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

Gillian L. Sowden, Daniel J. Drucker, David Weinshenker and Steven J. Swoap

Am J Physiol Regulatory Integrative Comp Physiol 292:962-970, 2007. First published Oct 12, 2006;
doi:10.1152/ajpregu.00405.2006

The American Journal of Physiology - Regulatory, Integrative and Comparative Physiology publishes original investigations that illuminate normal or abnormal regulation and integration of physiological mechanisms at all levels of biological organization, ranging from molecules to humans, including clinical investigations. It is published 12 times a year (monthly) by the American Physiological Society, 9650 Rockville Pike, Bethesda MD 20814-3991. Copyright © 2005 by the American Physiological Society. ISSN: 0363-6119, ESSN: 1522-1490. Visit our website at http://www.the-aps.org/.

Am J Physiol Regul Integr Comp Physiol 292: R962–R970, 2007.
First published October 12, 2006; doi:10.1152/ajpregu.00405.2006.

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

Gillian L. Sowden,[1] Daniel J. Drucker,[2] David Weinshenker,[3] and Steven J. Swoap[1]

[1]*Department of Biology, Williams College, Williamstown, Massachusetts;* [2]*Department of Medicine, Samuel Lunenfeld Research Institute, Mt. Sinai Hospital, Toronto, Canada; and* [3]*Department of Human Genetics, Emory University School of Medicine, Atlanta, Georgia*

Submitted 9 June 2006; accepted in final form 9 October 2006

Sowden GL, Drucker DJ, Weinshenker D, Swoap SJ. Oxyntomodulin increases intrinsic heart rate in mice independent of the glucagon-like peptide-1 receptor. *Am J Physiol Regul Integr Comp Physiol* 292: R962–R970, 2007. First published October 12, 2006; doi:10.1152/ajpregu.00405.2006.—Oxyntomodulin (OXM), a postprandially released intestinal hormone, inhibits food intake via the glucagon-like peptide-1 receptor (GLP-1R). Although 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 wild-type mice, raising HR by >200 beats/min to a maximum of 728 ± 11 beats/min. To determine the extent to which the sympathetic nervous system mediates the tachycardia of OXM, we delivered this hormone to mice deficient in dopamine-β-hydroxylase [*Dbh*(−/−) mice], littermate controls [*Dbh*(+/−) mice], and autonomically blocked C57Bl mice. OXM increased HR equally in all groups (192 ± 13, 197 ± 21, and 216 ± 11 beats/min, respectively), indicating that OXM elevated intrinsic HR. Intrinsic HR was also vigorously elevated by OXM in *Glp-1R*(−/−) mice (200 ± 28 beats/min). In addition, peripherally administered OXM inhibited food intake and activity levels in wild-type 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.

glucagon receptor; core body temperature; exendin-4; insulin; food intake; mean arterial pressure; rats; activity

OXYNTOMODULIN (OXM) is a 37-amino acid peptide, containing the entire 29-amino acid sequence of glucagon followed by an 8-amino acid carboxy-terminal 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. Although glucagon is the primary cleavage product of proglucagon in the pancreas, OXM and glucagon-like peptide-1 (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). Furthermore, 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-1 receptor (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-1R 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, because the pattern of neuronal activation observed after administration of peripheral OXM differs from the pattern seen after peripheral administration of GLP-1 (14).

Although 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 elevates heart rate (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 independently of catecholamines (4). Indeed, GLP-1 elevates intracellular cAMP in target cells that express the GLP-1R, including rat heart cells (43). Furthermore, 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 β_1-adrenergic receptor antagonist betaxonal (41).

In the absence of any nervous or hormonal influences, the murine heart will beat at ~510 beats/min (23). This intrinsic HR is modified by the activity of parasympathetic and/or sympathetic nerves at the sinoatrial 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-depen-

Address for reprint requests and other correspondence: S. J. Swoap, Dept. of Biology, Williams College, Williamstown, MA 01267 (e-mail: sswoap@williams.edu).

The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

dent manner, we used radiotelemetry to measure the HR, body temperature (T_b), and activity responses to OXM in *1*) wild-type mice with chemically induced type 1 diabetes, *2*) mice lacking the sympathetic neurotransmitters norepinephrine and epinephrine due to deficiency in the enzyme dopamine-β-hydroxylase [*Dbh*(−/−) mice] (39), *3*) mice deficient in the GLP-1R [*Glp-1R*(−/−) mice] (33), and *4*) mice treated with pharmacological agents to block autonomic signaling at the heart.

MATERIALS AND METHODS

Animals. Female C57Bl mice weighing 22–24 g (3 mo old) were obtained from Harlan and used in OXM dose-response experiments, as well as the streptozotocin (STZ)-induced diabetes experiment. Female *Dbh*(−/−) mice and *Dbh*(+/−) controls (3–4 mo old) were shipped from Emory University. *Glp-1R*(−/−) mice (~6 mo old) were shipped from the University of Toronto to Williams College for physiological assessment. Age-matched female C57Bl mice (from Harlan) served as controls for *Glp-1R*(−/−) 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 12:12-h light-dark cycle. Studies were approved by each of the local Institutional Animal Care and Use Committees.

Reagents. Porcine OXM and Ex-4 were obtained from California Peptide Research (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 h following the surgery and then housed individually at 28–30°C for 1 wk 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, T_b, and activity levels were collected every minute for 5 s for the 1 h preceding and 2 h following each injection of peptide or vehicle. Activity levels were measured as described previously (35, 36) with the use of software from Data Sciences International that 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, we injected animals intraperitoneally 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 the other half received peptide on any given day. If mice were injected with peptide on *day 1*, they were injected with saline on *day 2* (and vice versa) to allow for paired comparisons between effects of peptide and saline. Unless stated otherwise, mice were injected with 15 μg of OXM, 2.5 μg of Ex-4, or saline; rats were injected with 100 μg of OXM, 7.5 of μg Ex-4, or saline. These dosages were chosen on the basis of 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 intraperitoneal injection of STZ (200 mg/kg body wt). The effects of OXM and saline were tested 10 days after STZ treatment. An autonomic block was induced by intraperitoneal injection of metoprolol (12 mg/kg) and atropine (5 mg/kg) 20 min before administration of OXM or saline. A SNS block was confirmed by the absence of a HR response to intraperitoneal injection of dobutamine (1 mg/kg), a specific β_1-adrenergic receptor agonist.

Blood samples and assays. Approximately 0.5 ml of blood was drawn directly from the heart of anesthetized STZ-treated mice, which were then immediately killed. Blood was centrifuged at 10,000 *g* at 4°C for 5 min, and plasma was stored at −80°C. Plasma glucose was measured using a glucose oxidase kit (Sigma), whereas insulin was measured using 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 h, consisting of the last 3 h of the light cycle and the entire dark cycle. Food was given at the onset of the light cycle immediately after intraperitoneal administration of peptides or vehicle and was weighed at regular time intervals thereafter. Experiments were conducted in three separate trials with at least 4 days between trials such that every mouse was injected with OXM (22 μg), Ex-4 (2.5 μg), 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 ± 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 2 × 2 design, followed by a post hoc Bonferroni test for statistical significance. A repeated-measures ANOVA with a 3 × 2 design was used to assess differences among 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 wild-type mice. HR increased after administration of vehicle, from baseline levels of 473 ± 11 to 605 ± 25 beats/min. OXM at all doses tested (1.5 μg, $n = 11$; 15 μg, $n = 9$; 45 μg, $n = 4$) elevated HR above vehicle level ($P < 0.05$) 5 min after administration of peptide or vehicle, reaching a maximum of 728 ± 11 beats/min (Fig. 1). HR was significantly elevated above saline for 40 min after administration of 1.5 μg OXM, for 60 min after administration of 15 μg OXM, and for 80 min after administration of 45 μg OXM (Fig. 1).

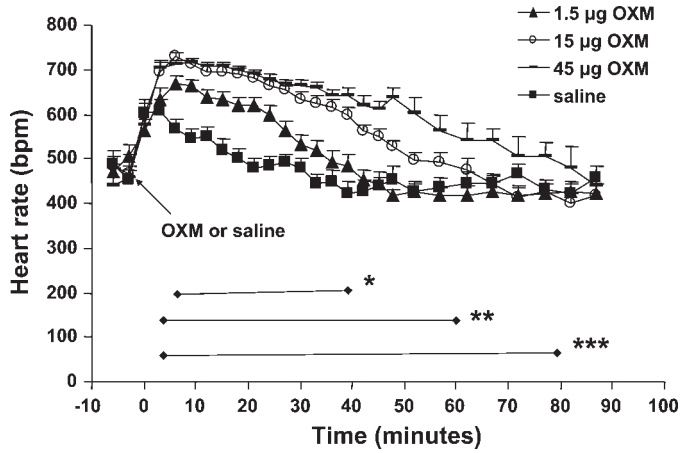

Fig. 1. Peripheral oxyntomodulin (OXM) increased heart rate (HR) in the mouse. Three-month-old C57Bl mice were injected intraperitoneally at the onset of the light cycle with saline or OXM at doses of 1.5, 15, or 45 μg. bpm, Beats/min. *$P < 0.05$, 1.5 μg OXM vs. saline. **$P < 0.05$, 15 μg OXM vs. saline. ***$P < 0.05$, 45 μg OXM vs. saline.

Table 1. *Baseline physiological characteristics*

Mouse Model	Heart Rate, beats/min		T_b, °C		Activity, arbitrary units	
	Dark cycle	Light cycle	Dark cycle	Light cycle	Dark cycle	Light cycle
C57Bl (3 mo old)	453±7‡	379±7	37.8±0.1‡	36.7±0.1	16.9±2.5‡	7.3±0.9
STZ-treated C57Bl	403±8*‡	332±13*	37.1±0.1*‡	35.9±0.3*	7.6±1.2*‡	3.1±0.4*
Dbh (+/−)	545±22‡	486±9	36.9±0.3‡	36.4±0.3	5.0±0.5‡	2.8±0.3
Dbh (−/−)	404±13†	385±2†	36.2±0.4†	36.2±0.1†	5.7±0.5	4.2±0.6†
C57Bl (6 mo old)	495±19‡	424±12	36.8±0.7‡	35.5±0.8	11.9±2.5‡	4.1±0.8
Glp-1R (−/−)	484±11‡	432±9	37.7±0.1‡	36.5±0.1	13.9±2.5‡	3.4±0.3

*C57Bl (3 mo) significantly different from streptozotocin (STZ) treatment within the same light-dark cycle. †*Dbh* (+/−) mice significantly different from *Dbh* (−/−) mice within the same light-dark cycle. ‡Light cycle significantly different from dark cycle within the same group.

OXM-induced tachycardia is independent of insulin. To test the hypothesis that the tachycardia induced by OXM was mediated through insulinotropic effects, we treated the same C57Bl mice used in the dose-response experiment ($n = 9$ at the 15-μg dose) 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 ± 40 mg/dl. Baseline HR values in both the dark and light cycles were lower in these diabetic mice than before treatment with STZ (Table 1 and Fig. 2), as has been shown 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 beats/min in these diabetic mice to 542 ± 23 beats/min (Fig. 2).

OXM elevates intrinsic HR. To determine whether the effects of OXM were mediated via the SNS, we determined the effect of OXM on HR in *Dbh*(−/−) mice ($n = 6$), which lack the SNS transmitters norepinephrine and epinephrine (39), and their littermate controls, *Dbh*(+/−) mice ($n = 6$). Baseline average HR, T_b, and activity levels obtained the day before the injection are shown in Table 1. As we have shown previously (36), *Dbh*(−/−) 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*(−/−) mice had no effect on HR (Fig. 3*A*), indicating the lack of a HR response to animal handling. However, administration of OXM increased HR in *Dbh*(−/−) mice by 192 ± 13 beats/min (Fig. 3*A*). This increase is similar to the increase in HR observed in *Dbh*(+/−) mice after OXM administration (197 ± 21 beats/min). To confirm that OXM

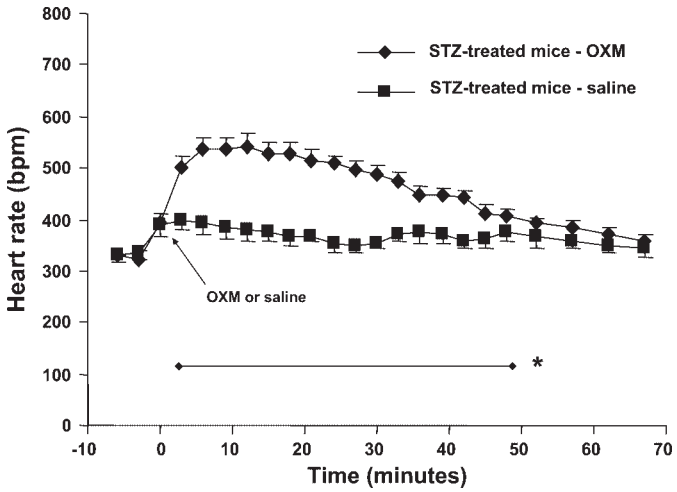

Fig. 2. OXM increased HR independently of insulin. C57Bl mice were treated with streptozotocin (STZ) 10 days before the OXM injection. Saline induced a minimal HR response in these STZ-treated mice, whereas administration of OXM increased HR by >200 beats/min. *$P < 0.05$ vs. saline.

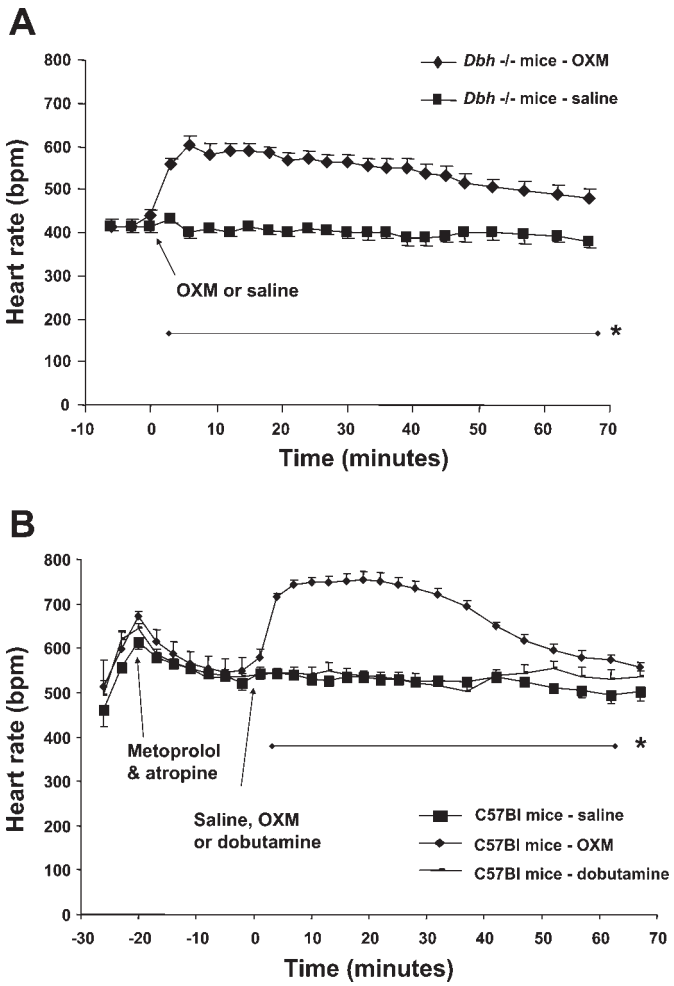

Fig. 3. OXM increased intrinsic HR. *A*: dopamine-β-hydroxylase-deficient [*Dbh*(−/−)] mice, lacking norepinephrine and epinephrine, were injected with either saline or OXM. Whereas saline had no effect on HR, OXM increased HR by 192 ± 13 beats/min in these mice. *$P < 0.05$ vs. saline. *B*: pretreatment of C57Bl mice with metoprolol and atropine blocks both inputs of the autonomic system to the heart and results in an intrinsic HR. Whereas saline and dobutamine (a β_1-adrenergic receptor agonist) did not influence HR in these autonomically blocked mice, OXM increased intrinsic HR. *$P < 0.05$ vs. saline.

works independently of the SNS in wild-type mice, we investigated the effects of OXM in mice in which both sympathetic and parasympathetic input to the heart were chemically blocked. A new set of 6-mo-old C57Bl mice ($n = 6$) was treated with metoprolol, a β_1-adrenergic receptor antagonist, and atropine, a muscarinic receptor antagonist, 20 min before peptide or vehicle administration. Blocking both inputs of the autonomic nervous system (ANS) to the heart resulted in an intrinsic HR of 525 ± 11 beats/min, similar to the intrinsic HR of mice measured by others (23). As shown in Fig. 3*B*, OXM increased intrinsic HR by 181 ± 9 beats/min. As a control, autonomically blocked mice were injected with either dobutamine, a β_1-receptor agonist, or vehicle. Neither of these affected HR in autonomically blocked C57Bl mice, as expected (Fig. 3*B*).

OXM-induced tachycardia is not mediated through the GLP-1R. To determine whether OXM-induced tachycardia is mediated via the GLP-1R, we measured the physiological responses to OXM in *Glp-1R*(−/−) mice ($n = 6$) and their age-matched controls (C57Bl; $n = 6$). (These C57Bl mice are the same mice tested in *OXM elevates intrinsic HR*.) Baseline physiological parameters of these two sets of mice are shown in Table 1. *Glp-1R*(−/−) mice exhibit equivalent resting HR, T_b, and activity levels compared with 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 ± 45 beats/min in *Glp-1R*(−/−) mice. Surprisingly, injection of OXM into *Glp-1R*(−/−) mice elevated intrinsic HR just as vigorously as in C57Bl mice (200 ± 28 beats/min; Fig. 4), indicating that the GLP-1R does not mediate the tachycardia induced by OXM.

Glucagon has effects similar to those of OXM on HR in mice. Previous studies have shown that glucagon has chronotropic effects independent of the SNS (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, we pretreated mice with metoprolol and atropine 20 min before intraperitoneal administration of glucagon or OXM at 15, 1.5, or 0.3 μg. As with resting HR in unblocked mice (Fig. 1), OXM induced a dose-dependent increase in intrinsic HR in these blocked mice (Fig. 5) with no

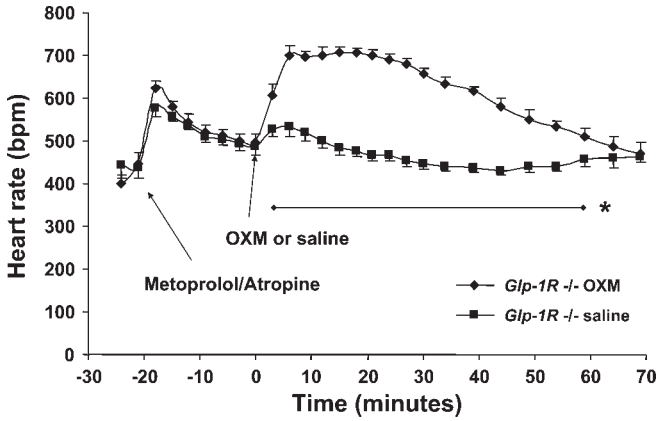

Fig. 4. OXM mediates tachycardia independently of the glucagon-like peptide-1 receptor (GLP-1R). GLP-1R-deficient [*Glp-1R*(−/−)] mice were treated with metoprolol and atropine to measure intrinsic HR. Saline induced a minimal response in intrinsic HR of *Glp-1R*(−/−) mice, whereas OXM elevated intrinsic HR ~200 beats/min. This finding indicates that OXM does not exert its HR effect via the GLP-1R. *$P < 0.05$ vs. saline.

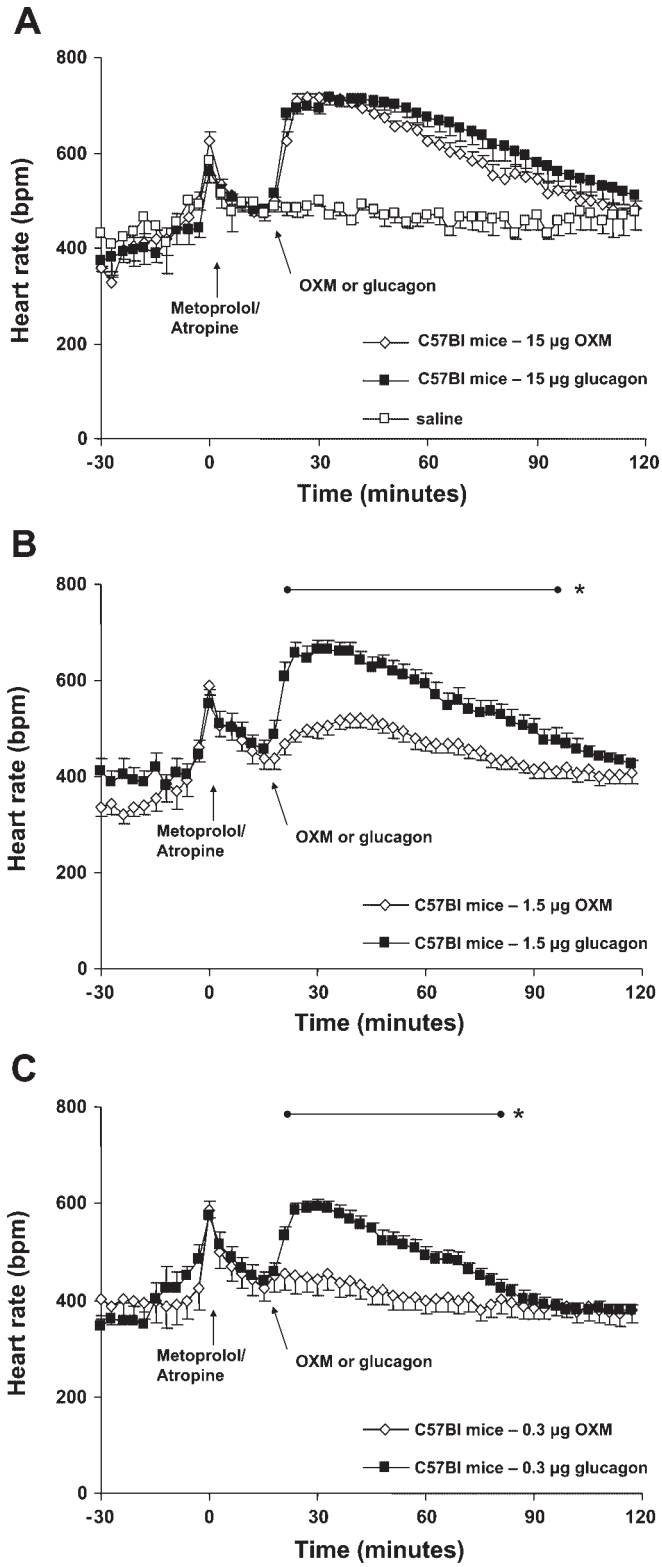

Fig. 5. Glucagon elevates intrinsic HR in mice at lower doses to a greater extent than OXM. C57Bl mice were pretreated with metoprolol and atropine to measure intrinsic HR. The mice were injected with either glucagon or OXM at 1 of 3 doses: 15 μg (*A*), 1.5 μg (*B*), or 0.3 μg (*C*). *$P < 0.05$ vs.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

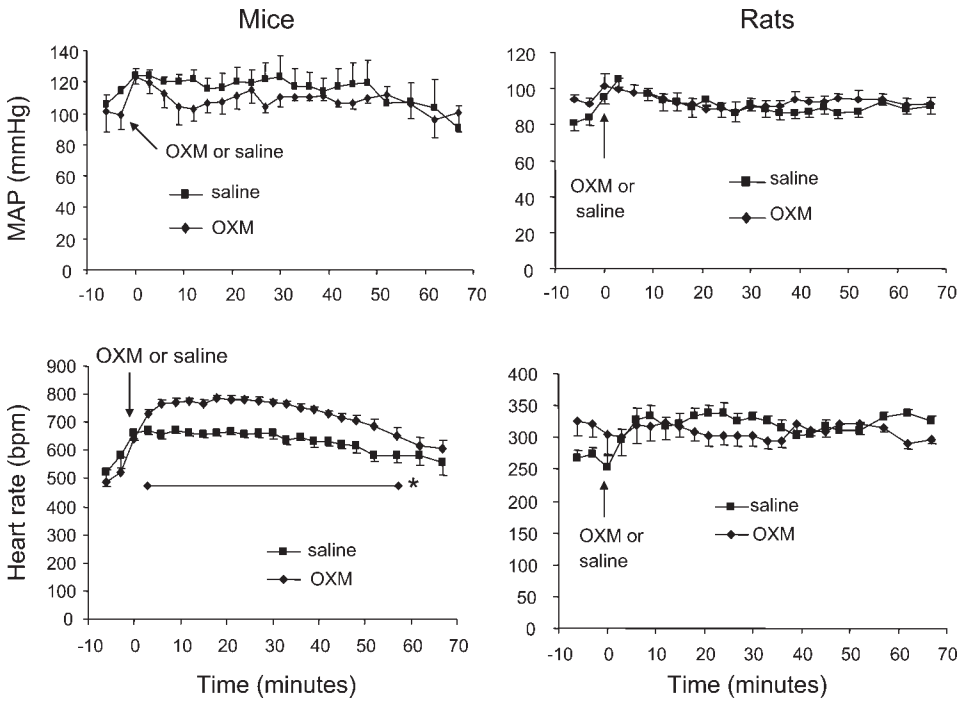

Fig. 6. OXM does not alter blood pressure in mice or rats. C57Bl mice and Sprague-Dawley rats were implanted with blood pressure telemeters to measure mean blood pressure and HR. OXM did not significantly alter blood pressure in either mice or rats. OXM elevated HR only in mice, not in rats. MAP, mean arterial blood pressure. *$P < 0.05$ vs. saline.

detectable response at the lowest dose used (0.3 μg). Glucagon (15 μg) also increased intrinsic HR in mice by over 200 beats/min in a similar manner to that of OXM (Fig. 5*A*). At the lower concentrations of 1.5 and 0.3 μg, glucagon elicited a higher intrinsic HR than OXM (Fig. 5, *B* and *C*).

OXM has no effect on blood pressure in mice or rats. To determine whether OXM can influence blood pressure, we implanted a new set of 6-mo-old C57Bl mice ($n = 6$) with blood pressure telemeters. Although HR was elevated in mice injected with OXM (Fig. 6) as shown previously, mean blood pressure (Fig. 6) was not different when mice were injected with OXM or saline. The mean blood pressure and HR of Sprague-Dawley rats ($n = 5$) were also measured with telemetry. Surprisingly, OXM had no effect on either HR or blood pressure of these rats (Fig. 6).

Ex-4 effects are rat specific. Because others have shown that GLP-1 agonists influence HR in rats (4, 48), we wanted to confirm this using Ex-4, a potent GLP-1 agonist, in both mice and rats. In rats, the intrinsic HR of 302 ± 9 beats/min was elevated by 55 ± 15 beats/min after injection with Ex-4 (Fig. 7). We found that the HR of mice did not respond to Ex-4 (Fig. 7), further supporting the hypothesis that the GLP-1R 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 ANS (Fig. 8, *top*). However, OXM induced a significant drop in T_b of 0.7 ± 0.2°C in autonomically blocked 6-mo-old C57Bl mice (Fig. 8, *bottom*) and autonomically blocked 3-mo-old C57Bl (data not shown). This hypothermic effect was also observed in *Dbh*(−/−) mice, with a drop in core T_b of 1.1 ± 0.3°C (Fig. 8, *bottom*). Importantly, the fall in T_b in response to OXM administration was not observed in autonomically blocked *Glp-1R*(−/−) mice (Fig. 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 12–24 min after peripheral OXM treatment, independently of whether they were autonomically

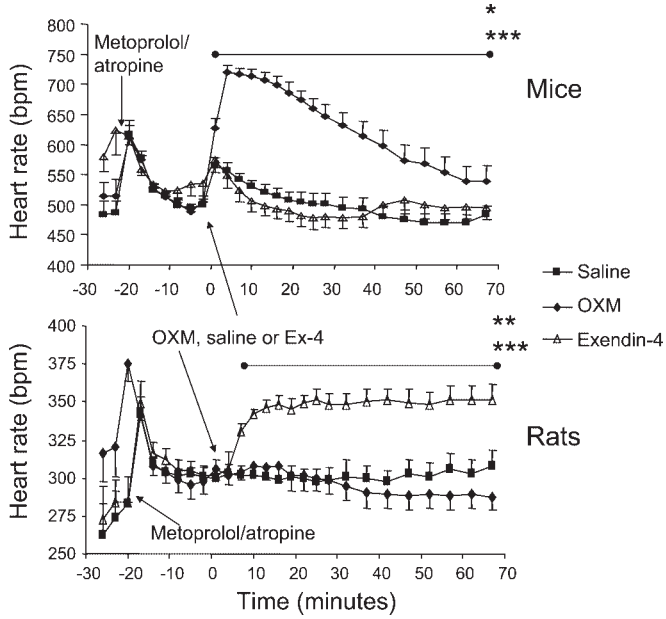

Fig. 7. Exendin-4 (Ex-4) only alters intrinsic HR in rats. Mice and rats were pretreated with metoprolol and atropine to measure intrinsic HR. After 20 min, animals were injected with saline, OXM, or Ex-4. Whereas OXM elevated intrinsic HR in mice, as shown previously, it had no effect on HR in rats. Similarly, Ex-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.

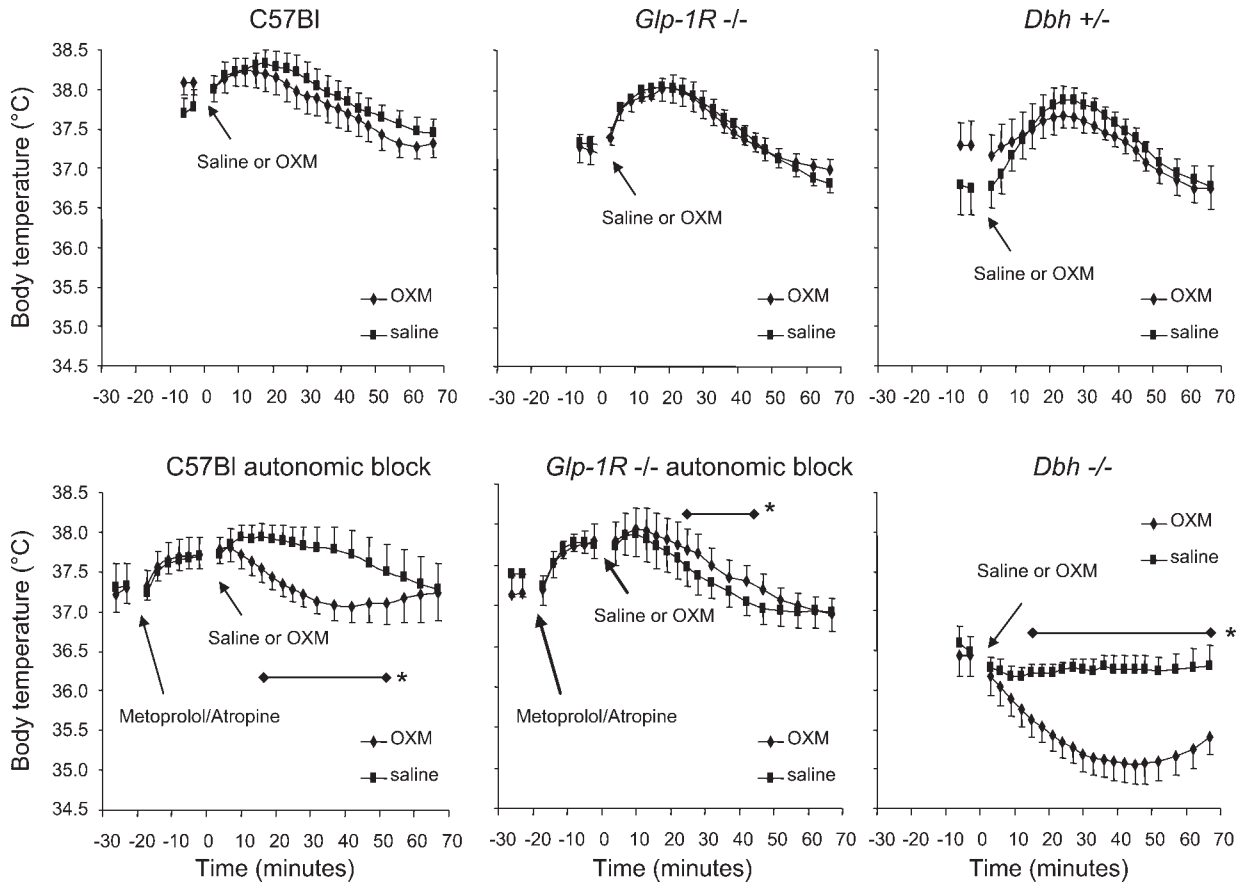

Fig. 8. OXM-induced hypothermia requires the GLP-1R. *Top*: OXM or saline was administered to C57Bl, *Glp-1R*(−/−), and *Dbh*(+/−) mice. Both saline and OXM induced a transient elevation in core body temperature (T_b), followed by a fall in T_b. Core T_b after OXM or saline was not significantly different in these mice. *Bottom*: OXM or saline was administered to autonomically blocked C57Bl and autonomically blocked *Glp-1R*(−/−) mice, as well as to *Dbh*(−/−) mice, which are deficient in sympathetic nervous system neurotransmitters. OXM induced significant hypothermia in autonomic nervous system-blocked wild-type and *Dbh*(−/−) mice but not in *Glp-1R*(−/−) mice, suggesting that OXM-induced hypothermia is mediated via the GLP-1R. *$P < 0.05$ vs. saline.

blocked (Fig. 9*A*) or not (data not shown). Importantly, OXM-induced inactivity was not observed in *Glp-1R*(−/−) mice (Fig. 9*B*), suggesting that this, like the drop in T_b, is mediated through the GLP-1R. Ex-4 also induced inactivity in C57Bl mice but not in *Glp-1R*(−/−) mice (Fig. 9*B*).

Peripheral OXM inhibits food intake in mice. Previous research has shown that intraperitoneal administration of OXM does not impact cumulative food intake over a period of 2 h (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 intraperitoneal administration of OXM in mice over shorter time periods. *Glp-1R*(−/−) mice and their control C57Bl mice received 22 μg of OXM or vehicle intraperitoneally after a 15-h fast. Food intake was significantly suppressed for 60 min after administration of OXM compared with after vehicle in wild-type mice, which was not observed in *Glp-1R*(−/−) mice (Fig. 10). Ex-4 (2.5 μg) invoked a similar but more potent suppression of food intake in C57Bl mice (Fig. 10), as has been shown previously (2). This effect of Ex-4 also was not observed in *Glp-1R*(−/−) mice. At the lower dose of 15 μg of OXM, food intake was significantly inhibited in C57Bl mice for 30 min (data not shown).

DISCUSSION

Although 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 have shown that peripheral OXM vigorously increases intrinsic HR in mice independently of the GLP-1R. 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 μg) results in physiological, subpharmacological, or pharmacological levels of circulating OXM compared with postprandial levels. The dose chosen is within the range reported by others using peripheral injections in animals studies (14). Furthermore, a low dose of 1.5 μg, which is similar to that chosen for weight loss in human studies (45), evoked a cardiovascular response greater than that achieved with saline (Fig. 1).

Because GLP-1 elevates HR in rats independently of the SNS (3), we hypothesized that OXM would have a similar tachycardic effect on HR in mice independently of the SNS. Since the HR effects of OXM are seen in wild-type mice

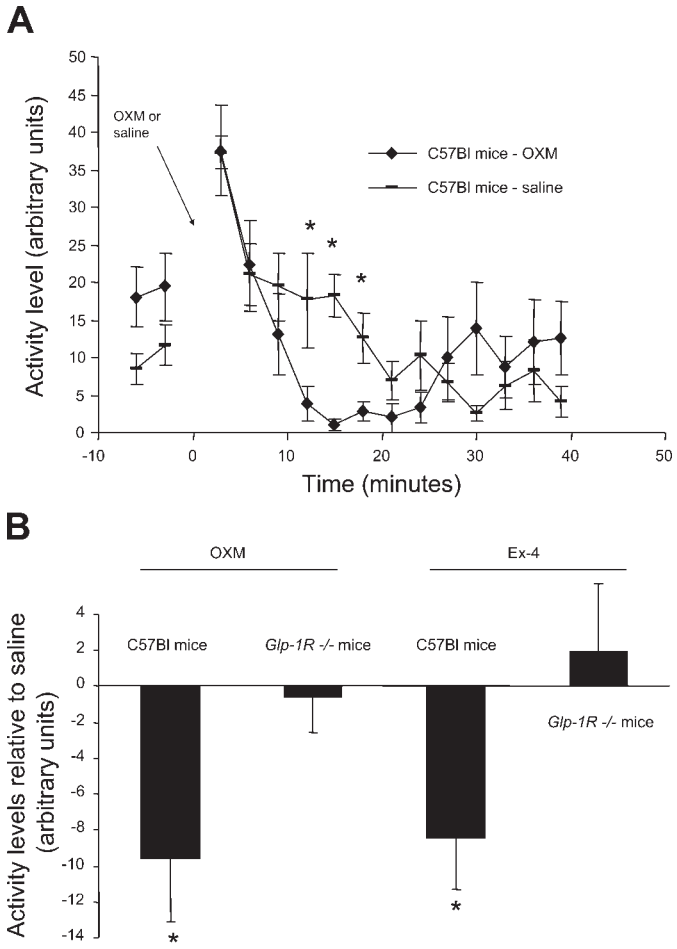

Fig. 9. Transient inactivity induced by OXM requires the GLP-1R. *A*: general cage activity levels in C57Bl mice were quantified using Data Sciences International software. These mice demonstrated significantly lower activity levels in the first 12–24 min after OXM injection than after saline injection. *$P < 0.05$ vs. saline. *B*: average activity levels in C57Bl and *Glp-1R*(−/−) mice were quantified over this same time frame after OXM or Ex-4 injection. Cage activity was significantly less after OXM and Ex-4 administration compared with saline in C57Bl mice but not in *Glp-1R*(−/−) mice, indicating that these peptides suppress activity via the GLP-1R. *$P < 0.05$ vs. saline.

pretreated with metoprolol and atropine, as well as in *Dbh*(−/−) 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 also can 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. Furthermore, we found the magnitude of the HR change in response to OXM was similar (~200 beats/min) 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 >200 beats/min (~40%) in only a few minutes, OXM almost certainly binds receptors directly on the heart. The GLP-1R appears not to be that receptor, because the HR of *Glp-1R*(−/−) mice responded just as robustly as the HR of C57Bl mice to OXM. Given that mice exhibit tachycardia independently of the GLP-1R and that Ex-4 action is likely mediated through the GLP-1R (5), it is not surprising that the HR of mice tested did not respond to Ex-4 (Fig. 7). These data support the notion that OXM functions independently of the GLP-1R at the heart and that the GLP-1R has 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 and 10% affinity for the GCGR compared with that of glucagon (2, 6). Third, we have shown in this study that at the highest dose tested (Fig. 5*A*), the effects of glucagon on intrinsic HR in the mouse are nearly identical in both magnitude and duration to those of OXM on intrinsic HR. Importantly, at the lower doses tested (Fig. 5, *B* and *C*), glucagon had a much greater effect on intrinsic HR than OXM, consistent with the decreased affinity of OXM for the GCGR. These data are a strong indication that OXM elevates intrinsic HR through the GCGR. Contrary to this hypothesis, however, is the fact that rats express the GCGR in the heart (9, 20) but that OXM has no effect on either HR or intrinsic HR of the rat (Figs. 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.

Whereas the HR of rats responds robustly to Ex-4 (Fig. 7 and Refs. 5, 48), the murine HR does not respond to Ex-4 (Fig. 7). However, Ex-4 does evoke other physiological effects in the mouse, including diminished feeding (2, 38) and reversal of hepatic steatosis (16). Similarly, whereas the HR of mice responds vigorously to OXM, the HR of rats does not respond to OXM (Fig. 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

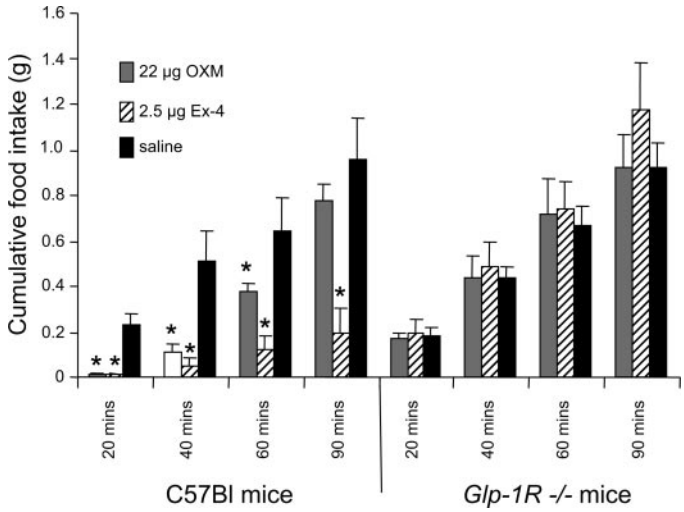

Fig. 10. Peripheral OXM transiently decreases food intake in C57Bl mice and is dependent on the GLP-1R. C57Bl and *Glp-1R*(−/−) mice were fasted for 15 h and injected with 22 μg of OXM, 2.5 μg of Ex-4, or saline at the onset of the light cycle. OXM and Ex-4 significantly inhibited food intake for 60 and 90 min, respectively, in C57Bl mice but had no effect on food intake in *Glp-1R*(−/−) mice. *$P < 0.05$ vs. saline

OXM extends to humans. Recently, Wynne et al. (45) have shown that OXM does not influence HR or blood pressure in humans. 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). In this study we have extended these findings 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 because of the lack of catecholamines (40), and it may be that the metoprolol used to block β_1 receptors on the heart also blocked β_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 (Fig. 9*A*) or the lack of food intake (Fig. 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 (Sowden GL, 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. Although it is clear that OXM increases intrinsic HR independently of the GLP-1R, we expect that further investigation 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

We thank Molly Gutilla and Auyon Mukharji for technical assistance with the experiments in this manuscript.

GRANTS

This work was supported by National Heart, Lung, and Blood Institute Grant R15 HL081101-01 (to S. J. Swoap), grants from the Canadian Institutes of Health Research and the Heart and Stroke Foundation of Canada (to D. J. Drucker), and in part by a Howard Hughes Medical Institute grant to Williams College.

REFERENCES

1. **Anini Y, Jarrousse C, Chariot J, Nagain C, Yanaihara N, Sasaki K, Bernad N, Nguyen DL, Bataille D, Roze C.** Oxyntomodulin inhibits pancreatic secretion through the nervous system in rats. *Pancreas* 20: 348–360, 2000.
2. **Baggio LL, Huang Q, Brown TJ, Drucker DJ.** Oxyntomodulin and glucagon-like peptide-1 differentially regulate murine food intake and energy expenditure. *Gastroenterology* 127: 546–558, 2004.
3. **Barragan JM, Eng J, Rodriguez R, Blazquez E.** Neural contribution to the effect of glucagon-like peptide-1-(7–36) amide on arterial blood pressure in rats. *Am J Physiol Endocrinol Metab* 277: E784–E791, 1999.
4. **Barragan JM, Rodriguez RE, Blazquez E.** Changes in arterial blood pressure and heart rate induced by glucagon-like peptide-1-(7–36) amide in rats. *Am J Physiol Endocrinol Metab* 266: E459–E466, 1994.
5. **Barragan JM, Rodriguez RE, Eng J, Blazquez E.** Interactions of exendin-(9–39) with the effects of glucagon-like peptide-1-(7–36) amide and of exendin-4 on arterial blood pressure and heart rate in rats. *Regul Pept* 67: 63–68, 1996.
6. **Bataille D, Gespach C, Coudray A, Rosselin G.** Enteroglucagon: a specific effect on gastric glands isolated from the rat fundus. Evidence for an "oxyntomodulin" action. *Biosci Rep*: 151–155, 1981.
7. **Bataille D, Tatemoto K, Gespach C, Jörnvall H, Rosselin G, Mutt V.** Isolation of glucagon-37 (bioactive enteroglucagon/oxyntomodulin) from porcine jejuno-ileum: characterization of the peptide. *FEBS Lett* 146: 79–86, 1982.
8. **Bullock B, Heller R, Habener J.** Tissue distribution of messenger ribonucleic acid encoding the rat glucagon-like peptide-1 receptor. *Endocrinology* 137: 2968–2978, 1996.
9. **Burcelin R, Li J, Charron MJ.** Cloning and sequence analysis of the murine glucagon receptor-encoding gene. *Gene* 164: 305–310, 1995.
10. **Clark C Jr.** Evidence that cyclic AMP is not involved in the chronotropic action of glucagon in the adult mouse heart. *Endocrinology* 99: 23–29, 1976.
11. **Cohen MA, Ellis SM, Le Roux CW, Batterham RL, Park A, Patterson M, Frost GS, Ghatei MA, Bloom SR.** Oxyntomodulin suppresses appetite and reduces food intake in humans. *J Clin Endocrinol Metab* 88: 4696–4701, 2003.
12. **Collie NL, Zhu Z, Jordan S, Reeve JR.** Oxyntomodulin stimulates intestinal glucose uptake in rats. *Gastroenterology* 112: 1961–1970, 1997.
13. **Dakin CL, Gunn I, Small CJ, Edwards CMB, Hay DL, Smith DM, Ghatei MA, Bloom SR.** Oxyntomodulin inhibits food intake in the rat. *Endocrinology* 142: 4244–4250, 2001.
14. **Dakin CL, Small CJ, Batterham RL, Neary NM, Cohen MA, Patterson M, Ghatei MA, Bloom SR.** Peripheral oxyntomodulin reduces food intake and body weight gain in rats. *Endocrinology* 145: 2687–2695, 2004.
15. **Dakin CL, Small CJ, Park AJ, Seth A, Ghatei MA, Bloom SR.** Repeated ICV administration of oxyntomodulin causes a greater reduction in body weight gain than in pair-fed rats. *Am J Physiol Endocrinol Metab* 283: E1173–E1177, 2002.
16. **Ding X, Saxena NK, Lin S, Gupta N, Anania FA.** Exendin-4, a glucagon-like protein-1 (GLP-1) receptor agonist, reverses hepatic steatosis in ob/ob mice. *Hepatology* 43: 173–181, 2006.
17. **Druce MR, Ghatei M.** Oxyntomodulin. *Curr Opin Endocrinol Diabetes* 13: 49–55, 2006.
18. **Foley CM, McAllister RM, Hasser EM.** Thyroid status influences baroreflex function and autonomic contributions to arterial pressure and heart rate. *Am J Physiol Heart Circ Physiol* 280: H2061–H2068, 2001.
19. **Gordon CJ.** *Temperature Regulation in Laboratory Rodents.* New York: Cambridge University Press, 1993.
20. **Hansen LH, Abrahamsen N, Nishimura E.** Glucagon receptor mRNA distribution in rat tissues. *Peptides* 16: 1163–1166, 1995.
21. **Hicks KK, Seifen E, Stimers JR, Kennedy RH.** Effects of streptozotocin-induced diabetes on heart rate, blood pressure and cardiac autonomic nervous control. *J Auton Nerv Syst* 69: 21–30, 1998.
22. **Holst JJ.** Enteroglucagon. *Annu Rev Physiol*: 257–271, 1997.
23. **Janssen BJA, Smits JFM.** Autonomic control of blood pressure in mice: basic physiology and effects of genetic modification. *Am J Physiol Regul Integr Comp Physiol* 282: R1545–R1564, 2002.
24. **Jarrousse C, Audousset-Puech MP, Dubrasquet M, Niel H, Martinez J, Bataille D.** Oxyntomodulin (glucagon-37) and its C-terminal octapeptide inhibit gastric acid secretion. *FEBS Lett* 188: 81–84, 1985.
25. **Jarrousse C, Bataille D, Jeanrenaud B.** A pure enteroglucagon, oxyntomodulin (glucagon 37), stimulates insulin release in perfused rat pancreas. *Endocrinology* 115: 102–105, 1984.
26. **Landsberg L.** Insulin-mediated sympathetic stimulation: role in the pathogenesis of obesity-related hypertension (or, how insulin affects blood pressure, and why). *J Hypertens* 19: 523–528, 2001.
27. **Lowell BB, Bachman ES.** β-Adrenergic receptors, diet-induced thermogenesis, and obesity. *J Biol Chem* 278: 29385–29388, 2003.
28. **Osaka T, Endo M, Yamakawa M, Inoue S.** Energy expenditure by intravenous administration of glucagon-like peptide-1 mediated by the lower brainstem and sympathoadrenal system. *Peptides* 26: 1623–1631, 2005.
29. **O'Shea DGI, Chen X, Bloom S, Herbert J.** A role for central glucagon-like peptide-1 in temperature regulation. *Neuroreport* 7: 830–832, 1996.

30. **Safa-Tisseront V, Ponchon P, Laude D, Elghozi JL.** Contribution of the autonomic nervous system to blood pressure and heart rate variability changes in early experimental hyperthyroidism. *Eur J Pharmacol* 352: 247–255, 1998.
31. **Schjoldager BT, Baldissera FG, Mortensen PE, Holst JJ, Christiansen J.** Oxyntomodulin: a potential hormone from the distal gut. Pharmacokinetics and effects on gastric acid and insulin secretion in man. *Eur J Clin Invest* 18: 499–503, 1988.
32. **Schjoldager BT, Mortensen PE, Myhre J, Christiansen J, Holst JJ.** Oxyntomodulin from distal gut. Role in regulation of gastric and pancreatic functions. *Dig Dis Sci* 34: 1411–1419, 1989.
33. **Scrocchi LA, Brown TJ, MaClusky N, Brubaker PL, Auerbach AB, Joyner AL, Drucker DJ.** Glucose intolerance but normal satiety in mice with a null mutation in the glucagon-like peptide 1 receptor gene. *Nat Med* 2: 1254–1258, 1996.
34. **Shughrue P, Lane M, Merchenthaler I.** Glucagon-like peptide-1 receptor (GLP1-R) mRNA in the rat hypothalamus. *Endocrinology* 137: 5159–5162, 1996.
35. **Swoap SJ.** Altered leptin signaling is sufficient, but not required, for hypotension associated with caloric restriction. *Am J Physiol Heart Circ Physiol* 281: H2473–H2479, 2001.
36. **Swoap SJ, Weinshenker D, Palmiter RD, Garber G.** Dbh(−/−) mice are hypotensive, have altered circadian rhythms, and have abnormal responses to dieting and stress. *Am J Physiol Regul Integr Comp Physiol* 286: R108–R113, 2004.
37. **Takayama K, Yuhki Ki Ono K, Fujino T, Hara A, Yamada T, Kuriyama S, Karibe H, Okada Y, Takahata O, Taniguchi T, Iijima T, Iwasaki H, Narumiya S, Ushikubi F.** Thromboxane A_2 and prostaglandin $F_{2\alpha}$ mediate inflammatory tachycardia. *Nat Med* 11: 562–566, 2005.
38. **Talsania T, Anini Y, Siu S, Drucker DJ, Brubaker PL.** Peripheral exendin-4 and peptide YY3–36 synergistically reduce food intake through different mechanisms in mice. *Endocrinology* 146: 3748–3756, 2005.
39. **Thomas SA, Matsumoto AM, Palmiter RD.** Noradrenaline is essential for mouse fetal development. *Nature* 374: 643–646, 1995.
40. **Thomas SA, Palmiter RD.** Thermoregulatory and metabolic phenotypes of mice lacking noradrenaline and adrenaline. *Nature* 387: 94–97, 1997.
41. **Uesaka T, Yano K, Sugimoto S, Ando M.** Glucagon-like peptide isolated from the eel intestine: effects on atrial beating. *J Exp Biol* 204: 3019–3026, 2001.
42. **Uttenthal LOTA, Blazquez E.** Autoradiographic localization of receptors for glucagon-like peptide-1 (7–36) amide in rat brain. *Neuropeptides* 21: 143–146, 1992.
43. **Vila Petroff MG, Egan JM, Wang X, Sollott SJ.** Glucagon-like peptide-1 increases cAMP but fails to augment contraction in adult rat cardiac myocytes. *Circ Res* 89: 445–452, 2001.
44. **Wei Y, Mojsov S.** Tissue-specific expression of the human receptor for glucagon-like peptide-I: brain, heart and pancreatic forms have the same deduced amino acid sequences. *FEBS Lett* 358: 219–224, 1995.
45. **Wynne K, Park AJ, Small CJ, Meeran K, Ghatei MA, Frost GS, Bloom SR.** Oxyntomodulin increases energy expenditure in addition to decreasing energy intake in overweight and obese humans: a randomised controlled trial. *Int J Obes* 30: 1729–1736, 2006.
46. **Wynne K, Park AJ, Small CJ, Patterson M, Ellis SM, Murphy KG, Wren AM, Frost GS, Meeran K, Ghatei MA, Bloom SR.** Subcutaneous oxyntomodulin reduces body weight in overweight and obese subjects: a double-blind, randomized, controlled trial. *Diabetes* 54: 2390–2395, 2005.
47. **Wynne K, Stanley S, McGowan B, Bloom S.** Appetite control. *J Endocrinol* 184: 291–318, 2005.
48. **Yamamoto H, Lee CE, Marcus JN, Williams TD, Overton JM, Lopez ME, Hollenberg AN, Baggio L, Saper CB, Drucker DJ, Elmquist JK.** Glucagon-like peptide-1 receptor stimulation increases blood pressure and heart rate and activates autonomic regulatory neurons. *J Clin Invest* 110: 43–52, 2002.