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Acute hypoglycemia causes depressive-like behaviors in mice

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ABSTRACT

Reports in humans advocate a link between hypoglycemia and altered mood. Such observations, however, have not been mechanistically explored. Here we examined depressive-like behaviors in mice resulting from acute hypoglycemia. Mice were fasted for 12 hours and then administered intraperitoneal insulin to induce a blood glucose nadir of 50 mg/dL at 0.75 hour after injection that by 2 hours postinjection had returned to normal. The behaviors of locomotion, forced swim, saccharin preference, and novel object recognition were subsequently examined. Mice made hypoglycemic showed depressive-like behaviors 24 hours after resolution of hypoglycemia as evidenced by increased immobility in the forced swim test (FST) and reduced saccharin preference. Movement and memory were not impacted by hypoglycemia 24 hours after its resolution. By 48 hours posthypoglycemia, depressive-like behaviors resolved. In contrast, neither peripheral insulin administration without resultant hypoglycemia nor intracerebroventricular insulin administration altered performance in the FST. The antidepressants fluoxetine and desipramine prevented hypoglycemia-induced immobility in the FST, as did the antiadrenergic agents phentolamine, metoprolol, and butoxamine. Epinephrine and norepinephrine administration caused increased immobility in the FST at 24 hours postadministration that subsequently resolved by 48 hours. These data indicate that, in mice, acute hypoglycemia through adrenergic pathways caused depressive-like behaviors that exist well beyond the resolution of hypoglycemia.

1. Introduction

Hypoglycemia is the most common adverse effect of insulin treatment [1,2]. Whereas much is known about the acute features of insulin-induced hypoglycemia, especially as related to neuroglycopenia [3], little is known about the noncognitive brain-based complications of hypoglycemia. Studies by Gold et al [4,5] in the early 1990s reported changes

Authors' contributions: MJP: designed and executed study, analyzed data, and wrote paper. SWY: executed study. BSC: executed study. RD: designed study. GGF: designed study, analyzed data, and wrote paper.

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in mood during the course of insulin-induced hypoglycemia. Gold et al found that, in healthy volunteers, acute hypoglycemia caused negative mood states including tense-tiredness, a reduction in hedonic tone, an increase in tense arousal and a decrease in energetic arousal that lasted for at least 30 minutes after return to euglycemia [5]. Curiously, limited work has been done in this area since, despite hypoglycemia remaining a considerable barrier to the use of insulin therapy in diabetes [6,7]. In contrast, cognitive decline and depression associated with diabetes have generated considerable interest [8,9]; and diabetes-associated neurodegeneration, particularly Alzheimer disease–type neurodegeneration, has been coined by some as *type 3 diabetes* [10].

Conceptually, a link between hypoglycemia and depression seems apparent. Hypoglycemia triggers a robust activation of the autonomic nervous system [11,12] with marked changes in circulating [13] and brain-based catecholamines [14]. In turn, dysfunction in the monoamine systems of the brain involving serotonin, dopamine, and norepinephrine is well tied to mood disorders and major depression [15], with acute severe stress a notable perceived antecedent to a variety of psychiatric disorders [16]. The symptoms elicited by acute severe stress overlap those of hypoglycemia because both manifest, in part, from adrenergic signals delivered by the sympathetic nervous system [17]. In addition, analogous regions of the brain appear to be involved in both hypoglycemia and mood, as the ventromedial hypothalamus is important to glucose sensing [18], energy homeostasis [19], and anxiety [20].

We have previously shown that hypoglycemia-dependent activation of the autonomic nervous system is associated with social withdrawal in mice and that this impact of hypoglycemia on social behavior resolves within 4 hours after a return to euglycemia. In addition, this catecholamine-mediated loss of social exploration requires a β-2 adrenergic receptor (AR)–dependent pathway and not an α or β-1 AR pathway [21]. These findings relate to human disease because catecholamines, especially epinephrine, cause restlessness and apprehension [22]. Traditionally, such feelings of discomfort are often linked to the impact of epinephrine on the cardiovascular system, skeletal muscle, and/or intermediary metabolism [22] and not directly associated to an effect of catecholamines in the central nervous system. Given the intersection of symptoms, neurotransmitters, and brain areas and that nearly one third of young adults with type 1 diabetes mellitus experience psychological distress that often revolves around iatrogenic hypoglycemia [23], we sought to show that hypoglycemia caused depressive-like symptoms in mice that depend on adrenergic signals.

2. Materials and methods

2.1. Materials

All reagents and chemicals were purchased from Sigma-Aldrich (St Louis, MO) except for Humulin R (insulin), which was purchased from Eli Lilly (Indianapolis, IN).

2.2. Animals, fasting and testing for glucose, epinephrine, and norepinephrine

Mouse use was conducted in accordance with Institutional Animal Care and Use Committee–approved protocols at the University of Illinois. Male C57BL/6J mice 3 to 4 weeks of age were purchased from Jackson Laboratories. Mice were housed (4 per cage) in standard shoebox cages (length, 28 cm; width, 17 cm; height, 12.5cm) and allowed water and food ad libitum. Mice were fed pelleted food (NIH 5K52; LabDiet; Purina Mills, St Louis, MO). Housing temperature (23°C) and humidity (45%-55%) were controlled, as was a 12/12-hour reversed dark-light cycle (8:00 PM-8:00 AM). Male 10- to 12-week-old animals were used for all experiments. When 12-hour fasting was performed (8:00 PM-8:00 AM), mice were transferred to a new cage and singly housed. Fasted mice were provided water ad libitum. If insulin was administered to a 12-hour–fasted mouse, food was reintroduced 0.75 hour after insulin administration. Mouse tail blood glucose was recorded using a FreeStyle Freedom blood glucose monitor (Abbott, Abbott Park, IL) after the tail was cleaned with 70% ethanol and lanced with a sterile 18-gauge hypodermic needle (BD, Franklin Lakes, NJ). Plasma for catecholamine testing was derived from left ventricular blood after mice were deeply anesthetized with sodium ketamine hydrochloride:xylazine hydrochloride (80 mg/mL:12 mg/mL, ketamine:xylazine) at 5 mL/kg body weight. Catecholamine (epinephrine and norepinephrine) detection was performed at the Mouse Metabolic Phenotyping Center at Vanderbilt University by high-performance liquid chromatography. Measurement of catecholamines was performed on cohorts of mice independent from those being examined for blood glucose and behavior.

2.3. Intracerebroventricular cannula placement

Mice were anesthetized using an intraperitoneal (IP) sodium ketamine hydrochloride/xylazine hydrochloride solution delivering 80 mg/kg ketamine and 12 mg/kg xylazine at 1.5 mL/kg body weight. A Kopf stereotaxic instrument (David Kopf Instruments, Tujunga, CA) was used to place a mouse-specific brain infusion cannula (Plastics One, Roanoke, VA) at the following coordinates: 0.6 mm posterior, 1.5 mm lateral to the bregma, and 2.5 mm ventral from the skull surface, using The Mouse Brain in Stereotaxic Coordinates [24]. Cannulas were affixed to the skull with cyanoacrylate gel adhesive (Plastics One) and protected by a plastic guard. Animals were allowed 1 week for recovery.

2.4. Treatments

Insulin was administered IP at 0.8 U/kg per mouse or intracerebroventricular (ICV) at 0.4 U per mouse. Epinephrine and norepinephrine were administered IP at 1.5 mg/kg. The pan-α AR antagonist phentolamine (1 mg/kg per mouse), the β-1 AR antagonist metoprolol (10 mg/kg per mouse), or the β-2 AR antagonist butoxamine (5 mg/kg per mouse) was administered IP twice, 30 minutes before insulin injection and 30 minutes before the forced swim test (FST). Antidepressant fluoxetine (10 mg/kg per mouse) or desipramine (5 mg/kg per mouse) was administered IP 30 minutes before the FST.

Insulin, epinephrine, and norepinephrine were administered at the beginning of the dark cycle (8:00 AM).

2.5. Forced swim test

Testing was initiated by individually transferring mice from their home cage to a clean novel white cylindrical PVC container (diameter, 16 cm; height, 31 cm) containing 20 cm of water maintained at 25°C ± 1°C. Total swim duration was 6 minutes, and immobility was evaluated from the video record using EthoVision XT 7 (Noldus Information Technology, Leesburg, VA) encompassing the final 5 minutes of the swim. After testing, mice were allowed to dry in a warmed environment (28°C ± 1°C) for 10 minutes and then returned to their home cage. Testing occurred 2 hours after the beginning of the dark cycle (10:00 AM). Video recording of animal behavior was performed under red light using a HDR-XR500V night shot–capable video camera (Sony, New York, NY). Forced swim testing was performed on cohorts of mice independent from those being examined for all other behaviors. Where indicated, mice were examined at both 24 and 48 hours after insulin administration.

2.6. Saccharin preference test

Three days before insulin administration (adaptation phase), mice were singly housed in standard cages adapted for 2-bottle water access. Bottles contained either saccharin as a 0.4% sodium saccharin solution or water (saccharin and water were randomized to right vs left bottles). After adaptation, mice were administered insulin with or without pretreatments as indicated and returned to the cage in which they were adapted in the presence of both water and saccharin (testing phase). Fluid consumption was recorded every 24 hours. Fluid consumption was determined by bottle weight. Saccharin preference testing was performed on cohorts of mice independent from those being examined for all other behaviors.

2.7. Movement/exploration

Mice were examined in their home cage by video recording for 5 minutes. Movement was quantified using EthoVision XT 7 (Noldus Information Technology). Testing occurred 3 hours after the beginning of the dark cycle (11:00 AM). Video recording of animal behavior was performed under red light using a night shot–capable video camera. Movement/exploration, line crossings, and novel object recognition testing were performed as a test battery on single cohorts of mice.

2.8. Line crossings

Mice were examined in their home cage by video recording for 5 minute. The cage was divided into 4 identical rectangles and line crossing quantified by a trained observer blind to experimental treatments. A line was crossed if the fore and hind limbs of a mouse entered a new rectangle. Testing occurred 3 hours after the beginning of the dark cycle (11:00 AM). Video recording of animal behavior was performed under red light using a night shot–capable video camera.

2.9. Novel object recognition

One hour before testing, mice were individually removed from their home cage and placed for 5 minutes in a novel arena (home cage sized with light bedding) containing 2 identical objects positioned 10 cm apart at the short-side wall end 5 cm from the short side wall and 6.5 cm from the long-side wall. After training, mice were returned to their home cage for 1 hour. Testing was initiated by returning mice to the testing arena where one of the identical objects (familiar object) was replaced (randomized to right or left) by an unfamiliar object (novel object). Investigative behavior was video recorded for 5 minutes and evaluated from the video record using EthoVision XT 7 (Noldus Information Technology). Percentage investigation was calculated by dividing the time spent examining each object by the total time spent investigating both objects. Testing occurred 4.5 hours after the beginning of the dark cycle (12:00 PM). Video recording of animal behavior was performed under red light using a night shot–capable video camera.

2.10. Statistical analysis

Data are presented as mean ± SEM. The experimental design for behavioral experiments was a completely randomized design. All data were analyzed using SAS (Cary, NC). Where appropriate, the experimental design for behavioral experiments was a completely randomized design with a 2 x 2 factorial arrangement of treatment (2 levels of pretreatment and 2 levels of treatment). The statistical model in the behavior experiments included the effects of insulin, agonist/antagonist, and time. Post hoc comparisons of individual group means were performed with the Tukey test. Experimental data, where appropriate, were analyzed by analysis of variance (ANOVA). Statistical significance was denoted at $P < .05$.

3. Results

3.1. Depressive-like behaviors but not movement or memory deficits exist 24 hours after insulin-induced hypoglycemia

Table 1 demonstrates that IP insulin administration in mice fasted for 12 hours caused hypoglycemia. Refeeding 0.75 hour after insulin administration restored blood glucose within 1.25 hours of hypoglycemia. Fig. 1A shows that 24 hours

Table 1 – Blood glucose (milligrams per deciliter) before fasting (–12 hours), after fasting (0 hour), and after insulin administration and refeeding (0.75-24 hours)

	–12 h	0 h	0.75 h	2 h	4 h	24 h
Saline	152 ± 6[a]	99 ± 4[b]	105 ± 4[b]	172 ± 5[a]	150 ± 10[a]	152 ± 7[a]
Insulin	149 ± 6[a]	103 ± 3[b]	50 ± 2[c]	166 ± 4[a]	148 ± 10[a]	145 ± 8[a]

Results are expressed as mean ± SEM; n = 9 per group. Two-way ANOVA revealed main effects of time ($P < .001$), treatment ($P = .002$), and time-treatment interaction ($P < .001$). Results without a common superscript letter are different ($P < .05$).

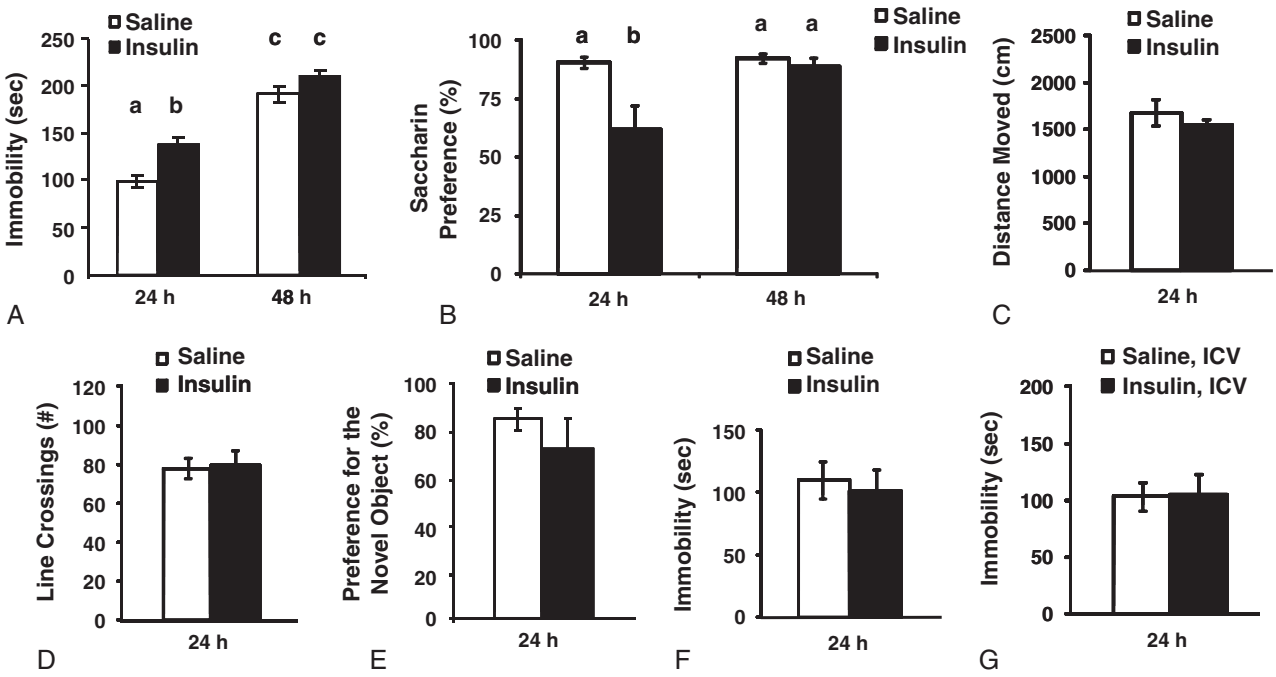

Fig. 1 – Depressive-like behaviors but not movement or memory deficits exist 24 hours after insulin-induced hypoglycemia. A, After a 12-hour fast, mice were administered either saline or insulin IP. Immobility in the FST was measured at 24 and 48 hours after saline or insulin injection. Results are expressed as mean ± SEM; n = 14 to 15 per group. Two-way ANOVA revealed main effects of treatment ($P = .003$) and time ($P < .001$). Bars without a common superscript letter are different ($P < .05$). B, After a 12-hour fast, mice were administered either saline or insulin IP. Saccharin preference was measured at 24 and 48 hours after saline or insulin injection. Results are expressed as mean ± SEM; n = 4 per group. Two-way ANOVA revealed main effects of treatment ($P = .0482$), time ($P = .0118$), and treatment-time interaction ($P = .0196$). Bars without a common superscript letter are different ($P < .05$). C, After a 12-hour fast, mice were administered either saline or insulin IP. Locomotor activity was measured 24 hours after saline or insulin injection. Results are expressed as mean ± SEM; n = 4 to 8 per group. D, After a 12-hour fast, mice were administered either saline or insulin IP. Line crossings were measured 24 hours after saline or insulin injection. Results are expressed as mean ± SEM; n = 4 to 8 per group. E, After a 12-hour fast, mice were administered either saline or insulin IP. Novel object recognition was measured 24 hours after saline or insulin injection. Results are expressed as mean ± SEM; n = 4 to 8 per group. F, Unfasted mice were administered either saline or insulin IP. Immobility in the FST was measured at 24 hours after saline or insulin injection. Results are expressed as mean ± SEM; n = 3 to 4 per group. G, After a 12-hour fast, mice were administered either saline or insulin ICV. Immobility in the FST was measured at 24 hours after saline or insulin injection. Results are expressed as mean ± SEM; n = 3 to 4 per group.

after insulin-induced hypoglycemia, mice had increased immobility in the FST (138 ± 9 vs 99 ± 6 seconds). This difference resolved by 48 hours after insulin administration. Fig. 1B shows that 24 hours after insulin-induced hypoglycemia, mice had decreased preference for saccharin (62% ± 10% vs 91% ± 2%, insulin vs saline) and that this difference resolved by 48 hours after insulin administration. Figs. 1C-E demonstrate that 24 hours after insulin-induced hypoglycemia, mouse locomotor activity, line crossings, and novel object recognition were unaffected by antecedent hypoglycemia. Table 2 demonstrates that if mice were provided food ad libitum and were not fasted for 12 hours, blood glucose after insulin administration did not fall below 85 mg/dL. Fig. 1F shows that if mice were provided food ad libitum and were not fasted for 12 hours, insulin administration did not impact immobility in the FST 24 hours after insulin injection. Table 3 shows that ICV insulin administration in mice fasted for 12 hours did not cause hypoglycemia. Fig. 1G demonstrates that if mice were fasted for 12 hours, ICV insulin had no impact on immobility in the FST 24 hours post–insulin administration.

3.2. *Insulin-induced immobility in the FST is prevented by antidepressants*

Fig. 2 shows that insulin-induced immobility in the FST was prevented by pretreatment with fluoxetine or desipramine

Table 2 – Blood glucose (milligrams per deciliter) after insulin administration without fasting

	0 min	10 min	20 min	40 min	60 min	80 min
Saline	141 ± 6^a	152 ± 12^a	167 ± 7^a	177 ± 7^a	172 ± 3^a	166 ± 5^a
Insulin	131 ± 15^a	99 ± 11^b	101 ± 6^b	122 ± 1^b	168 ± 10^a	172 ± 10^a

Results are expressed as mean ± SEM; n = 3 to 8 per group. Two-way ANOVA revealed main effects of time ($P < .001$), treatment ($P < .001$), and time-treatment interaction ($P < .001$). Results without a common superscript letter are different ($P < .05$).

Table 3 – Blood glucose (milligrams per deciliter) after Insulin ICV Injection

	-12 h	0 h	0.75 h	2 h	4 h	24 h
Saline, ICV	161 ± 13[a]	114 ± 3[b]	116 ± 8[b]	132 ± 5[a]	131 ± 13[a]	142 ± 13[a]
Insulin, ICV	156 ± 9[a]	108 ± 6[b]	113 ± 9[b]	141 ± 13[a]	136 ± 7[a]	151 ± 14[a]

Results are expressed as mean ± SEM; n = 3 to 4 per group. Two-way ANOVA revealed main effect of time ($P < .001$). Insulin ICV at 0.2 U per mouse per 2 μL injection. Results without a common superscript letter are different ($P < .05$).

(148 ± 16 vs 82 ± 14 seconds, insulin vs saline; 148 ± 16 vs 41 ± 14 seconds, insulin vs saline + fluoxetine; 148 ± 16 vs 58 ± 12 seconds, insulin vs saline + desipramine; 148 ± 16 vs 65 ± 21 seconds, insulin vs insulin + fluoxetine; 148 ± 16 vs 65 ± 12 seconds, insulin vs insulin + desipramine).

3.3. *Catecholamines induce immobility in the FST*

Fig. 3A demonstrates that 24 hours after epinephrine or norepinephrine administration, mice had increased immobility in the FST (108 ± 13 vs 71 ± 5 seconds, epinephrine vs saline; 127 ± 13 vs 71 ± 5 seconds, norepinephrine vs saline). This difference resolved by 48 hours after catecholamine administration. Fig. 3B shows that mouse locomotor activity after catecholamine administration was unaffected at 24 hours.

3.4. *Adrenergic antagonists block insulin-induced immobility and loss of saccharin preference*

Fig. 4A shows that insulin-induced immobility in the FST was prevented by pretreatment with the pan-α AR antagonist phentolamine, the β-1 AR antagonist metoprolol, or

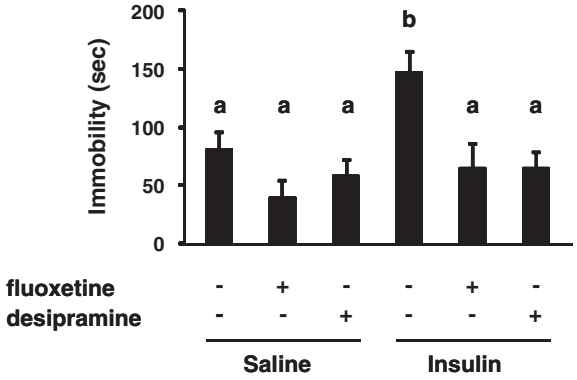

Fig. 2 – Insulin-induced immobility in the FST is prevented by antidepressants. After a 12-hour fast, mice were administered either saline or insulin IP. Immobility in the FST was measured at 24 hours after saline or insulin injection in the presence or absence of the indicated antidepressants. Results are expressed as mean ± SEM; n = 9 per group. Two-way ANOVA reveals main effect of pretreatment ($P < .001$) and treatment ($P = .0121$). Bars without a common superscript letter are different ($P < .05$).

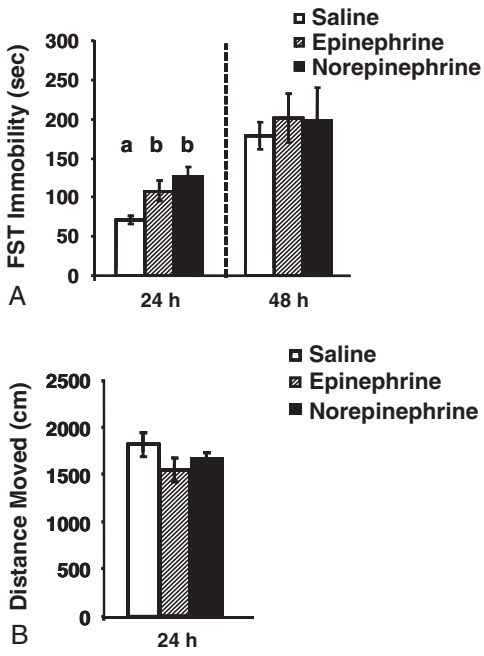

Fig. 3 – Catecholamines induce immobility in the FST. A, After a 12-hour fast, mice were administered saline, epinephrine, or norepinephrine IP. Immobility in the FST was measured at 24 and 48 hours after saline, epinephrine, or norepinephrine injection. Results are expressed as mean ± SEM; n = 4 per group. One-way ANOVA revealed main effect of treatment ($P = .007$). B, After a 12-hour fast, mice were administered saline, epinephrine, or norepinephrine IP. Locomotor activity was measured 24 hours after saline, epinephrine, or norepinephrine injection. Results are expressed as mean ± SEM; n = 4 per group.

the β-2 AR antagonist butoxamine (109 ± 15 vs 80 ± 6 seconds, insulin vs saline; 109 ± 15 vs 54 ± 7 seconds, insulin vs insulin + phentolamine; 109 ± 15 vs 46 ± 9 seconds, insulin vs insulin + metoprolol; 109 ± 15 vs 19 ± 5 seconds, insulin vs insulin + butoxamine). Fig. 4B shows that insulin-induced loss of saccharin preference was prevented by pretreatment with the pan-α AR antagonist phentolamine, the β-1 AR antagonist metoprolol, or the β-2 AR antagonist butoxamine (64% ± 8% vs 92% ± 1%, insulin vs saline; 64% ± 8% vs 82% ± 3%, insulin vs insulin + phentolamine; 64% ± 8% vs 88% ± 3%, insulin vs insulin + metoprolol; 64% ± 8% vs 89% ± 1%, insulin vs insulin + butoxamine). Table 4 shows that epinephrine and norepinephrine levels were not significantly impacted by insulin 24 hours after insulin administration.

4. Discussion

Hypoglycemia precipitates a range of symptoms that become more severe with decreasing plasma glucose. In general, epinephrine secretion is triggered at a plasma glucose concentration between 65 and 70 mg/dL, with neurogenic and neuroglycopenic symptoms first becoming manifest at plasma glucose concentrations between 50 and 55 mg/dL [25]. As

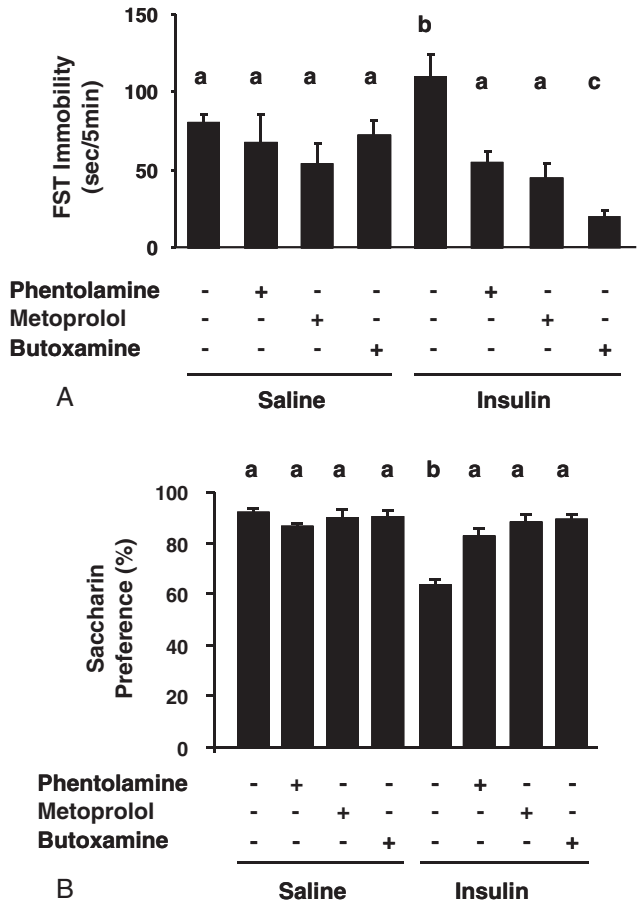

Fig. 4 – Adrenergic antagonists block insulin-induced immobility and loss of saccharin preference. A, After a 12-hour fast, mice were administered either saline or insulin IP. Immobility in the FST was measured at 24 hours after saline or insulin injection in the presence or absence of the indicated adrenergic antagonists. Results are expressed as mean ± SEM; n = 8 per group. Two-way ANOVA revealed main effects of pretreatment ($P < .001$) and pretreatment-treatment interaction ($P = .008$). Bars without a common superscript letter are different ($P < .05$). B, After a 12-hour fast, mice were administered either saline or insulin IP. Saccharin preference was measured at 24 hours after saline or insulin injection in the presence or absence of the indicated adrenergic antagonists. Results are expressed as mean ± SEM; n = 8 per group. Two-way ANOVA revealed main effects of pretreatment ($P = .0022$), treatment ($P < .001$), and pretreatment-treatment interaction ($P < .001$). Bars without a common superscript letter are different ($P < .05$).

we have previously shown, mice with insulin-induced hypoglycemia near 50 mg/dL develop almost complete loss of social exploration and movement at 0.75 hour after insulin administration that resolves at about 3 hours after refeeding [21]. Here we achieved a blood glucose of 50 mg/dL at 0.75 hour that with refeeding rebounded to normal within 1.25 hours of refeeding. At 24 hours postreturn to euglycemia, mouse movement and learning/memory were normal; but immobility in the FST was increased, indicative of depressed behavior. The

Table 4 – Plasma catecholamines (picograms per milliliter) 24 hours after saline or insulin

	Saline (IP)	Insulin (IP)
Epinephrine	1471 ± 743	2345 ± 1034
Norepinephrine	1317 ± 304	1961 ± 490x

Results are expressed as mean ± SEM; n = 7 to 8 per group.

FST is a powerful tool for demonstrating depressive-like behavior and is currently the most widely and frequently used experimental paradigm for detecting antidepressant activity [26]. To confirm the presence of depressive-like behavior, we used saccharin preference testing [27]. The reason tail-suspension testing was not used as a corroborative test is that C57Bl/6J mice are significantly motoric with an aggressive tail-climbing phenotype that tends to confound and invalidate the tail-suspension test in these animals [26]. Notably, initial development of hypoglycemia was a key to the behaviors seen because insulin administration (peripheral or central) without adjacent hypoglycemia was not sufficient to induce depressive-like behaviors. This was not surprising because we have previously shown that central (ICV) administration of insulin to mice increased by as much as 50% social exploratory activity that lasts for more than 22 hours post–insulin administration [28]. Interestingly, Robertson et al [29] have just shown that insulin can downregulate surface expression of the high-affinity norepinephrine transporter in mouse hippocampal slices and superior cervical ganglion neuron boutons. Whereas reduced brain norepinephrine and/or norepinephrine signaling may be tied to anxious depression [17], the limbic circuit rather than the hippocampus is probably more relevant to this type of mood imbalance. Furthermore, Robertson et al [29] did not report on behavioral manifestations that result from their identified decline in norepinephrine transporters.

Pharmacologic validation of depression was achieved with either the selective serotonin reuptake inhibitor fluoxetine or the tricyclic desipramine. Given that both were equally effective at reducing immobility in the FST, it is difficult to speculate on the monoamine pathways essential to hypoglycemia-associated depression. In addition, it was not surprising that both antidepressants were effective because each has been shown to impact both serotonin and norepinephrine pathways [30,31]. It was surprising that epinephrine and norepinephrine caused increased depressive-like behavior measurable 24 hours after administration. It has been shown that drugs that reduce central catecholamines (more so than serotonin) impact the FST [32]. In addition, it was our initial thought that hypoglycemia might trigger focal transient norepinephrine depletion in the hypothalamus due to activity of glucose-sensing neurons. With such depletion, the potential would exist for depressive-like behavior to manifest. However, because both epinephrine and norepinephrine induced depressive-like behavior, such behavior may be more reliant on increases rather than decreases in catecholamine-dependent neuronal pathways. Our work lends support to that of Tanaka et al [33] who demonstrated that rats exposed to a variety of stressful and fear-inducing conditions had increased norepinephrine release in the hypothalamus,

amygdale, and locus coeruleus that when mitigated reduced anxiety. Evidence for catecholamine involvement in mood change has been widely reported [34], and individuals with mood disorders often present with some degree of increased plasma catecholamines [35-37]. More recently, the brainstem noradrenergic system has gained credence as a potential regulator of stress, anxiety, and depression [38].

Finally, administration of α and β AR antagonists blocked hypoglycemia-associated depressive behavior, indicating that adrenergic pathways are important to this process. Based on our previous findings, these data were somewhat unexpected because hypoglycemia-associated social withdrawal is blocked specifically by the β-2 receptor antagonist butoxamine and not by the pan-α AR antagonist phentolamine or the β-1 AR antagonist metoprolol [21]. However, given the complex pathways involved in depressive behaviors, we should have expected that a multiplicity of ARs were involved. Even within well-defined norepinephrine pathways, pre- and postsynaptic α and β ARs can impact a cornucopia of neurotransmitters and neurotrophic factors [39]. Therefore, it was quite significant that hypoglycemia-associated immobility in the FST and loss of saccharin preference could be blocked by peripheral administration of AR antagonists. Why such depressive behaviors manifest or persist after resolution of the triggering event is not clear, as we have reviewed [40], but is a very active area research. Whereas we did not detect a statistically significant difference in plasma epinephrine or norepinephrine at 24 hours after insulin administration, there was a trend toward catecholamine elevation; and as noted above, such findings are frequently seen in the individual presenting with depression. In sum, our results underscore the importance of acute adrenergic stress to depressive behaviors and suggest that the reluctance of some individuals to continue insulin therapy for the treatment of diabetes may be more than conditioned aversion due to iatrogenic hypoglycemia but generalized loss of motivation due to depression. In addition, peripherally administered AR antagonists may have a role in the management of stressful events that are considered depressogenic.

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Conflict of Interest

No conflict of interest for any authors.

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