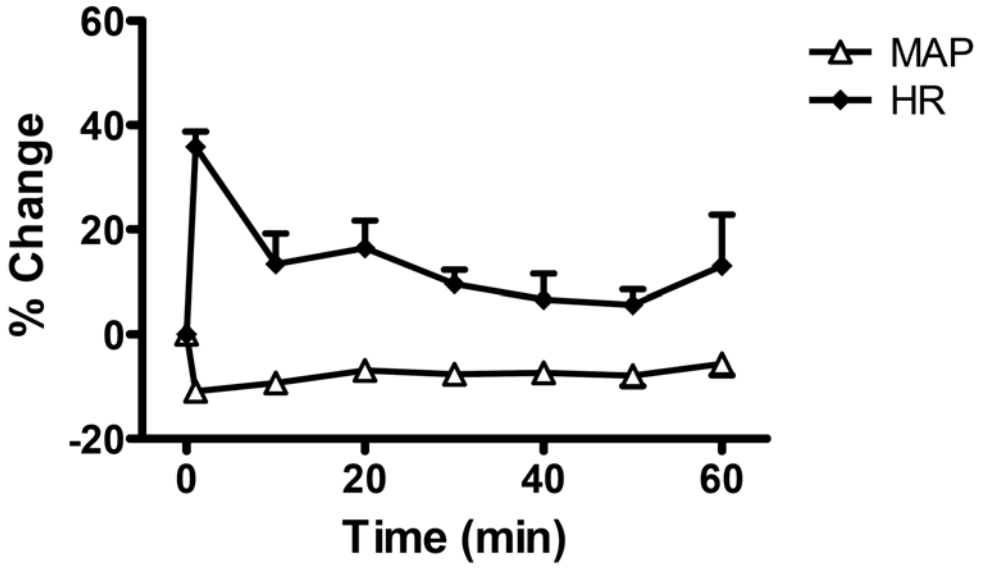
SUPPLEMENTARY DATA

Supplementary Figure 1. **Effect of phentolamine on mean arterial pressure and heart rate.** Mean % change in mean arterial pressure (MAP) and heart rate (HR) in adult male Wistar rats that received an i.v. bolus (0.2 mg/kg) followed by continuous infusion (5 μg/kg/min) of the α-adrenergic receptor antagonist, phentolamine.



SUPPLEMENTARY DATA

Frequently sampled intravenous glucose tolerance test (FSIGT)

After an overnight fast, multiple blood samples were obtained from unrestrained, conscious animals via an arterial catheter, as previously described (20-23). Baseline samples were obtained at -10 and -1 min. A bolus of 50% dextrose (1 g/kg body weight) was then injected i.v. over a period of 15 sec beginning at t = 0 min. Blood (100 μl) was sampled for measurement of both glucose (using a hand-held glucometer; Nova Max plus, Nova Diabetes Care, Billercia, MA) and for subsequent assay of plasma insulin levels at time points 2, 5, 8, 12, 20, 30 and 60 min after glucose injection. Additional samples were obtained for blood glucose measurement at 1, 3, 4, 6, 10, 14, 16, 18, 25, 40 and 50 min.

Minimal Model Analysis and Calculations

Plasma insulin and blood glucose levels generated from the FSIGTs were analyzed using MinMod software to quantify S_G and S_I as previously described (20-23). The AIR_G was calculated as the mean increment above basal of insulin values measured between t = 0 - 4 min, and the disposition index (DI, which provides a measure of the overall contribution made by insulin to disposal of a glucose load) was computed as the product of S_I and AIR_G. All S_I calculations are multiplied by 10^4. For comparison purposes, we also estimated the insulin response to glucose as the incremental area under the insulin curve during the FSIGT ($AUC_{insulin}$). The basal insulin effect (BIE) was calculated as the product of basal insulin (I_{basal}; mU/L) and S_I, while glucose effectiveness at zero insulin (GEZI) was calculated as S_G minus the BIE. Intravenous glucose tolerance was estimated from the incremental area under the glucose curve ($AUC_{glucose}$) during the FSIGT and the glucose disappearance rate constant (K_G) calculated as the slope of the natural logarithm of blood glucose values from t = 4 - 20 min, expressed as percent change per minute (20,23,24).

Euglycemic-Hyperinsulinemic Clamp

Adult male Wistar rats bearing catheters to the right jugular vein and left carotid artery were subjected to an euglycemic-hyperinsulinemic clamp as previously described (21,22). Briefly, animals were exposed to the cold (5°C) for 28h without access to food and placed into a clear animal enclosure with bedding and connected to a rat infusion system (Instech Solomon, Plymouth Meeting, PA) to allow simultaneous sampling from the artery and infusion into the vein in conscious, unrestrained animals. A 24μCi prime of [3-^{3}H] glucose was given at t = -90 min for 3 min followed by a continuous 0.2μCi/min infusion for 90 min. At t = -30 min, animals received a continuous i.v. infusion of either phentolamine (8 μg/kg/min) or its vehicle. Following the basal period, a primed continuous infusion of regular human insulin (16 mU/kg bolus followed by 2 mU/kg/min HumulinR; EliLilly, Indianapolis, IN) was administered at t = 0 min. The [3-^{3}H] glucose infusion was increased to 0.3μCi/min (at t = 0 min) for the remainder of the experiment (t = 120 min) to keep specific activity constant. Blood glucose levels were determined every 10 min using a handheld glucometer during the clamp period (Nova Max plus, Nova Diabetes Care, Billercia, MA) and a 50% dextrose solution was infused as required to maintain euglycemia. The glucose infusion rate (GIR) needed to maintain euglycemia is a reflection of insulin action. Plasma insulin levels and glucose turnover rates were calculated from 80-μl blood samples drawn at 10-min intervals during the last 30 min of both the basal period and the clamp period.