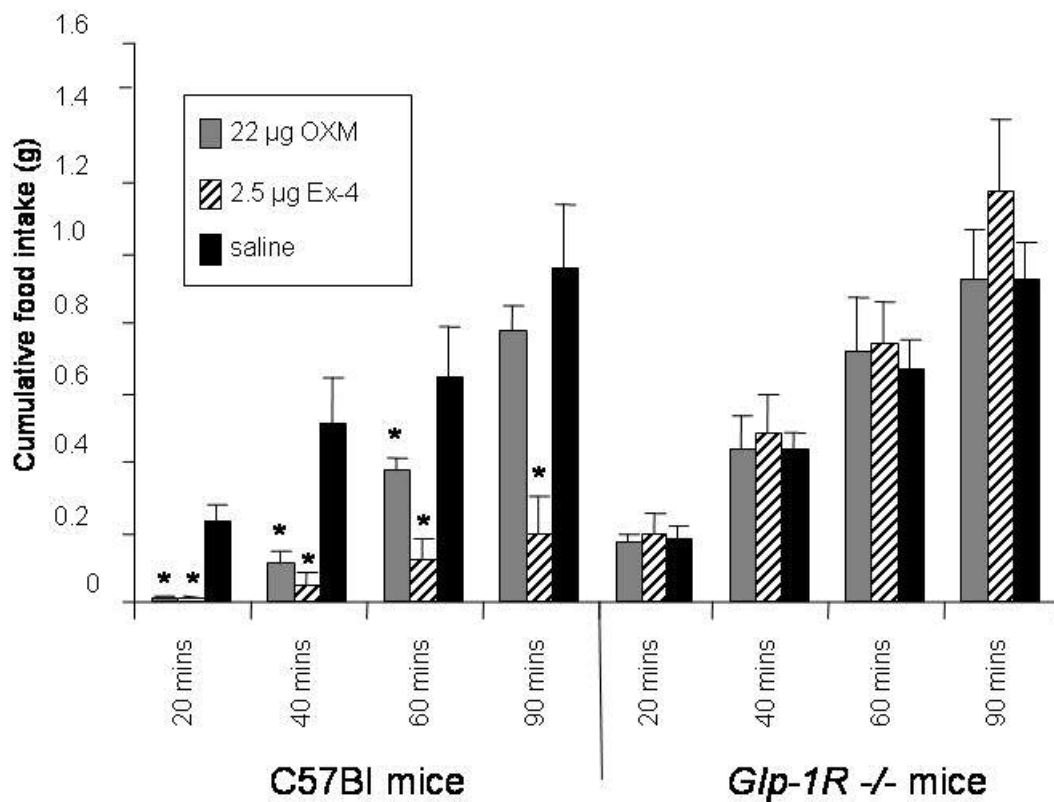


Figure 10. Peripheral OXM transiently decreases food intake in C57Bl mice and is dependent on the GLP-1R. C57Bl mice and *Glp-1R*<sup>-/-</sup> mice were fasted for 15 hours and injected with 22  $\mu$ g OXM, 2.5  $\mu$ g Ex-4 or saline at the onset of the light cycle. OXM and Ex-4 significantly inhibited food intake for 60 minutes and 90 minutes, respectively, in C57Bl mice, but had no effect on food intake in *Glp-1R*<sup>-/-</sup> mice. \*  $P < 0.05$  vs. saline



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