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Effects of Antecedent GABA A Receptor Activation on Counterregulatory Responses to Exercise in Healthy Man

The aim of this study was to determine whether antecedent stimulation of γ-aminobutyric acid (GABA) A receptors with the benzodiazepine alprazolam can blunt physiologic responses during next-day moderate (90 min) exercise in healthy man. Thirty-one healthy individuals (16 male/15 female aged 28 ± 1 year, BMI 23 ± 3 kg/m^2) were studied during separate, 2-day protocols. Day 1 consisted of morning and afternoon 2-h hyperinsulinemic-euglycemic or hypoglycemic clamps with or without 1 mg alprazolam given 30 min before a clamp. Day 2 consisted of 90-min euglycemic cycling exercise at 50% VO$_{2max}$. Despite similar euglycemia (5.3 ± 0.1 mmol/L) and insulinemia (46 ± 6 pmol/L) during day 2 exercise studies, GABA A activation with alprazolam during day 1 euglycemia resulted in significant blunting of plasma epinephrine, norepinephrine, glucagon, cortisol, and growth hormone responses. Lipolysis (glycerol, nonesterified fatty acids) and endogenous glucose production during exercise were also reduced, and glucose infusion rates were increased following prior euglycemia with alprazolam. Prior hypoglycemia with alprazolam resulted in further reduction of glucagon and cortisol responses during exercise. We conclude that prior activation of GABA A pathways can play a significant role in blunting key autonomous nervous system, neuroendocrine, and metabolic physiologic responses during next-day exercise in healthy man.

Exercise is a cornerstone of diabetes management, improving insulin sensitivity and reducing the risk of cardiovascular disease, and is beneficial for weight management (1–4). Nevertheless, exercise remains a significant risk for and cause of hypoglycemia in individuals receiving insulin secretagogues and/or insulin (5,6). During exercise, homeostatic (counterregulatory) responses are activated to provide glucose and fat to the working muscles and maintain normal plasma glucose levels. These counterregulatory responses include inhibition of insulin release and enhanced catecholamine (autonomous nervous system [ANS]) and neuroendocrine hormone (glucagon) secretion (7). The result is increased hepatic glucose production through glycogenolysis and gluconeogenesis (8). These mechanisms function so efficiently that healthy individuals do not become hypoglycemic during all but prolonged exercise.

Counterregulatory defenses against falling plasma glucose in exercise are similar to the ANS and neuroendocrine mechanisms activated during hypoglycemia (8,9). Studies in rats and primates have demonstrated that activation of γ-aminobutyric acid (GABA) A receptors reduces major counterregulatory responses (epinephrine, glucagon) during hypoglycemia (10,11). Our laboratory has also reported similar effects in nondiabetic healthy human subjects (12), demonstrating that prior activation of GABA A receptors with the benzodiazepine alprazolam reduces autonomic, neuroendocrine, and metabolic counterregulatory responses during next-day hypoglycemia.

Studies investigating the integrated effects of activation of GABA A receptors on neuroendocrine, ANS, and metabolic counterregulatory responses to subsequent exercise are lacking. The aim of the current study was to test the hypothesis that antecedent pharmacologic activation of GABA A receptors with alprazolam can result in neuroendocrine, ANS, and/or metabolic counterregulatory failure during next-day moderate exercise in healthy humans.

RESEARCH DESIGN AND METHODS

Subjects

Thirty-one healthy individuals (16 males/15 females aged 28 ± 1 years, BMI 23 ± 3 kg/m^2) were studied. Subjects were nonsmokers, had no family history of diabetes, and

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COMPLICATIONS

participated in moderate recreational exercise, but no elite athletes were studied (mean VO_{2max} 39 ± 2 mL/kg/min). All individuals had normal liver, renal, and hematological parameters. Studies were approved by the Vanderbilt University human subjects institutional review board, and all subjects gave informed written and verbal consent.

VO_{2max} Testing
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. Rates of ventilation, VO_2, and VCO_2 were continuously monitored using a computerized, open-circuit indirect calorimetry cart (Parvo Medics, Sandy, UT) with a mouthpiece and nose clip system. Heart rate and surface electrocardiogram were monitored continuously before, during, and after exercise through electrodes placed on the anterior chest. Blood pressure was monitored every 2–3 min using a manual cuff.

Experimental Design
Individuals participated in four separate, single-blind, 2-day experiments with differing day 1 protocols separated by at least 2 months (Fig. 1). All subjects were instructed to avoid intense exercise and alcohol and to consume their usual weight-maintaining diet for 3 days before each study. Each subject was admitted to the Vanderbilt University Clinical Research Center (CRC) the evening before an experiment. The next morning, after an overnight 10-h fast, subjects had intravenous cannulae placed under local 1% lidocaine anesthesia. One cannula was placed in a retrograde fashion into a vein in the back of the hand. This hand was placed in a heated box (55–60°C) so that arterialized blood could be obtained (13). The other cannula was placed in the arm for infusions of

dextrose solution, insulin, potassium chloride, and labeled glucose. As a safety measure, an electrocardiogram was recorded continuously throughout the 2-h hyperinsulinemic clamps and day 2 exercise studies.

Day 1 consisted of four different antecedent challenges (Fig. 1). Protocol 1 consisted of day 1 morning and afternoon hyperinsulinemic-euglycemic clamps ($n = 20$). 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 (2.9 ± 0.1 mmol/L) ($n = 16$). Protocol 4 involved morning and afternoon hyperinsulinemic hypoglycemia (2.9 ± 0.1 mmol/L) with alprazolam ($n = 10$). Four individuals participated in three studies, 21 participated in two studies, and 6 participated in one study. Some of the subjects had participated and provided data in a previous study (14).

Day 1 began with a baseline period (0–120 min) followed by a 2-h hyperinsulinemic experimental clamp period (120–240 min). At the start of the experimental period, a primed continuous infusion of insulin (Eli Lilly, Indianapolis, IN) was administered at a rate of 9 pmol/kg/min for 120 min. Potassium chloride (5 mmol/h) was also infused during the clamp period to reduce insulin-induced hypokalemia. Plasma glucose levels were measured every 5 min, and a variable infusion of 20% dextrose was adjusted so that plasma glucose levels were held constant in the euglycemia studies (15). During hypoglycemia, the rate of fall of glucose was controlled (~0.08 mmol/min), and the hypoglycemic nadir (2.9 ± 0.1 mmol/L) was achieved and held constant using a modification of the glucose clamp technique (16). After completion of the initial 2-h clamp period, the insulin infusion was stopped, and a 2-h period of euglycemia
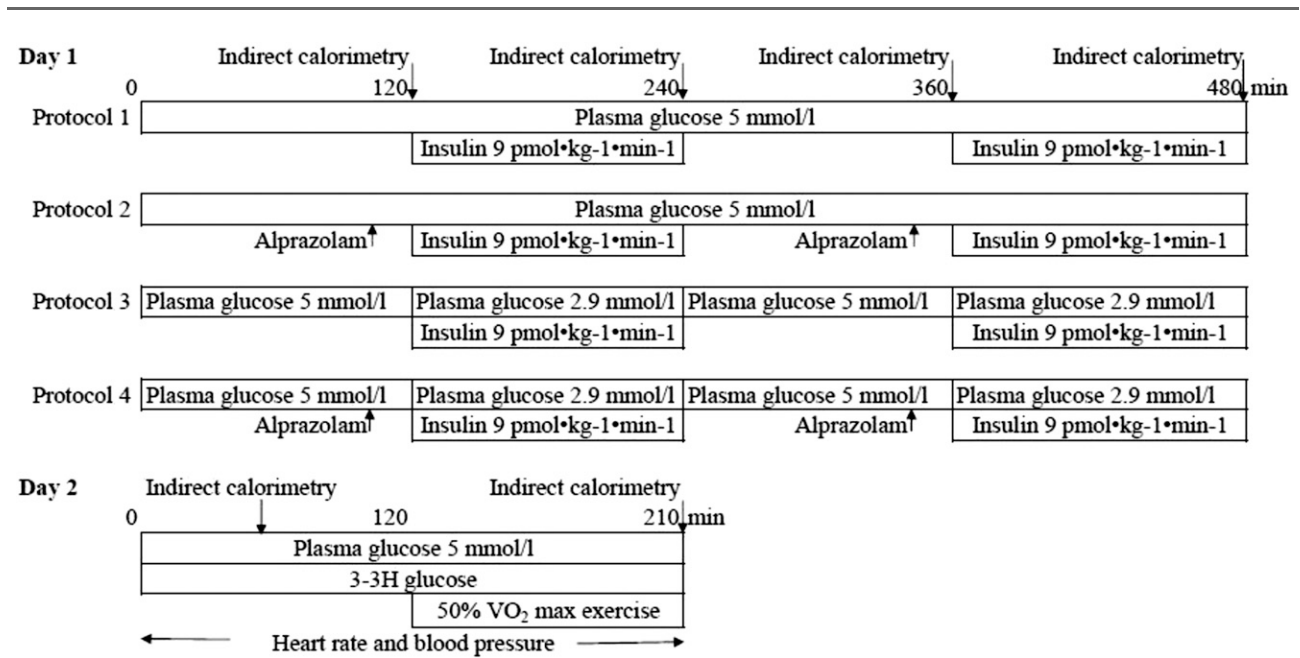

Figure 1—Diagram of study procedures.

was maintained using 20% dextrose infusion. At that point, insulin was restarted, and a second hyperinsulinemic-euglycemic clamp or hyperinsulinemic-hypoglycemic clamp (identical to the morning study) was performed (Fig. 1). At completion of the second glucose clamp, subjects consumed a standardized meal and a bedtime snack, and remained in the CRC.

Day 2 Exercise

Day 2 was identical for all four protocols and was started after an overnight 10-h fast. Each study consisted of a tracer equilibration period (0–120 min) and a 90-min experimental period (120–210 min). A primed (18-μCi) continuous infusion (0.18 μCi/min) of high-performance liquid chromatography–purified [3-3H]glucose (11.5 mCi/mmol/L; PerkinElmer Life Sciences, Boston, MA) was administered starting at 0 min and continued throughout the study to measure glucose kinetics. Exercise consisted of 90 min continuous cycling (at 60–70 rpm) on a bicycle ergometer at a relative work intensity of 50% of the individual's VO_{2max}. Twenty-percent dextrose was infused with a variable rate so that the euglycemic target (5.2–5.3 mmol/L) was held constant for the duration of the study.

Tracer Calculations

Endogenous glucose production (EGP) was calculated according to the method of Wall et al. (17) by determining the total rate of appearance, which comprises both EGP and any exogenous glucose infused to maintain the desired euglycemia, and subtracting from it the amount of exogenous glucose infused. It is now recognized that the original model described by Wall et al. and Steele et al. (18) is not fully quantitative because underestimates of total rate of appearance and rate of disappearance can be obtained. Using a highly purified tracer and taking measurements under steady-state conditions (i.e., constant specific activity) in the presence of low glucose flux eliminate most, if not all, of the problems. To minimize changes in specific activity, the tracer infusion rate was gradually doubled during the first 30 min of exercise. During the remaining 60 min of exercise, proportional additional increases of the tracer infusion rate were made commensurate with the changes in exogenous glucose infusion rate.

Analytical Methods

The collection and processing of blood samples have been previously described (19). Plasma glucose concentrations were measured in triplicate using the glucose oxidase method with a glucose analyzer (Beckman Coulter, Fullerton, CA). Glucagon was measured according to the method of Aguilar-Parada et al. (20) with an interassay coefficient of variation (CV) of 12%. Insulin was measured as previously described (21) with an interassay CV of 11%. Catecholamines were determined by high-performance liquid chromatography (22) with an interassay CV of 12% for epinephrine and 8% for norepinephrine. We made two modifications to the procedure for catecholamine determination: 1) We used a three-point rather than a one-point standard calibration

curve; and 2) we spiked the initial and final samples of plasma with known amounts of epinephrine and norepinephrine so that accurate identification of the relevant catecholamine peaks could be made. Growth hormone (23) (interassay CV = 8%), cortisol (Clinical Assays Gamma Coat Radioimmunoassay Kit, interassay CV = 6%), and pancreatic polypeptide (24) (interassay CV = 8%) were measured using radioimmunoassay techniques. Lactate and glycerol were measured from deproteinized whole blood using the method of Lloyd et al. (25). Nonesterified fatty acids (NEFAs) were measured using a kit from Wako Diagnostics (26).

Cardiovascular Parameters

Heart rate and systolic, diastolic, and mean arterial blood pressures were measured noninvasively by a Dinamap vitals monitor (Critikon, Tampa, FL) every 10 min.

Statistical Analysis

Data are expressed as mean ± SE and were analyzed using standard, parametric, one-, and two-way ANOVA with repeated measures where appropriate (GraphPad Software, Inc., San Diego, CA). Tukey post hoc analysis was used to delineate statistical significance within each group. $P < 0.05$ was accepted as statistically significant. Changes (responses) from baseline to the end of exercise on day 2 were compared. Baseline data were calculated as an average of two time points (110 and 120 min), and final 15-min data represent an average of two measurements taken during time 195 and 210 min.

RESULTS

Day 1 Glucose and Insulin

Plasma glucose levels were similar in the morning and afternoon during all day 1 euglycemic studies (5.3 ± 0.1 mmol/L) (Fig. 2). Plasma glucose levels during day 1 morning and afternoon hypoglycemia studies were similar with and without alprazolam (2.9 ± 0.05 mmol/L) (Fig. 2). Plasma insulin levels during all hyperinsulinemic day 1 studies were similar among all groups (500 ± 48 pmol/L).

Day 2 Exercise

Glucose and Insulin

During day 2 exercise, plasma glucose was similar (5.3 ± 0.09 mmol/L) in all four groups (Fig. 2). Plasma insulin was suppressed by a greater amount during exercise following day 1 euglycemia (20 ± 5 pmol/L) compared with day 1 hypoglycemia (33 ± 4 pmol/L) and day 1 alprazolam studies (euglycemia 35 ± 4 pmol/L, hypoglycemia 34 ± 6 pmol/L, $P < 0.02$) (Fig. 2).

ANS Responses

Baseline values of ANS-mediated hormones were similar at the start of all day 2 exercise studies (Table 1). Increases in day 2 plasma epinephrine responses (Fig. 3) were significantly lower ($P < 0.005$) during day 2 exercise following day 1 euglycemia and alprazolam (Δ 183 ± 37 pmol/L), day 1 hypoglycemia (Δ 277 ± 58 pmol/L), and day 1 hypoglycemia and alprazolam (Δ 225 ± 53 pmol/L)

Day 1 Plasma Glucose

Day 2 Plasma Glucose

Day 2 Insulin

- prior eugly/eugly alprazolam
- prior eugly/eugly control (no alprazolam)
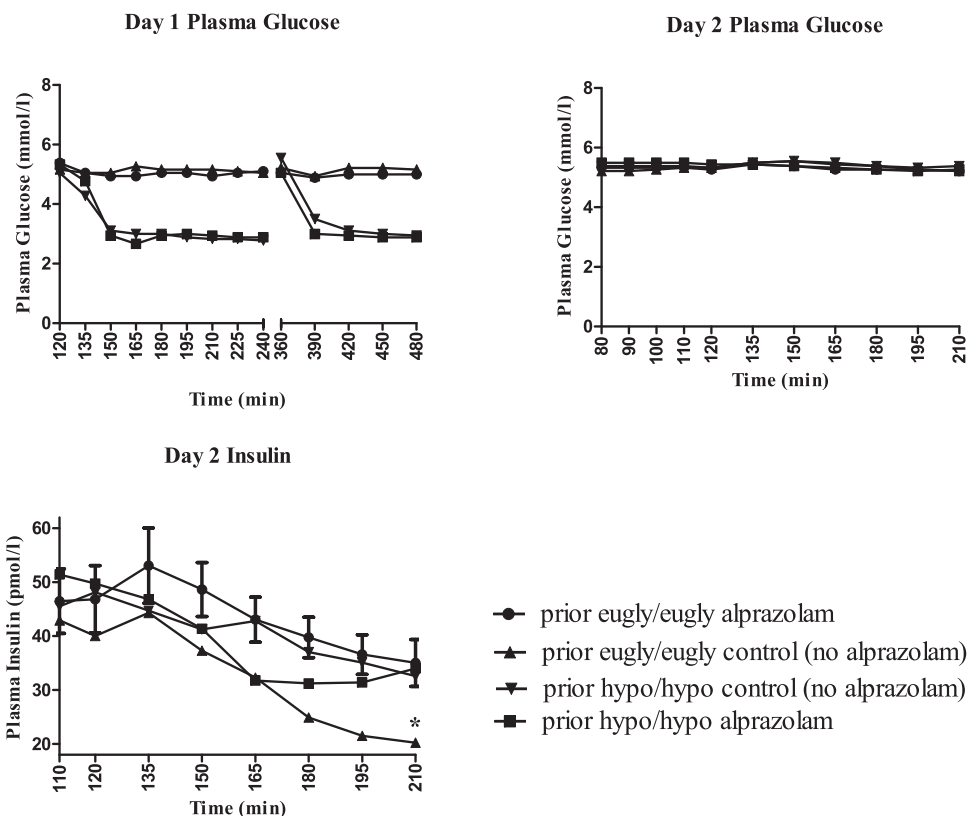- prior hypo/hypo control (no alprazolam)
- prior hypo/hypo alprazolam

Figure 2—Plasma glucose (mean ± SE) levels during day 1 and day 2 studies and plasma insulin levels (mean ± SE) during day 2 studies. *$P < 0.001$ compared with prior eugly/eugly alprazolam, prior hypo/hypo control, and prior hypo/hypo alprazolam. eugly, euglycemia; hypo, hypoglycemia.

compared with day 1 euglycemia (Δ 769 ± 213 pmol/L). Day 2 norepinephrine responses (Fig. 3) were also significantly lower ($P < 0.0001$) following day 1 euglycemia and alprazolam (Δ 1.3 ± 0.3 nmol/L) and day 1 hypoglycemia and alprazolam (Δ 1.8 ± 0.4 nmol/L) compared with day 1 euglycemia (Δ 4.8 ± 0.6 nmol/L). Day 2 pancreatic polypeptide (Fig. 3) responses were lower ($P < 0.003$) following day 1 euglycemia and alprazolam (Δ 43.6 ± 18 ng/L), day 1 hypoglycemia (Δ 53 ± 15 ng/L), and day 1 hypoglycemia and alprazolam (Δ 17.3 ± 15 ng/L) compared with day 1 euglycemia (Δ 109 ± 16 ng/L).

Neuroendocrine Counterregulatory Hormones
Baseline values of neuroendocrine counterregulatory hormones were similar at the start of all day 2 exercise studies (Table 1). Increases in plasma glucagon levels on day 2 (Fig. 3) were significantly blunted ($P < 0.0001$) following day 1 euglycemia and alprazolam (Δ 4.3 ± 2.2 ng/L) and day 1 hypoglycemia (Δ 4.2 ± 1.4 ng/L) compared with day 1 euglycemia (Δ 13 ± 1.6 ng/L). Day 1 hypoglycemia and alprazolam resulted in a greater reduction ($P < 0.05$) in day 2 glucagon during exercise responses (Δ −4.9 ± 4.3 ng/L) compared with the other groups.

Day 2 growth hormone responses (Fig. 4) were also blunted ($P < 0.006$) following day 1 euglycemia and alprazolam (Δ 0.7 ± 1.7 μg/L), day 1 hypoglycemia (Δ 3.2 ± 1.0

μg/L), and day 1 hypoglycemia and alprazolam (Δ 1.2 ± 3.0 μg/L) compared with day 1 euglycemia (Δ 10.2 ± 2.2 μg/L). Day 2 plasma cortisol responses (Fig. 5) were also lower ($P < 0.002$) following day 1 euglycemia and alprazolam (Δ −76 ± 37 nmol/L) and day 1 hypoglycemia and alprazolam (Δ −32.6 ± 34.8 nmol/L) compared with day 1 euglycemia (Δ 195 ± 48 nmol/L) and day 1 hypoglycemia (Δ 131.0 ± 53.6 nmol/L).

Glucose Kinetics
Baseline rates of glucose kinetics were similar at the start of all day 2 exercise studies (Table 1). Rates of EGP were reduced ($P < 0.0001$) during the final 15 min of day 2 exercise following day 1 euglycemia and alprazolam (13 ± 1.5 μmol/kg/min), day 1 hypoglycemia (10.7 ± 1.5 μmol/kg/min), and day 1 hypoglycemia and alprazolam (Δ 14.6 ± 2.2 μmol/kg/min) compared with day 1 euglycemia (20 ± 1.1 μmol/kg/min) (Fig. 5). Glucose infusion rates were increased ($P < 0.0001$) during the final 15 min of day 2 exercise following day 1 euglycemia and alprazolam (9.6 ± 1.9 μmol/kg/min), day 1 hypoglycemia and alprazolam (11.8 ± 2.0 μmol/kg/min), and day 1 hypoglycemia (7.1 ± 1.5 μmol/kg/min) compared with 1.2 ± 0.4 μmol/kg/min following day 1 euglycemia. Rates of glucose disposal were similar among the four groups (Fig. 5).

Table 1—Day 2 baseline neuroendocrine, EGP, and intermediary metabolite values in overnight-fasted healthy individuals following each day 1 experimental condition

Baseline values	Prior eugly control (no alprazolam)	Prior eugly alprazolam	Prior hypo control (no alprazolam)	Prior hypo alprazolam
Epinephrine (pmol/L)	191 ± 39	196 ± 38	139 ± 43	158 ± 54
Norepinephrine (nmol/L)	1.4 ± 0.2	1.6 ± 0.1	1.4 ± 0.2	1.5 ± 0.2
Glucagon (ng/L)	42 ± 3	46 ± 9	44 ± 3	46 ± 4
Growth hormone (μg/L)	2.7 ± 2	2.9 ± 1	2.4 ± 0.6	2.2 ± 0.1
Cortisol (nmol/L)	306 ± 55	419 ± 82	286 ± 83	333 ± 55
Pancreatic polypeptide (pmol/L)	18 ± 3	21 ± 1	19 ± 2	21 ± 6
EGP (μmol/kg/min)	10.4 ± 0.5	9.3 ± 1.6	11 ± 0.5	8.8 ± 0.5
NEFA (μmol/L)	332 ± 50	325 ± 62	337 ± 57	273 ± 52
Lactate (mmol/L)	1.07 ± 0.1	1.01 ± 0.1	1.0 ± 0.2	0.7 ± 0.1
Glycerol (μmol/L)	66 ± 10	76 ± 13	51 ± 12	54 ± 7

Data are mean ± SE. eugly, euglycemia; hypo, hypoglycemia.

Intermediary Metabolism

Baseline levels of lactate, glycerol, and NEFA were similar at the start of all day 2 exercise studies (Table 1). Blood lactate responses were significantly reduced ($P < 0.008$) during day 2 following day 1 euglycemia and alprazolam (Δ 0.18 ± 0.1 mmol/L), day 1 hypoglycemia (Δ 0.11 ± 0.09 mmol/L), and day 1 hypoglycemia and alprazolam (Δ 0.07 ± 0.06 mmol/L) compared with day 1 euglycemia (Δ 0.7 ± 0.2 mmol/L) (Fig. 4). Day 2 plasma NEFA responses were also reduced ($P < 0.003$) following both day 1 alprazolam groups (Δ 123 ± 23 and 119 ± 28 μmol/L) compared with day 1 euglycemia (Δ 319 ± 48 μmol/L) (Fig. 4). Day 2 glycerol responses (Fig. 4) were significantly lower ($P < 0.05$) following day 1 euglycemia and alprazolam (Δ 66.3 ± 15.6 μmol/L) and
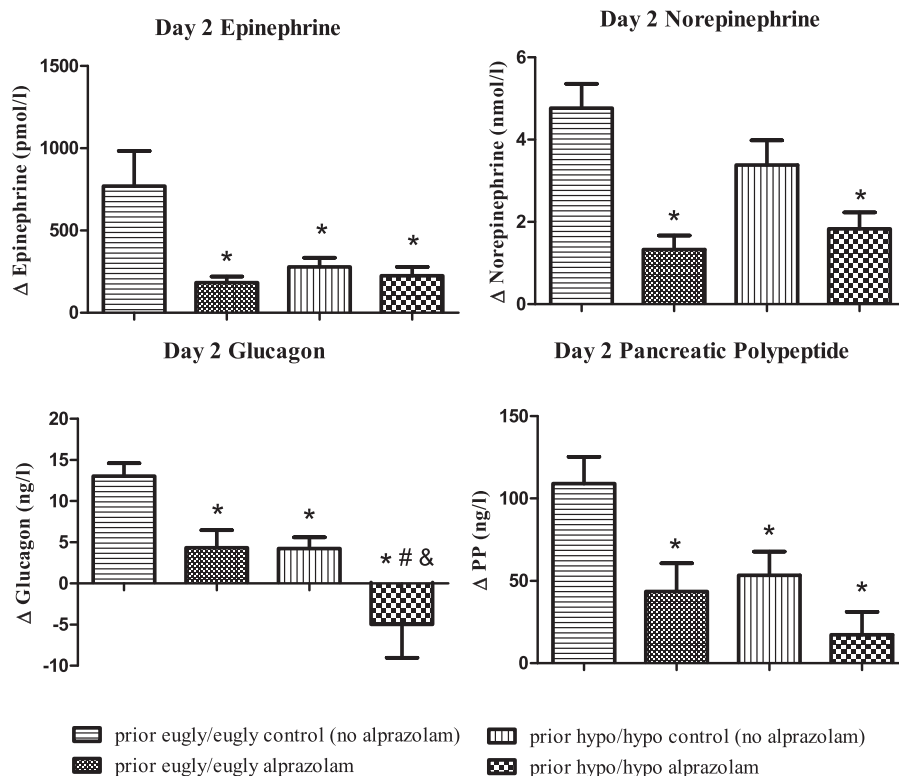

Figure 3—Day 2 epinephrine, norepinephrine, glucagon, and pancreatic polypeptide (PP) (change from baseline to final 15 min of day 2 clamps) in overnight-fasted healthy individuals following either day 1 euglycemia, day 1 euglycemia and alprazolam, day 1 hypoglycemia, or day 1 hypoglycemia and alprazolam. *$P < 0.006$–0.0001 compared with eugly/eugly control (no alprazolam); #$P < 0.04$ compared with eugly/eugly alprazolam; &$P < 0.02$ compared with hypo/hypo control (no alprazolam). eugly, euglycemia; hypo, hypoglycemia.
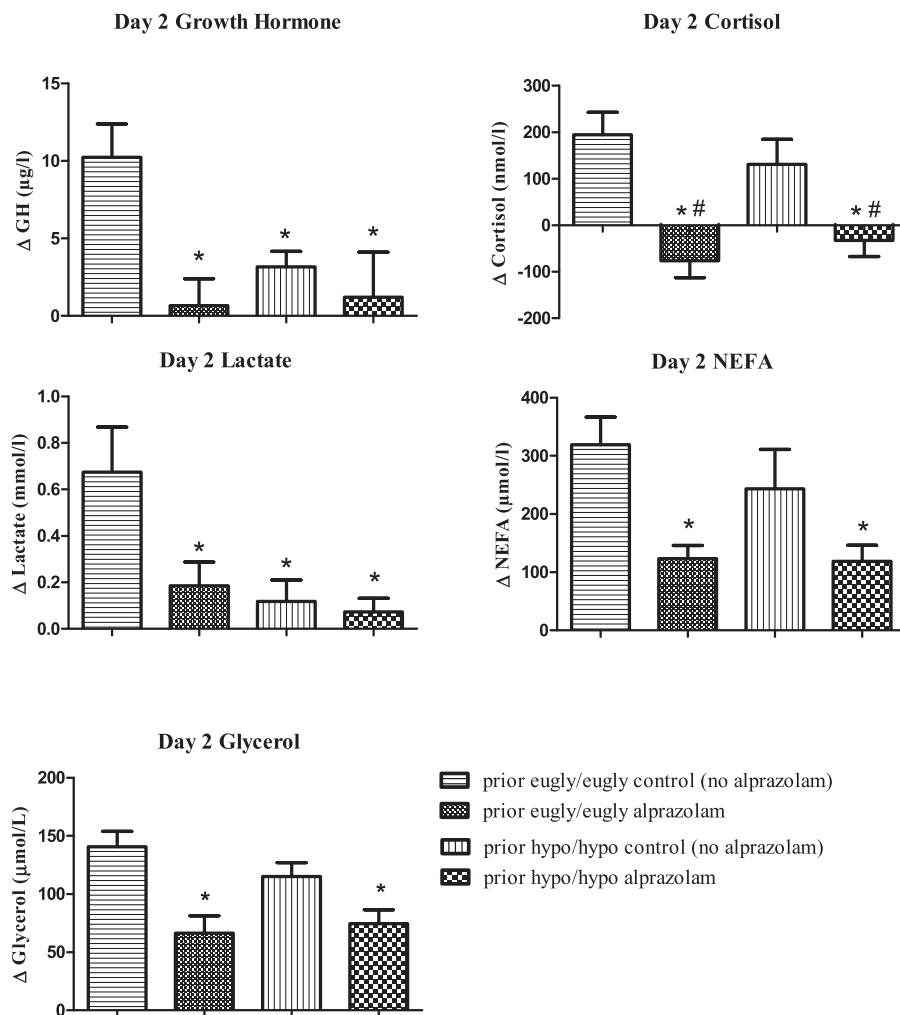
Figure 4—Day 2 growth hormone (GH), cortisol, lactate, NEFA, and glycerol levels (change from baseline to final 15 min of day 2 clamps) in overnight-fasted healthy individuals following either day 1 euglycemia, day 1 euglycemia and alprazolam, day 1 hypoglycemia, or day 1 hypoglycemia and alprazolam. *$P < 0.02–0.0009$ compared with prior eugly/eugly control (no alprazolam); #$P < 0.05–0.006$ compared with prior hypo/hypo control (no alprazolam). eugly, euglycemia; hypo, hypoglycemia.

day 1 hypoglycemia and alprazolam (Δ 74.5 $\pm$ 12.7 μmol/L) compared with day 1 euglycemia (Δ 141 $\pm$ 13 μmol/L).

Cardiovascular Responses

There were similar changes in systolic and diastolic blood pressure, mean arterial pressure, and heart rate in all groups (Table 2).

DISCUSSION

The current study determined the effects of prior GABA A activation with the benzodiazepine alprazolam on a background of euglycemia or hypoglycemia on homeostatic (counterregulatory) responses during next-day exercise. The euglycemic clamp technique was used to maintain identical glycemia during all day 2 exercise studies. The results demonstrate that day 1 activation of GABA A receptors can blunt a wide spectrum of key neuroendocrine (glucagon, cortisol, growth hormone), ANS (epinephrine, norepinephrine), and metabolic (endogenous

glucose production, lipolysis, glycogenolysis) counterregulatory responses during 90 min of next-day moderate exercise in healthy humans.

In addition to being an important homeostatic neuromodulator, GABA is a major inhibitory neurotransmitter. Activation of the GABA A receptor subtype commonly occurs after benzodiazepine and alcohol use (27,28). Despite the importance and frequent activation of GABA A receptors, very little is known about the effects of stimulation of these receptors on subsequent exercise. Only three studies appear to have addressed this question (29–31), all of which were performed during maximal exercise and reported that alprazolam could blunt catecholamine (29) or pituitary/adrenal (ACTH, cortisol) (30,31) responses in healthy humans. However, the effects of prior GABA A activation on integrated ANS, neuroendocrine, and metabolic counterregulatory responses during clamped euglycemia under moderately prolonged submaximal exercise

Day 2 EGP

Day 2 GIR

Day 2 Rd

prior eugly/eugly control
prior eugly/eugly alprazolam
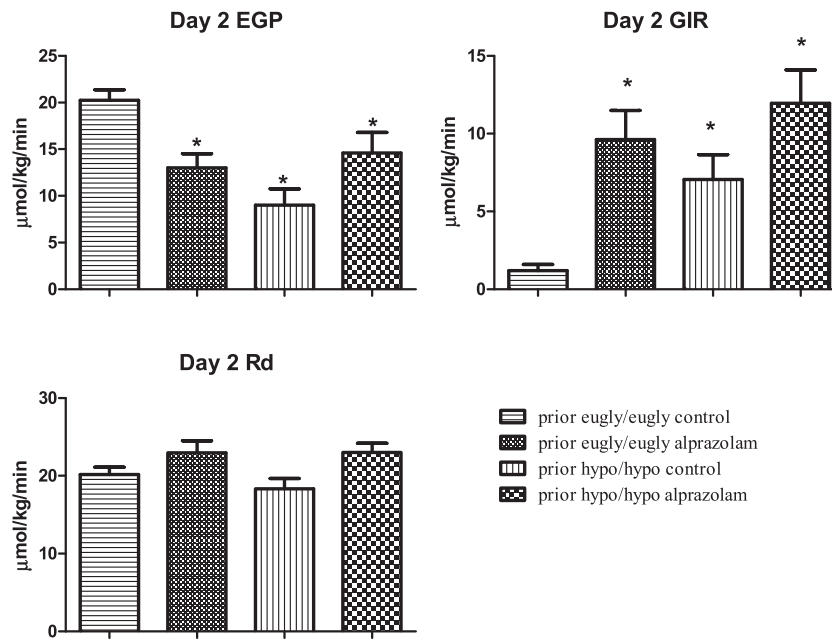prior hypo/hypo control
prior hypo/hypo alprazolam

Figure 5—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 following either day 1 euglycemia (eugly), day 1 eugly and alprazolam, day 1 hypoglycemia (hypo), or day 1 hypo and alprazolam. *$P < 0.04–0.0001$ compared with eugly/eugly control (no alprazolam).

conditions appears to be unknown. An important feature of the present experimental design was the use of the glucose clamp technique, which allowed equivalent day 1 euglycemia or hypoglycemia to be created and to maintain identical glucose levels during day 2 exercise studies. This is relevant because during exercise only small reductions in fasting glucose can amplify, whereas increases in glycemia can inhibit, neuroendocrine responses (32).

After prior alprazolam or day 1 hypoglycemia, glucagon levels were substantially suppressed. Additionally, combined day 1 alprazolam and hypoglycemia further suppressed glucagon responses below baseline during day 2 exercise. Insulin levels fell during all day 2 exercise protocols, but, of note, the fall was blunted following day 1 alprazolam and prior hypoglycemia protocols compared with the day 1 euglycemic control studies. Adrenal medullary (epinephrine), sympathetic neural (norepinephrine), and parasympathetic

nervous system (pancreatic polypeptide) responses were also blunted during day 2 exercise by day 1 alprazolam and/or hypoglycemia. The diffuse blunting of ANS responses following GABA A receptor activation suggests reductions in central (central nervous system) sympathetic and parasympathetic outflow (33) and/or reductions of epinephrine, norepinephrine, and pancreatic polypeptide release from the adrenal medulla and pancreas (34,35). Additionally, the reduced sympathetic nervous system drive (both direct and circulating catecholamines) may have contributed to the blunted glucagon and insulin physiologic responses during day 2 exercise.

Cortisol and growth hormone responses were also blunted during day 2 exercise. Animal studies have determined that GABA A receptors are present in the pituitary gland and adrenal cortex (in addition to the adrenal medulla and pancreas) (36–38). Thus, the present findings extend previous

Table 2—Day 2 cardiovascular parameters in overnight-fasted healthy individuals following each day 1 experimental condition

Parameter	Prior eugly control (no alprazolam)		Prior eugly alprazolam		Prior hypo control (no alprazolam)		Prior hypo alprazolam	
	Baseline	Final	Baseline	Final	Baseline	Final	Baseline	Final
Systolic blood pressure (mmHg)	111 ± 7	145 ± 15*	107 ± 5	137 ± 7*	110 ± 6	148 ± 10*	111 ± 3	140 ± 3*
Diastolic blood pressure (mmHg)	65 ± 7	73 ± 3*	66 ± 4	75 ± 9*	65 ± 5	73 ± 3*	68 ± 1	72 ± 3*
Mean arterial pressure (mmHg)	80 ± 3	96 ± 10*	79 ± 3	96 ± 7*	81 ± 5	92 ± 9*	82 ± 4	94 ± 7*
Heart rate (beats/min)	63 ± 5	139 ± 7*	64 ± 3	132 ± 2*	60 ± 4	147 ± 8*	67 ± 3	135 ± 5*

Data are mean ± SE. eugly, euglycemia; hypo, hypoglycemia. *$P < 0.006–0.0001$, significantly increased compared with baseline value.

observations (29–31) and demonstrate the wide-ranging effects of GABA A on downregulating multiple key neuroendocrine responses during exercise.

As a result of the blunted neuroendocrine and ANS responses, key metabolic homeostatic responses also were significantly reduced during day 2 exercise. During submaximal exercise, the hepatic sinusoidal glucagon-to-insulin ratio is a key regulator of glucose production (32). In the current study, glucagon responses were reduced and insulin levels increased, thus contributing to substantially reduced glucose production rates during day 2 exercise. In fact, analysis of glucose kinetics during day 2 exercise demonstrated that the primary metabolic defect was indeed reduced EGP because rates of peripheral glucose uptake were maintained. Thus, the increased glucose infusion rates needed to maintain euglycemia during exercise following day 1 alprazolam and/or hypoglycemia were used to supplement the deficient (hepatic) EGP and meet the needs of the working muscles. Of note, because of the severe blunting of neuroendocrine and ANS responses, almost the entire physiologic increment of EGP during exercise was blunted and had to be replaced by an exogenous glucose infusion.

The reduced neuroendocrine and ANS responses also blunted glycogenolytic (lactate) responses from liver and skeletal muscle and lipolytic (glycerol, NEFA) responses from adipose tissue. All could have contributed to the deficient EGP response because lactate and glycerol are important gluconeogenic substrates, and NEFA produces energy to drive the process.

In addition to determining the effects of day 1 alprazolam on a background of euglycemia, we examined whether additional specific GABA A activation would alter the blunting effects of day 1 hypoglycemia on day 2 exercise. Most of the counterregulatory responses during exercise were not blunted further by the administration of alprazolam during day 1 hypoglycemia. The notable exceptions were glucagon and cortisol, indicating an additive blunting effect of prior hypoglycemia and alprazolam on selective neuroendocrine responses during subsequent exercise.

The dose of alprazolam (1 mg before each of the day 1 glucose clamps) was chosen on the basis of the average daily clinical dose of the drug (1–4 mg/day). Thus, we cannot comment on whether lower or higher doses of alprazolam would have had similar blunting effects on physiologic counterregulatory responses during exercise. Additionally, the half-life of alprazolam is 11.2 h, and the time from the last dose to exercise was ~21 h. Therefore, ~25% of the day 1 dose would still be pharmacologically active during day 2 exercise. Thus, we cannot fully determine how much of the blunting effects of alprazolam was directly due to day 1 administration or additional lingering effects on day 2.

Also of note, day 2 basal levels of neuroendocrine, ANS, and metabolic parameters were unaffected by prior GABA A activation. Thus, it would appear that prior GABA A stimulation reduces the exercise-induced stress response (i.e., ANS and neuroendocrine drive) rather than basal ANS and neuroendocrine constitutive tone.

We studied individuals with average fitness levels to avoid the possible confounding variables associated with the physiology of elite athletes. Submaximal moderate-intensity exercise (50% VO_{2max}) of 90 min duration was chosen so that individuals could complete the exercise and produce large, easily measurable neuroendocrine, ANS, and metabolic counterregulatory signals. Additionally, this duration of exercise represents common sporting activities (e.g., soccer, tennis).

In summary, the current study demonstrates that prior activation of GABA A receptors with the benzodiazepine alprazolam can result in widespread blunting of homeostatic neuroendocrine (glucagon, cortisol, growth hormone, insulin), ANS (epinephrine, norepinephrine, pancreatic polypeptide), and metabolic (EGP, lipolysis, glycogenolysis) responses during subsequent submaximal exercise in healthy humans. We conclude that prior activation of GABA A receptors can inhibit a broad range of neuroendocrine (pituitary, adrenal, pancreas), ANS (both sympathetic and parasympathetic branches), and metabolic (liver, adipose tissue, muscle) counterregulatory responses aimed at preserving glucose levels during subsequent exercise in healthy humans.

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