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Insulin attenuates the acquisition and expression of ethanol-induced locomotor sensitization in DBA/2J mice

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Abstract

Aims—Ethanol-induced locomotor sensitization is a behavioral manifestation of physiological responses to repeated ethanol exposures. While ethanol exerts direct effects on multiple neurotransmitter systems in the brain, ethanol-induced changes in metabolic state, including acute hyperglycemia and inhibition of insulin signaling, also have plausible roles in the expression of ethanol-related behaviors through direct and indirect effects on brain function. The current experiments examined whether insulin administration or the resultant hypoglycemia might attenuate the development of sensitization to the locomotor stimulant effect of ethanol.

Main methods—Male and female DBA/2J mice received daily injections of 5 or 10 IU/kg insulin before or after a stimulating dose of ethanol and subsequent testing in an automated activity monitor. Blood glucose levels were determined upon the completion of the experiments.

Key findings—Insulin injected prior to ethanol blunted the acute stimulant response as well as the acquisition and expression of locomotor sensitization, while insulin given after ethanol did not affect the development of the sensitized response. In a separate experiment, mice given glucose concurrently with insulin developed ethanol-induced locomotor sensitization normally.

Significance—These experiments suggest that insulin attenuates the development of ethanol-induced locomotor sensitization, and that blood glucose levels can largely account for this effect. Further studies of the role of ethanol-induced metabolic states should provide novel information on the expression of ethanol-related behaviors.

Keywords

alcohol; mice; sensitization; behavior; insulin; locomotion

Introduction

In studies of ethanol-related behaviors, the effects of ethanol on the metabolic state of the organism are generally considered secondary to direct effects of the drug on the brain. Acute ethanol administration in normally feeding rodents results in a mild but prolonged

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hyperglycemia (Oliveira de Souza and Masur, 1981; Souza and Masur, 1982, 1984; Risinger and Cunningham, 1991, 1992; Risinger, 2003), due in part to increased glycogenolysis and decreased glyconeogenesis (see van de Wiel, 2004 for review). Ethanol also acutely inhibits signaling through the insulin receptor (e.g. Xu et al., 1995; Xu et al., 2003; de la Monte et al., 2005; He et al., 2006), and physical dependence on ethanol is associated with increased incidence of cardiovascular disease and Type II diabetes (see van de Wiel, 2004 for review). While altered insulin signaling and deleterious effects on metabolism have generally been considered as effects of ethanol dependence, it is possible that they are also causally involved in the expression of ethanol-related behaviors.

Insulin receptors are expressed throughout the brain and regulate hippocampal long-term potentiation and depression (LTP and LTD), monoamine transporter expression, and behavior, including feeding and measures of learning and memory (Wozniak et al., 1993; Schulingkamp et al., 2000; Plum et al., 2005; Wada et al., 2005; Figlewicz and Benoit, 2009). However, very few studies have examined whether insulin signaling might affect ethanol-related behaviors. In one of these studies (Goas et al., 1979), insulin at 5 IU/kg produced a relatively selective decrease in ethanol intake in C57BL/6J mice, while a higher dose nonselectively reduced the intake of ethanol and water. Similarly, drugs that increase insulin release or increase receptor affinity including tolbutamide, phenformin, and glibenclamide have been reported to reduce ethanol consumption and preference in rats (Connelly et al., 1983; Grupp et al., 1997), while ablation of the insulin-producing pancreatic beta cells with streptozotocin has been shown to result in a non-significant increase in ethanol consumption (Connelly et al., 1983). These results are intriguing as systemically administered insulin produces a pronounced hyperphagia (Strubbe and Mein, 1977; Rowland and Engle, 1978; Carruba et al., 1985; Beczkowska and Bodnar, 1991; Obici et al., 2002), while insulin administered directly to the CNS decreases food intake (see Pliquet et al, 2006; Hillebrand et al 2002 for reviews), which might suggest that insulin differentially regulates the intake of food and ethanol. Finally, using experimenter-administered insulin and ethanol, Duncan (2001) suggested that insulin and ethanol interact to decrease locomotion in outbred rats, a finding that could be somewhat dissociated from insulin-induced hypoglycemia. These results might be indicative of a more causal role for insulin signaling in behavioral responses to ethanol.

In many mouse strains and some rat lines, low doses of ethanol produce locomotor stimulation, while higher doses result in sedation (e.g. Phillips, 1993; Agabio et al., 2001; Rodd et al., 2004; Meyer et al., 2009). As with traditional stimulants, such as cocaine and amphetamine, the acute locomotor stimulant response to ethanol sensitizes, resulting in dramatically increased locomotion in response to the same dose following repeated injections (e.g. Fee et al., 2007; Stevenson et al., 2008; see Camarini et al, 2010 and Phillips & Shen, 1996 for reviews).

Ethanol-induced locomotor sensitization reflects neurophysiological adaptations to chronic ethanol exposure, while allowing for repeated behavioral measurements on the same animal. If an ethanol-induced change in metabolic state, such as hyperglycemia, was involved in the development of the sensitized locomotor response, it might be expected that the hyperglycemic response would concomitantly sensitize with locomotion, evidenced as a correlation between ethanol-induced locomotion and blood glucose levels across trials. Such a finding might indicate that metabolic changes induced by ethanol partially underlie the observed behavioral effects, indicating a potential mechanism underlying locomotor sensitization. Further, blocking the response should antagonize the development of locomotor sensitization, an effect that occurs across or between the sensitization trials. The current experiments tested whether insulin or the resultant hypoglycemic state might attenuate the acquisition and expression of ethanol-induced locomotor sensitization in DBA/

2J mice, a mouse strain that shows robust ethanol-stimulated locomotion and develops locomotor sensitization (e.g. Melón & Boehm, 2011)

Materials and Methods

Subjects—Male and female DBA/2J mice, 50–80 days old, were obtained from The Jackson Laboratory (Bar Harbor, ME). The mice were housed 4–5 per cage and allowed to acclimate in the vivarium for a minimum of two weeks prior to the start of the experiments, with food (Purina 5001) and water freely available. The mice were maintained on a standard 12:12 light/dark cycle (lights on at 7:00 am) throughout the experiment, and behavior was measured in the mornings. Mice were pseudorandomly assigned to conditions such that when possible, all treatments were represented within each cage. In all experiments, each treatment group contained 9–11 mice, with approximately equal representation of males and females in each group. All experiments were approved by the Ernest Gallo Clinic and Research Center IACUC.

Statistics—The dependent measures from all experiments were initially analyzed by ANOVA (sex by insulin dose by ethanol dose). No significant interactions between sex and the other factors were found for any dependent variable measured, so the data from male and female mice were pooled to simplify the analysis. Results are presented separately for each experiment. Post hoc analysis of main effects and interactions used Tukey's HSD test. All effects were considered significant at $p < 0.05$.

Experiment 1: The effects of insulin given prior to ethanol on the acquisition and expression of ethanol-induced locomotor sensitization

Methods—A protocol similar to that described by Meyer and Phillips (2003) was used to induce ethanol-induced locomotor sensitization in mice. Mice were transported to the procedure room and allowed to acclimate for a minimum of one hour prior to testing. During each of the first three days of the experiment, the mice were weighed, injected with saline, and placed into an automated activity monitor (Accuscan Instruments, Columbus, OH). Locomotor activity (total distance traveled) was recorded for 15 min. Beginning on day 4 of the experiment, the mice were weighed, injected IP with saline or regular human insulin (5 or 10 IU/kg; Novolin; Novo Nordisk, Bagsværd, Denmark) 30 min prior to an injection of 0 or 2 g/kg ethanol (absolute ethanol, Gold Shield Chemical Co., Hayward, CA prepared as 20% v/v in saline) and placed into the activity monitors for 15 min. This procedure was repeated on days 7, 10, and 13 of the experiment. On the intervening days, the mice received the same injections but were placed back into the home cages. In this protocol, the acquisition of locomotor sensitization is evidenced as an increase in the stimulant effects of ethanol on day 13 (the final ethanol injection) minus that on day 4 (the first ethanol injection). This value is referred to as the sensitization score.

To determine whether insulin might also affect the expression of ethanol-induced locomotor sensitization, ethanol-sensitized and ethanol-naïve mice (both groups insulin-naïve) were given a single injection of 0, 5, or 10 IU/kg insulin 30-min prior to a 0 or 2 g/kg ethanol injection and placement in an activity monitor.

The expression test occurred on day 16 of the experiment, two days after the completion of the acquisition portion of the experiment. Additional groups of mice naïve and treated mice served as controls for the acute and chronic effects of ethanol and insulin, and are described in Results below. The experimental design for the expression test included all groups necessary to make the comparisons reported, but was not designed as a fully factorial experiment due to the large number of experimental groups required. The treatment each

group of mice was given during the acquisition and expression portions of the experiment were therefore combined and differences among the treatment groups analyzed by one-way ANOVA.

Following the expression test, the mice were placed in holding cages with no food or water, and a small sample of blood was collected from the tip of the tail 60 min after the insulin or saline injection (30 min after ethanol). This sample was then read on a hand-held, instant read glucose meter (Ascensia Elite XL; Bayer AG, Leverkusen, Germany) to determine blood glucose level.

Results

Effect of insulin on the acquisition of locomotor sensitization: The overall result for the effect of insulin on the acquisition of ethanol-induced locomotor sensitization is shown in Figure 1, and the summary sensitization score shown in Figure 2A. Overall, a robust increase in locomotor activity was seen in mice that received daily injections of ethanol, while locomotion in mice given saline did not change across days. Analysis of the sensitization score (activity on day 13 - day 4; see Methods) yielded main effects of ethanol ($F_{1, 122} = 5.69$; $p < 0.05$) and insulin ($F_{2, 122} = 16.83$; $p < 0.001$), as well as a significant interaction between the two ($F_{2, 122} = 8.62$; $p < 0.001$). This interaction was due in part to significantly lower sensitization scores in ethanol-treated mice given 5 or 10 IU/kg insulin compared with mice given ethanol alone ($ps < 0.0001$; see Figure 2A). Mice treated with insulin alone did not differ significantly from the saline group on this score ($ps > 0.8$). While the scores were somewhat lower by the fourth locomotor activity test (day 13 in Figure 1), the means of insulin-alone treated mice still did not differ from saline controls at either dose ($ps > 0.17$; see Figure 1).

Insulin also appeared to attenuate the acute locomotor stimulant response to ethanol (Day 4 in Figure 1), and this effect is shown in Figure 2B. Analysis of the results from this day revealed main effects of insulin ($F_{2, 126} = 14.12$; $p < 0.001$), ethanol ($F_{1, 126} = 33.5$; $p < 0.001$) and a significant insulin by ethanol interaction ($F_{2, 126} = 3.22$; $p < 0.05$). This interaction resulted in part from significantly decreased activity in ethanol-treated mice given 5 or 10 IU/kg insulin compared with mice given ethanol alone ($ps < 0.05$). Insulin alone at either dose did not significantly affect locomotion relative to the saline-only control ($ps > 0.52$).

Effect of insulin on the expression of locomotor sensitization: The effects of insulin on the expression of ethanol-induced locomotor sensitization are shown in Figure 3A. The treatments the mice were given during the acquisition of ethanol-induced locomotor sensitization are indicated across the top of the graph, and the treatments given during the expression test are shown along the X-axis. Analysis of the total distance traveled yielded a main effect of group ($F_{8, 82} = 11.49$; $p < 0.0001$). Post-hoc tests among the ethanol-sensitized groups revealed significantly lesser distances traveled by ethanol-sensitized mice given insulin (5 or 10 IU/kg) compared with insulin-naïve, sensitized mice ($ps < 0.01$). As was seen on day 4 of the acquisition experiment, post hoc tests also found that insulin at either dose blunted the acute stimulant response to ethanol ($ps < 0.01$), but insulin by itself did not decrease locomotor activity ($ps > 0.9$).

Effect of ethanol and insulin on blood glucose levels: Blood glucose concentrations assayed 1 hr after the initial insulin injection are shown in Figure 3B. Analysis of blood glucose levels revealed a main effect of group ($F_{8, 82} = 16.33$; $p < 0.0001$). From the graph, it is particularly evident that all groups that received insulin on the test day exhibited lower blood glucose levels than controls. This was supported by post-hoc testing, which revealed significant differences between insulin naïve mice (control or ethanol-sensitized) compared

with all other insulin-treated groups ($p < 0.01$). Blood glucose levels in mice given an acute ethanol injection were nominally higher compared with saline-injected mice, but his effect did not approach significance ($p > 0.9$).

Experiment 2: The effects of insulin given after ethanol on the acquisition of locomotor sensitization

Methods—In Experiment 1, insulin blocked the acquisition of locomotor sensitization across trials, but at the 5 IU/kg dose, only mildly attenuated the acute locomotor stimulant effect of ethanol. This finding might indicate that insulin have interfered with an inter-trial process necessary for the development of locomotor sensitization, such as memory consolidation. If this were the case, one might predict that insulin given between the ethanol conditioning trials should still block the development of locomotor sensitization. To test this possibility, male and female DBA/2J mice were treated identically as described for Experiment 1, except that insulin (0, 5, or 10 IU/kg) was administered immediately upon removal from the activity monitors on days 4, 7, 10, and 13, or 15-min after ethanol injection on the intervening days.

Results—The overall result from Experiment 2 is shown in Figure 4. As is evident from the graph, insulin given after ethanol did not alter the acquisition of ethanol-induced locomotor sensitization ($F_{2, 57} = 0.8$; $p > 0.45$), indicating that insulin must be on board at the time of the behavioral test in order to attenuate the development of locomotor sensitization to ethanol.

Experiment 3: The effects of glucose and insulin on the acquisition of locomotor sensitization

Methods—To determine whether the effect of insulin seen in Experiment 1 might be explained by insulin-induced hypoglycemia, glucose was co-administered with insulin prior to the ethanol injection. Pilot testing found that 4 g/kg glucose injected with 5 IU/kg insulin was sufficient to maintain normal blood sugar levels for at least 30 min, the time at which ethanol was administered. Experiment 3 was conducted identically to Experiment 1, with the exception that only a single dose of insulin was used, 5 IU/kg, which was administered with or without 4 g/kg glucose 30 min prior to the ethanol injection.

Results—One mouse in ethanol-only group failed to sensitize and its data were removed from the analysis. The overall result for Experiment 3 is depicted in Figure 5. Analysis of the acute ethanol response on day 4 revealed a main effect of ethanol ($F_{1, 69} = 29.76$; $p < 0.001$) and insulin ($F_{1, 69} = 5.93$; $p < 0.05$). No significant two- or three-way interactions were found ($F_s < 2.7$; $p_s > 0.11$; see Figure 5, Day 4).

Analysis of the sensitization score revealed a main effect of ethanol treatment ($F_{1, 69} = 29.98$; $p < 0.0001$), a significant interaction between ethanol and glucose ($F_{1, 69} = 6.54$; $p < 0.05$), and the three-way interaction of insulin, glucose, and ethanol ($F_{1, 69} = 4.06$; $p < 0.05$; see Figure 6A). Post-hoc testing found robust sensitization in mice treated with ethanol alone relative to saline controls ($p < 0.01$), and mice given ethanol and insulin showed a significantly lower sensitization score compared with mice that received ethanol alone ($p < 0.01$). Mice given daily injections of glucose and insulin developed ethanol-induced locomotor sensitization to a level that did not differ from the ethanol-alone or ethanol and glucose groups ($p > 0.5$), indicating that experimenter-administered glucose was sufficient to block the effects of insulin on the development of locomotor sensitization. Analysis of blood glucose levels taken after the test session on day 13 identified all three main effects ($F_{s1, 69} > 6.5$; $p_s < 0.05$), but no significant interactions ($F_{s1, 69} < 0.33$; $p_s > 0.56$; Figure 6B).

Experiment 4: The effects of insulin on ethanol metabolism

Methods—To determine whether altered ethanol metabolism could account for effects of insulin on ethanol-induced locomotor stimulation and sensitization, male DBA/2J mice were injected with 0 or 5 IU/kg insulin 30 min prior to 2 g/kg ethanol ($n = 6$ /insulin dose), and blood samples taken from the tail tip at 15, 30, 90, and 150 min following the ethanol injection. Brain uptake of ethanol was not determined in this experiment. Only the 5 IU/kg dose was examined for effects on ethanol metabolism because this dose was sufficient to block the development of ethanol-induced locomotor sensitization in Experiments 1 and 3, and the effects of this insulin dose were blocked by concurrent glucose administration. Samples were processed as described previously (Carnicella et al., 2009), and ethanol concentrations determined with an NAD/ADH enzymatic kit (Diagnostic Chemicals Limited; Charlottetown, Prince Edward Island, Canada).

Results—The result from Experiment 4 is shown in Figure 7. A repeated measures ANOVA found a main effect of time ($F_{3, 30} = 101.24$; $p < 0.0001$), with lower blood ethanol levels seen at the last time points, but no effect of insulin, or insulin by time interaction was found ($F_s < 1$, $p_s > 0.4$), indicating that insulin did not affect ethanol metabolism.

Discussion

The main findings from the current studies are that insulin attenuates the acute locomotor response to ethanol and blocks the acquisition and expression of ethanol-induced locomotor sensitization. This effect is dependent on the order in which ethanol and insulin are administered: insulin given before but not after ethanol blocks the development of the sensitized response. This effect also appears to result in part from an insulin-induced decrease in blood glucose levels, as experimenter-administered glucose was largely sufficient to restore ethanol-induced locomotor sensitization in insulin-treated mice. Collectively, these results show that the development and expression of ethanol-induced locomotor sensitization depends on the maintenance of normal blood glucose levels.

One hypothesis of the current experiments was that blood glucose levels might concomitantly sensitize with ethanol-induced locomotion. Consistent with previous data (Risinger and Cunningham, 1991, 1992; Risinger, 2003), an acute ethanol injection induced a nominal, but non-significant increase in blood glucose levels (see Figure 3B). Blood glucose levels did not increase with repeated ethanol injections, and if anything, were lower in sensitized mice compared with controls. Our experiments assayed blood glucose levels in DBA/2J mice following 2 g/kg ethanol administration, a lower dose of ethanol that was used in some of the previous studies. It is possible that testing a different strain of mice or higher ethanol dose would have resulted in a hyperglycemic response. It is also possible that a sensitized hyperglycemic effect of ethanol might have been observable sooner after the ethanol injections, such as at the time behavior was measured. Given the relative stability of ethanol-induced hyperglycemia (Risinger and Cunningham, 1991), this possibility seems unlikely. Alternatively, a sensitized hyperglycemic effect could have occurred earlier during the sensitization protocol, but decayed with further injections. Even if correct, this possibility would still rule out a direct role for blood glucose levels in determining the sensitized locomotor response to ethanol, as a somewhat linear relationship between the two measures would be required. These results suggest that any effect of ethanol on blood glucose levels is not likely related to ethanol-induced locomotion or locomotor sensitization in mice not treated with insulin.

One explanation for insulin's attenuation of ethanol-induced locomotor sensitization is that insulin, which promotes glucose uptake and the formation of more complex energy stores, made unavailable those nutrients required to show a normal locomotor response to ethanol.

In this model, glucose could be considered rate limiting for ethanol-stimulated locomotion, so an increase in the blood glucose levels in mice made hypoglycemic by insulin would be expected to restore ethanol-induced locomotion to normal levels. The results from Experiment 3 are consistent with this suggestion, since glucose given concurrently with insulin largely restored the development of locomotor sensitization in ethanol-treated mice.

Interestingly though, mice given 5 IU/kg insulin in Experiments 1 and 3 still showed a locomotor stimulant response to ethanol, although the response was attenuated relative to saline-treated controls. If insulin acted only to decrease ethanol-stimulated locomotion and not to attenuate the acquisition of ethanol-induced locomotor sensitization, these mice would still be expected to develop locomotor sensitization across trials which started and ended at a lower level, but paralleled that of mice treated ethanol alone. As shown for Experiments 1 and 3, however, the blunted acute stimulant response to ethanol did not increase across repeated trials, but rather, persisted at approximately the same level throughout the experiment, which might suggest that insulin impaired the locomotor response to ethanol within trials, as well as the development of ethanol-induced locomotor sensitization across trials.

Similarly, an attenuated acute ethanol stimulant response was also observed for mice that received insulin and glucose (Experiment 3). These mice developed ethanol-induced locomotor sensitization to a degree statistically equivalent to the groups given ethanol alone, or ethanol and glucose. This finding might indicate that blood glucose levels are more important for the acquisition of ethanol-induced locomotor sensitization, but are not as essential for the acute locomotor response to ethanol. However, stronger evidence for these suggestions would come from comparing the response to a single ethanol injection of mice given insulin and ethanol to those given ethanol alone. In this experiment, mice treated chronically with ethanol and insulin would be expected to show lesser locomotion in response to ethanol compared with mice treated with ethanol only. Since this treatment protocol was not included in the current experiments, it is unknown whether this effect would be observed.

An alternative explanation for the attenuation of the acute and sensitized response to ethanol is that insulin and/or hypoglycemia are perceived by brain regions that mediate the locomotor response to ethanol. Elevated levels of insulin or decreased circulating glucose might alter neural activity in these regions through actions at insulin (Wozniak et al., 1993; Schulingkamp et al., 2000; Stockhorst et al., 2004; Plum et al., 2005) or glucose receptors (Levin, 2002; Levin et al., 2004), resulting in an attenuated response to ethanol. However, if insulin acts on receptors in the brain to decrease the locomotor response to ethanol, these effects would likely be indistinguishable from insulin-induced hypoglycemia originating in the periphery, as decreased ethanol-stimulated locomotion would likely be expected to result in either case. The current experiments therefore cannot distinguish the effects of insulin-induced hypoglycemia from any direct effects that insulin might have had on insulin receptors in the brain. Experiments utilizing CNS administrations of insulin and/or receptor antagonists would be necessary to distinguish between these mechanisms.

If the acquisition of ethanol-induced locomotor sensitization is viewed as a learning-related phenomenon, in that locomotion changes across trials as a function of experience, attenuation of ethanol-induced locomotor sensitization by insulin might result from a glucose dependent memory-impairment mechanism. Previous studies have found a memory-impairing effect of peripheral insulin as measured in a passive avoidance task, an effect that might be related to central cholinergic activity. (Kopf and Baratti, 1995, 1999; Messier, 2004; Watson and Craft, 2004). Consistent with this, our study found that glucose given concurrently with insulin largely restored locomotor sensitization. However, in the passive

avoidance tasks, insulin and glucose were given after the conditioning session, while in Experiment 2, the current experiment more analogous to those studies, insulin given after ethanol did not affect the development of locomotor sensitization. These discrepancies might be attributable to the relatively extensive habituation procedure used in each of the current experiments (15 minutes a day for 3 days), which all mice received prior to beginning the chronic treatments, or to insulin's effects on context and state dependent memory (Santucci et al., 1990). Further experiments are necessary to address these possibilities.

In Experiment 3, systemic glucose administration was used to directly manipulate blood glucose levels. At the time blood glucose was measured, mice injected with insulin and glucose showed only modestly higher blood glucose levels compared with the insulin-alone group. The doses of insulin and glucose used were based largely on those described by Kopf and Baratti (Kopf and Baratti, 1995), who found that systemic administration of glucose up to 1 g/kg glucose attenuated the memory impairing effects of insulin (8 IU/kg) in a passive avoidance task. In pilot experiments, a 4 g/kg glucose dose was sufficient to block insulin's hypoglycemic effect at 30 min, the time at which ethanol was given in the sensitization experiments. The recombinant human insulin used in the current experiments produces a long-lasting decrease in circulating blood glucose level, while glucose injections would be expected to increase circulating levels only transiently. In another pilot study not reported here, we found that a dose of 200 mg/kg glucose, which has been reported to be sufficient to attenuate the reduction in memory recall produced by insulin in a passive avoidance task (Kopf and Baratti, 1995), did not restore ethanol-induced locomotor sensitization in insulin-treated mice. This observation, combined with our pilot data, makes it likely that the glucose injections increased circulating blood glucose levels at the time of ethanol injection, but because of the continued action of insulin, fell over the next 30 minutes to just above control levels. In any case, the glucose dose used was sufficient to largely restore ethanol-induced locomotor sensitization in insulin-treated mice.

Conclusions

In summary, the current studies show that systemic insulin administration can attenuate the acute stimulant effects of ethanol, and block the acquisition and expression of ethanol-induced locomotor sensitization. In this behavioral model, glucose can restore the sensitized ethanol response in insulin-treated mice to control levels. The mechanism of this effect and its site of action are currently unknown, but likely involves communication between the brain and peripheral energy stores. One possible mediator is glucose itself, which has been shown to alter neural activity through action at specific glucose receptors in the brain (e.g. Levin, 2002; Levin et al., 2004; McCrimmon, 2009). Inhibiting these glucose receptors in specific brain regions could differentiate between purely metabolic and neural mechanisms of regulation. Hypoglycemic states are also known to be transmitted directly to the brain via the vagus nerve, which could possibly provide a more direct and specific input than glucose itself (e.g. Briski et al., 2009). It is also possible that differing basal circulating glucose levels might underlie behavioral and metabolic responses to ethanol across inbred strains of mice. Chronic ethanol exposure exerts robust effects on many metabolic processes. Understanding how these physiological consequences of ethanol exposure might in turn regulate the expression of ethanol-related behaviors might lead to more targeted treatments for alcohol dependence.

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Figure 1.

The effects of insulin on the acquisition of ethanol-induced locomotor sensitization. Saline injections were administered across days 1–3, and the first insulin and ethanol injections given on day 4 and continued throughout the experiment (see legend for all treatments).

Figure 2.

A. Locomotor sensitization score calculated as the difference in locomotion on day 13 (the tenth ethanol injection) minus that on day 4 (the first ethanol injection). Insulin at either dose blocked the acquisition of the sensitized response. **B.** The effects of insulin on the acute locomotor stimulant response to ethanol. Insulin dose-dependently reduced locomotion in ethanol-, but did not saline-injected mice. Ins = 0, 5 or 10 IU/kg insulin, EtOH = 0 or 2 g/kg ethanol. * $p < 0.05$, and *** $p < 0.001$ relative to the ethanol-only group. ### $p < 0.001$ relative to the saline-only group.

Figure 3.

A. The effects of insulin on the expression of ethanol-induced locomotor sensitization. The treatment given during the expression test is shown on the abscissa, and the treatment given throughout the conditioning trials is indicated above the bars. The first group of bars depicts the effects of insulin in control, ethanol-naïve mice, the second group shows the effects of insulin on acute ethanol-induced locomotion, and the third shows the effects of insulin on the expression of locomotor sensitization in ethanol treated mice. **B.** The effects of insulin and ethanol on blood glucose levels measured one hour after insulin injection (30 min after ethanol). Ins = 0, 5, or 10 IU/kg insulin, EtOH = 0 or 2 g/kg ethanol. ## $p < 0.01$, ### $p < 0.001$ relative to saline-only control. ** $p < 0.01$, *** $p < 0.001$ relative to respective acute or chronic ethanol treatment groups.

Figure 4.

A. The effects of insulin given after ethanol on the development of ethanol-induced locomotor sensitization. The doses of ethanol and insulin given beginning on Day 4 of the experiment are indicated in the Figure Legend.

Figure 5.

The effects of insulin (5 IU/kg) and glucose (4 g/kg) on the acquisition of ethanol-induced locomotor sensitization. The treatments given beginning on Day 4 of the Experiment are indicated in the Figure Legend.

Figure 6.

A. The effects of insulin (5 IU/kg) and glucose (4 g/kg) on the ethanol-induced locomotor sensitization score. Experimenter administered glucose largely restored the acquisition of ethanol-induced locomotor sensitization in insulin treated mice. **B.** The effects of experimenter administered glucose, insulin, and ethanol on blood glucose levels. ^{##} $p < 0.01$ relative to saline-alone control. ^{**} $p < 0.01$ relative to ethanol-alone control.

Figure 7.

The effects of 5 IU/kg insulin on the metabolism of 2 g/kg ethanol. Insulin did not alter ethanol metabolism across this time course.