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Effects of γ-Aminobutyric Acid A Receptor Activation on Counterregulatory Responses to Subsequent Exercise in Individuals With Type 1 Diabetes

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The effects of γ-aminobutyric acid (GABA) A receptor activation on physiologic responses during next-day exercise in type 1 diabetes are unknown. To test the hypothesis that GABA A activation with the benzodiazepine alprazolam would blunt counterregulatory responses during subsequent exercise, 29 (15 male, 14 female) individuals with type 1 diabetes (HbA_{1c} 7.8 ± 1%) were studied during separate 2-day protocols. Day 1 consisted of morning and afternoon 2-h euglycemic or 2.9 mmol/L hypoglycemic clamps with or without 1 mg alprazolam given 30 min before each clamp. Day 2 consisted of a 90-min euglycemic cycling exercise at 50% VO_{2max}. Tritiated glucose was used to measure glucose kinetics. Despite equivalent day 2 insulin (93 ± 6 pmol/L) and glucose levels (5.3 ± 0.1 mmol/L), plasma epinephrine, norepinephrine, glucagon, cortisol, and growth hormone responses were similarly reduced after alprazolam or day 1 hypoglycemia compared with euglycemic control. Endogenous glucose production, lipolysis (glycerol, nonesterified fatty acid), and glycogenolysis (lactate) were also reduced during day 2 exercise after day 1 GABA A activation. We conclude that activation of GABA A receptors with alprazolam can result in widespread neuroendocrine, autonomic nervous system, and metabolic counterregulatory failure during subsequent submaximal exercise and may increase the risk of exercise-associated hypoglycemia in individuals with type 1 diabetes.

Hypoglycemia is substantially increased during and after physical activity in type 1 diabetes (1,2). Several mechanisms have been demonstrated to increase the risk for exercise-associated hypoglycemia, including *1*) relatively increased insulin levels due to exercise-induced changes in insulin sensitivity, *2*) inadequate carbohydrate intake coupled with an inability to replenish endogenous glycogen stores, and *3*) reduced physiologic homeostatic (counterregulatory) responses that can defend against falling plasma glucose levels during and after exercise (3).

Several studies have demonstrated that activation of γ-aminobutyric acid (GABA) A receptors (the key central inhibitory neurotransmitter) can blunt counterregulatory responses to subsequent stress in humans and animal models (4–7). Previous work has also demonstrated that antecedent activation of GABA A receptors with a commonly prescribed benzodiazepine (alprazolam) results in blunted autonomic nervous system (ANS), neuroendocrine, and metabolic counterregulatory responses during moderate exercise in healthy subjects (8).

GABA A receptor agonists such as alcohol and/or benzodiazepines are widely used in individuals with diabetes. However, studies of the effects of GABA A activation on ANS, neuroendocrine, and metabolic counterregulatory responses to subsequent submaximal exercise in individuals with type 1 diabetes are lacking. The aim of the current study, therefore, was to test the hypothesis that pharmacologic activation of GABA A receptors results in neuroendocrine, ANS, and/or metabolic counterregulatory failure during next-day moderate exercise in subjects with type 1 diabetes.

RESEARCH DESIGN AND METHODS

Subjects

Twenty-nine individuals with type 1 diabetes (15 male, 14 female, age 28 ± 1 years, BMI 23 ± 3 kg/m^2, A1C

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7.5 ± 1% [62 ± 8 mmol/mol], diabetes duration 9 ± 4 years, no tissue complications of the disease) were studied. Subjects were treated with either multiple daily injections of insulin or continuous subcutaneous insulin infusion through a pump. They were nonsmokers and had normal liver, renal, and hematological parameters. Subjects participated in moderate recreational exercise, but no elite athletes were studied (mean VO_{2max} 30 ± 7 mL/kg/min). Studies were approved by the Vanderbilt University and University of Maryland human subject institutional review boards, and all subjects gave informed written and verbal consent.

Experimental Design

An estimate of physical fitness and VO_{2max} was obtained 1–3 weeks before the initial study, using a graded maximal exercise test on a bicycle ergometer as previously described (9). Subjects participated in three separate, single-blind, 2-day experiments with differing day 1 protocols separated by at least 2 months. Preadmission criteria and experimental procedures have been previously described (10,11). Protocol 1 consisted of day 1 morning and afternoon hyperinsulinemic-euglycemic clamps (n = 13). Protocol 2 involved day 1 morning and afternoon hyperinsulinemic-euglycemic clamps with 1 mg alprazolam administered 30 min before each clamp (n = 14). Protocol 3 consisted of day 1 morning and afternoon hyperinsulinemic hypoglycemia (n = 15). Fifteen subjects completed two protocols, and 14 completed one protocol. Data from historic control subjects participating in protocols 1 and 3 have been reported in a previous study (9). After a 10-h overnight fast, day 2 consisted of a 90-min euglycemic cycling exercise at 50% VO_{2max} and was identical for all three protocols, as previously described (12–14).

Analytical Methods

Endogenous glucose production (EGP) was calculated according to the method of Wall et al. (11) with modifications as previously described (8,9). The collection and processing of blood samples have been previously described (15–21). Assay procedures for epinephrine, norepinephrine, glucagon, growth hormone, cortisol, pancreatic polypeptide, nonesterified fatty acid (NEFA), glycerol, lactate, and glucose kinetics were similar and had equivalent quality control and coefficients of variance compared with those used in Bao et al (9). Heart rate and systolic, diastolic, and mean arterial blood pressure were measured noninvasively by a Dinamap vital signs monitor (Critikon, Tampa, FL) every 10 min during all studies.

Statistical Analysis

Data are expressed as mean ± SE and were analyzed using standard parametric one-way ANOVA (GraphPad Software, San Diego, CA). Tukey post hoc analysis was used to delineate statistical significance within each group. Data were also analyzed using unpaired two-tailed t tests. Changes (responses) from baseline to the end of exercise (final 15 min) on day 2 were compared.

RESULTS

Glucose and Insulin

Plasma glucose levels were similar in the morning and afternoon during all day 1 euglycemic (5.3 ± 0.1 mmol/L) and hypoglycemic (2.9 ± 0.05 mmol/L) studies. Plasma insulin levels were also similar during day 1 studies (500 ± 48 to 534 ± 59 pmol/L). End of clamp day 2 exercise plasma glucose (5.3 ± 0.1 mmol/L) and plasma insulin (93 ± 6 pmol/L) levels were similar in all three groups.

ANS Responses

Baseline values of epinephrine, norepinephrine, and pancreatic polypeptide were similar at the start of all day 2 exercise studies (Table 1 and Fig. 1). Day 2 plasma epinephrine responses were lower (P = 0.007) during day 2 exercise after day 1 euglycemia and alprazolam (Δ 329 ± 52 pmol/L) and day 1 hypoglycemia (Δ 278 ± 46 pmol/L) than day 1 euglycemia (Δ 582 ± 97 pmol/L).

Table 1—Day 2 baseline neuroendocrine, intermediary metabolite, and EGP values in overnight-fasted subjects with type 1 diabetes after either day 1 euglycemia and alprazolam, day 1 euglycemia, or day 1 hypoglycemia

Baseline	Prior euglycemia control (no alprazolam)	Prior euglycemia and alprazolam	Prior hypoglycemia control (no alprazolam)
Epinephrine (pmol/L)	152 ± 21	145 ± 49	174 ± 38
Norepinephrine (nmol/L)	0.8 ± 0.1	1 ± 0.1	0.7 ± 0.1
Glucagon (ng/L)	43 ± 3	45 ± 5	44 ± 4
Growth hormone (μg/L)	2.6 ± 1.7	2.4 ± 0.8	1.9 ± 0.9
Cortisol (nmol/L)	387 ± 55	441 ± 99	359 ± 55
Pancreatic polypeptide (pmol/L)	54 ± 7	55 ± 18	53 ± 19
EGP (μmol/kg/min)	8.8 ± 1.1	9.3 ± 0.6	8.8 ± 1.1
Rd (μmol/kg/min)	9.3 ± 0.5	9.9 ± 1.1	9.3 ± 0.5
NEFA (μmol/L)	168 ± 27	225 ± 51	226 ± 40
Lactate (mmol/L)	0.8 ± 0.1	0.6 ± 0.1	0.7 ± 0.1
Glycerol (μmol/L)	50 ± 11	58 ± 11	62 ± 12

Rd, rate of glucose disposal.

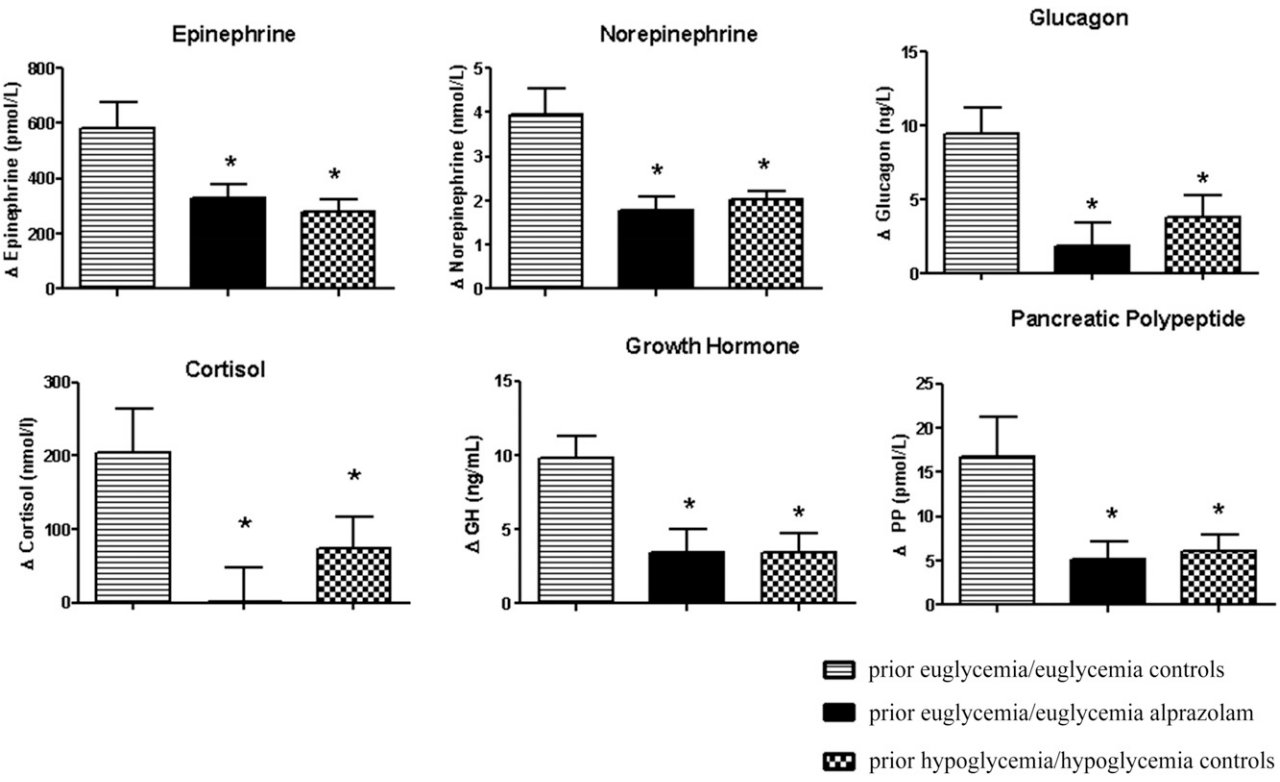

Figure 1—Day 2 epinephrine, norepinephrine, glucagon, pancreatic polypeptide (PP), growth hormone (GH), and cortisol responses (change from baseline to final 15 min of day 2 clamps) in overnight-fasted healthy individuals after either day 1 euglycemia, day 1 euglycemia and alprazolam, or day 1 hypoglycemia. *P < 0.01–0.002 significantly reduced compared with prior euglycemia/euglycemia control.

Day 2 norepinephrine responses were also lower (P = 0.0005) after day 1 euglycemia and alprazolam (Δ 1.8 ± 0.3 nmol/L) and day 1 hypoglycemia (Δ 2 ± 0.2 nmol/L) than day 1 euglycemia (Δ 4 ± 0.6 nmol/L). Day 2 pancreatic polypeptide responses were lower ($P < 0.01$) after day 1 euglycemia and alprazolam (Δ 5.2 ± 2 pmol/L) and day 1 hypoglycemia (Δ 6.1 ± 2 pmol/L) than day 1 euglycemia (Δ 16.7 ± 4 pmol/L).

Neuroendocrine Counterregulatory Hormones

Baseline values of glucagon, cortisol, and growth hormone were similar at the start of all day 2 exercise studies (Table 1 and Fig. 1). Plasma glucagon responses on day 2 were blunted ($P < 0.008$) after day 1 euglycemia and alprazolam (Δ 1.9 ± 1.5 ng/L) or day 1 hypoglycemia (Δ 3.8 ± 2 ng/L) compared with day 1 euglycemia (Δ 9.4 ± 2 ng/L). Day 2 growth hormone responses were also blunted ($P <$ 0.006) after day 1 euglycemia and alprazolam (Δ 3.5 ± 1.5 μg/L) or day 1 hypoglycemia (Δ 3.4 ± 1.3 μg/L) compared with day 1 euglycemia (Δ 9.8 ± 1.5 μg/L). Day 2 plasma cortisol responses were also lower ($P < 0.002$) after day 1 euglycemia and alprazolam (Δ 1 ± 47 nmol/L) or day 1 hypoglycemia (Δ 74 ± 42 nmol/L) than day 1 euglycemia (Δ 205 ± 60 nmol/L).

Intermediary Metabolism

Baseline levels of lactate, glycerol, and NEFA were similar at the start of all day 2 exercise studies (Table 1 and Fig. 2). Blood lactate, glycerol, and plasma NEFA responses were reduced ($P < 0.04$–0.003) during day 2 exercise after day 1 euglycemia and alprazolam and day 1 hypoglycemia compared with day 1 euglycemia.

Glucose Kinetics

Baseline rates of glucose kinetics were similar at the start of all day 2 exercise studies (Table 1 and Fig. 2). Rates of EGP were reduced ($P < 0.0001$) during the final 15 min of day 2 exercise after day 1 euglycemia and alprazolam (13 ± 2.7 μmol/kg/min) compared with day 1 euglycemia (20.7 ± 2.2 μmol/kg/min). Glucose infusion rates were increased after day 1 euglycemia and alprazolam (22.4 ± 5.4 μmol/kg/min) or day 1 hypoglycemia (17 ± 1.9 μmol/kg/min) compared with 10.7 ± 2.1 μmol/kg/min after day 1 euglycemia ($P < 0.04$). Rates of glucose disposal were similar among the three groups.

Cardiovascular Responses

Baseline values were similar, and similar changes in blood pressure (systolic, diastolic, and mean arterial pressure) and heart rate during day 2 exercise were seen in all groups (Table 2).

DISCUSSION

The results of this study demonstrate that GABA A activation with the benzodiazepine alprazolam in the presence of euglycemia results in a widespread substantial

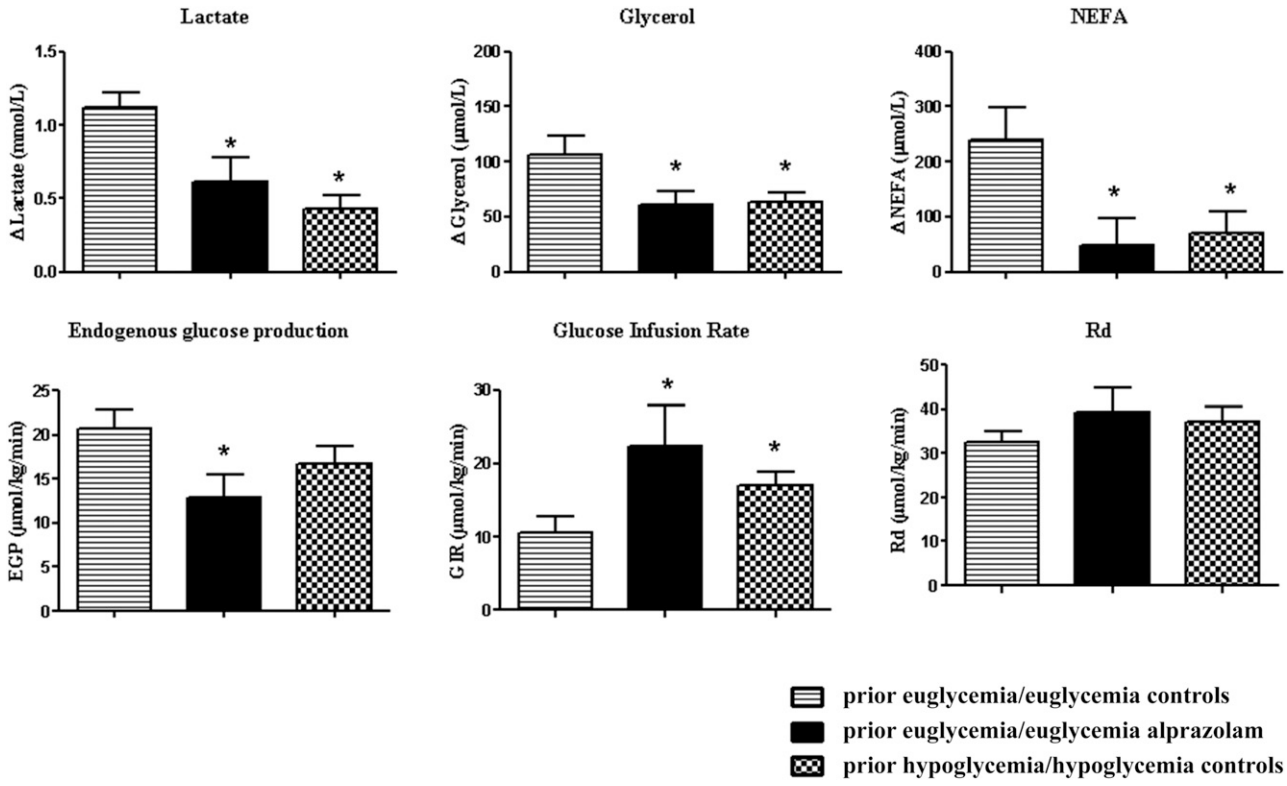

Figure 2—Day 2 lactate, NEFA, and glycerol responses (change from baseline to final 15 min of day 2 clamps) and day 2 EGP, glucose infusion rate (GIR), and rate of glucose disposal (Rd) (final 15 min of day 2 clamps) in overnight-fasted healthy individuals after either day 1 euglycemia, day 1 euglycemia and alprazolam, or day 1 hypoglycemia. *P < 0.04–0.003 significantly reduced compared with euglycemia/euglycemia control.

blunting of key neuroendocrine (glucagon, cortisol, growth hormone), ANS (epinephrine, norepinephrine), and metabolic (EGP, lipolysis, glycogenolysis) homeostatic responses during 90 min of next-day submaximal exercise in individuals with type 1 diabetes. During exercise, relatively mild hyperglycemia can blunt neuroendocrine responses, whereas relatively small reductions in plasma glucose can amplify ANS and neuroendocrine responses. Therefore, we used the glucose clamp technique to produce equivalent glycemia during day 2 exercise. Similarly, overnight plasma glucose control before each clamp study allowed the subjects to have similar and normalized baseline glucose levels and, thus, removed the potential uncontrolled and confounding effects of differing glycemia on metabolic (glucose kinetics, lipolysis) and neuroendocrine responses.

Day 1 alprazolam or moderate hypoglycemia similarly suppressed ANS (epinephrine, norepinephrine, pancreatic polypeptide) responses during day 2 exercise compared with day 1 euglycemic control. GABA A activation resulted in a diffuse reduction of ANS responses, including sympathetic neural (norepinephrine), sympathetic adrenal (epinephrine), and parasympathetic (pancreatic polypeptide) nervous system components, suggesting that GABA

Table 2—Day 2 cardiovascular parameters in overnight-fasted subjects with type 1 diabetes after either day 1 euglycemia and alprazolam, day 1 euglycemia, or day 1 hypoglycemia

	Prior euglycemia control (no alprazolam)		Prior euglycemia alprazolam		Prior hypoglycemia control (no alprazolam)	
	Baseline	Final	Baseline	Final	Baseline	Final
Systolic BP (mmHg)	111 ± 4	145 ± 15*	117 ± 5	132 ± 4*	112 ± 4	136 ± 4*
Diastolic BP (mmHg)	67 ± 3	68 ± 9	66 ± 4	73 ± 3	67 ± 3	68 ± 5
Mean arterial pressure (mmHg)	80 ± 3	88 ± 2*	85 ± 5	98 ± 3*	82 ± 3	91 ± 4*
Heart rate (beats/min)	64 ± 3	136 ± 3*	71 ± 5	136 ± 4*	64 ± 5	136 ± 2*

BP, blood pressure. *P < 0.05, significantly increased compared with the baseline value.

A receptors exert their actions through central nervous system (CNS) effects (6) either alone or possibly in combination with actions on specific endocrine organs, such as the adrenal medulla and pancreas (13,22). Glucagon, cortisol, and growth hormone responses were also reduced during day 2 exercise after day 1 GABA A activation or hypoglycemia. Because GABA A receptors are present in the pancreas, adrenal cortex, and pituitary, the reduced neuroendocrine responses could also have resulted from a combination of CNS and/or specific endocrine organ GABA A effects. Furthermore, the blunted sympathetic nervous system (SNS) drive could have contributed to the reduced glucagon response during day 2 exercise.

The present data add to and complement previous studies in moderate- and high-intensity exercise in healthy subjects (8,23–25) and work investigating CNS GABA A regulation of counterregulatory responses to hypoglycemia in animals (5,6). The reduced counterregulatory hormone and SNS responses resulted in substantial blunting of key metabolic responses during exercise. During submaximal exercise, EGP is primarily regulated by the hepatic sinusoidal insulin/glucagon ratio. Because critical insulin deficiency occurs in type 1 diabetes, insulin was exogenously and equivalently replaced during all day 2 studies. In fact, peripheral insulin levels were also reduced in all groups to simulate the usual physiologic fall during exercise in individuals without diabetes. The blunted day 2 exercise glucagon responses after day 1 GABA A activation, therefore, created an increased hepatic sinusoidal insulin-to-glucagon ratio, which would have been a powerful inhibitory signal for EGP.

The blunted neuroendocrine and SNS responses would also have contributed to the reduced NEFA, glycerol, and lactate responses after GABA A activation or hypoglycemia. Thus, the blunted day 2 lipolytic (NEFA, glycerol) and glycogenolytic (lactate) responses would have diminished the energy for and the flow of precursors for gluconeogenesis during day 2 exercise. GABA A activation appeared to have selective effects on glucose production because peripheral glucose uptake was similar in all groups. In part, the relatively normalized peripheral insulin levels, combined with reduced NEFA levels and preserved noninsulin-mediated glucose uptake mechanisms, may have maintained muscle glucose uptake during exercise. However, the rates of exogenous glucose infusion used to maintain euglycemia during day 2 exercise were substantially increased after day 1 alprazolam or hypoglycemia. Thus, the increased exogenous glucose infusion compensated for the decreased EGP and provided sufficient glucose substrate for the working muscles.

Similar to the study in healthy individuals (8) the dose of alprazolam (1 mg before day 1 euglycemic clamps) was used to represent the average daily clinical dose of the drug (1–4 mg/day). Day 1 alprazolam was administered ~24.5 and ~21.5 h before the start of day 2 exercise. The half-life of alprazolam is ~11 h. Therefore, ~25% of the day 1 dose would still be pharmacologically active during day 2 exercise. Thus, we are unsure about how much of the blunting effects of GABA A activation were due to day 1 alprazolam administration and/or carryover effects on day 2.

Day 2 baseline neuroendocrine, ANS, and metabolic biomarkers were similar in all three groups before exercise. This would indicate that similar to healthy individuals, prior GABA A activation does not affect basal physiologic tone but has a specific effect on reducing ANS and neuroendocrine stress responses during exercise.

The study subjects participated in regular exercise activities but were not elite athletes. Each subject could easily complete the 90 min of submaximal moderate exercise (50% VO_{2max}), which was set relative to each individual's exercise tolerance and maximal workload. With the advent of new basal insulin analogs and continuous subcutaneous insulin pump technology, the insulin levels created in this study reflect those occurring in typical clinical type 1 diabetes practice. Additionally, because type 1 diabetes is a state of critical endogenous insulin deficiency/absence, the insulin levels in this study would also simulate the typically reversed portal: peripheral insulin gradient occurring in type 1 diabetes.

In summary, this report appears to be the first of GABA A receptor activation on next-day moderate exercise in type 1 diabetes. Antecedent hypoglycemia or GABA A activation resulted in widespread substantial blunting of homeostatic counterregulatory responses during next-day exercise. Taken together, GABA A activation appears to have a greater aggregate blunting of counterregulatory responses due to similar effects to reduce neuroendocrine and ANS hormones as prior hypoglycemia but an even greater action to blunt EGP. Antecedent hypoglycemia may induce exercise-associated counterregulatory failure in part by activating GABA A pathways. However, this study demonstrates that pharmacologic activation of GABA A pathways independently results in a widespread blunting of neuroendocrine (glucagon, cortisol, growth hormone), ANS (epinephrine, norepinephrine, pancreatic polypeptide), and metabolic (EGP, lipolysis) glucoprotective mechanisms during subsequent moderate-intensity exercise in type 1 diabetes.

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