

Portal Vein Glucose Sensors Do Not Play a Major Role in Modulating Physiological Responses to Insulin-Induced Hypoglycemia in Humans

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OBJECTIVE—Experimental data from animal studies indicate that portal vein glucose sensors play a key role in the responses to slow-fall hypoglycemia. However, their role in modulating these responses in humans is not well understood. The aim of the present study was to examine in humans the potential role of portal vein glucose sensors in physiological responses to insulin-induced hypoglycemia mimicking the slow fall of insulin-treated diabetic subjects.

RESEARCH DESIGN AND METHODS—Ten nondiabetic subjects were studied on two different occasions during intravenous insulin ($2 \text{ mU} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$) plus variable glucose for 160 minutes. In both studies, after 60 min of normal plasma glucose concentrations, hypoglycemia (47 mg/dl) was induced slowly (60 min) and maintained for 60 min. Hypoglycemia was preceded by the ingestion of either oral placebo or glucose (28 g) given at 30 min.

RESULTS—Plasma glucose and insulin were not different with either placebo or glucose ($P > 0.2$). Similarly, counterregulatory hormones, substrates, and symptoms were not different with either placebo or glucose. The Stroop color and colored words subtest of the Stroop test deteriorated less ($P < 0.05$) with glucose than placebo.

CONCLUSIONS—In contrast to animals, in humans, prevention of portal hypoglycemia with oral glucose from the beginning of insulin-induced slow-fall hypoglycemia has no effect on sympathetic and symptomatic responses to hypoglycemia. *Diabetes* 58:194–202, 2009

It has been suggested that glucose sensors in the portal area are necessary to monitor glucose derived from the gut (1). In fact, when exogenous glucose is infused directly in the portal vein (2,3) or in the duodenum (4) or ingested orally as glucose load (5–7), a portal-arterial glucose gradient is generated with glucose concentrations higher in the portal vein than in arterial circulation. Such portal-arterial glucose gradient generates a portal signal that is probably dependent on glucose-sensitive nerves in the portal veins, the firing rate of which

is inversely proportional to the portal glucose concentration (8). The signal then moves through the hepatic vagal afferences to modulate the function of different tissues (e.g., liver, pancreatic β -cells) involved in the control of glucose homeostasis (9). In addition, signals enter the central nervous system to regulate hypothalamic functions, such as feeding and satiety (10). Recent evidence indicates that GLUT2 transporter is essential for glucose sensing by the portal glucose sensor (11,12) and also that glucagon-like peptide 1 (GLP-1) receptor is required for the function of the portal glucose sensor in mice (9).

Earlier studies in animals have shown that portal-arterial glucose gradient is involved in the control of intake of food (10) and stimulation of net hepatic glucose uptake (2,13). In addition, portal glucose sensors modulate the sympathetic responses to hypoglycemia (14,15). However, how portal glucose sensors may affect sympathetic responses to hypoglycemia and how their activity integrates with that of glucose-sensitive areas in the brain is not well understood (16). In fact, several studies in animals indicate that the brain is the prominent center for the sensing of hypoglycemia. In dogs in which insulin-induced hypoglycemia was allowed to occur peripherally while brain euglycemia was maintained by glucose infusions in carotid and vertebral arteries bilaterally, the responses of counterregulatory hormones decreased nearly completely compared with dogs with brain neuroglycopenia (17,18). In rats, the ventromedial hypothalamus (VMH) appears to be necessary to trigger counterregulation during hypoglycemia. In fact, bilateral lesions of the VMH in conscious rats suppresses glucagon and catecholamine responses during hypoglycemia (19,20), suggesting that the VMH is one of the most important sites acting as a glucose sensor (21,22). However, there is evidence that in rats, activation of portal glucose sensors by glucose may be the most important modulators of sympathetic response to hypoglycemia, resulting in a significant suppression of this response (23–25).

Recent studies in rats have established that portal vein glucose sensors, responsible for hypoglycemic detection, extend beyond the portal vein being placed also in the superior mesenteric vein and that their role is essential in detecting slow, but not fast, fall in blood glucose (26).

Limited knowledge is available about the potential role of portal glucose sensors in humans. Only three studies (5–7) have addressed the question, with conflicting results. In fact, counterregulatory hormone responses to hypoglycemia have been found potentiated (5), reduced (6), or reduced in early phase and potentiated in late phase (7) after ingestion of oral glucose (5,6) or orange juice (7). It is likely that methodological differences account, at least in part, for these divergent results.

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See accompanying commentary, p. 21.

So far, no study has investigated in humans the role of portal glucose sensors on counterregulation, symptoms, and cognitive function during hypoglycemia induced slowly, to mimic the hypoglycemia of the clinical situation (27). It is worthy of note that in the postprandial state, a condition characterized by glucose arriving in the portal vein from the gut, sympathetic responses and some aspects of cognitive function are affected by the rate of fall of blood glucose (27). However, in the postprandial condition, it is not only glucose that enters in the portal system but also other substrates that may suppress sympathoadrenal responses to hypoglycemia (28).

The aim of the present study was to examine in humans the potential effects of portal glucose sensors on hormonal counterregulatory responses and responses of symptoms and cognitive function in a model of slow-fall insulin-induced hypoglycemia. For this purpose, healthy subjects were studied during hypoglycemia preceded by ingestion of either oral glucose to prevent portal hypoglycemia or placebo.

RESEARCH DESIGN AND METHODS

Institutional review board approval was obtained for these studies. Ten healthy nondiabetic volunteers (five men, age 30 ± 5 years, BMI 22 ± 1.5 kg/m², means \pm SD) were studied. The study was carried out according to the Helsinki Declaration after obtaining written informed consent by all subjects. All subjects were studied on two different occasions in a random order, computer-generated sequence, at 2- to 3-week intervals, with the hyperinsulinemic-hypoglycemic glucose clamp technique. Both subjects and investigators were blind to treatments.

On the morning of the studies, subjects were admitted to the general clinical research center of the Department of Internal Medicine at ~0700 h. A hand vein of the nondominant arm was cannulated retrogradely and maintained in a hot box (~60°C) for sampling of arterialized-venous blood (29). A superficial vein of the ipsilateral arm was also cannulated for infusion of insulin using a syringe pump (Harvard Apparatus, Ealing, South Natick, MA) and glucose (see below). The two veins were maintained patent by means of 0.9% NaCl infusion (0.5 ml/min).

At time 0900 h (time 0 min), intravenous insulin at the rate of $2 \text{ mU} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ was started and continued until the end of the study (i.e., 160 min). Intravenous glucose at variable rate was also infused. Plasma glucose was allowed to decrease over a period of 60 min from 60 min to the target plasma glucose of 47 mg/dl at 120 min. This hypoglycemic plateau was maintained for the next 40 min, that is, until the end of the study.

On one occasion, at time 0930 h (time 30 min), subjects ingested a 150-ml drink containing either glucose (28 g) or a seemingly identical placebo containing aspartame (1 g). All drinks were prepared and administered by a research nurse not involved in the further execution of the study.

Pilot study results indicated that plasma glucose would increase by 10–15 mg/dl in the following 30 min after ingestion of 28 g glucose. To maintain the double-blind design of the study, glucose infusions rates were varied from 30 to 60 min to produce increments in the magnitude of 10–15 mg/dl in both study conditions.

In all studies, blood samples were drawn at 5- to 10-min intervals for bedside plasma glucose measurement and at 30-min intervals for measurement of plasma insulin, C-peptide, pancreatic polypeptide, counterregulatory hormone, and nonglucose substrate concentrations.

A semiquantitative symptom questionnaire (30) was administered every 30 min. Subjects were asked to score from 0 (none) to 5 (severe) on each of the following symptoms: seven autonomic/neurogenic (adrenergic: heart pounding, tremor, anxiety, and irritability; cholinergic: sweating, hunger, and tingling), five neuroglycopenic (difficulty in thinking, weakness, dizziness, blurred vision, and drowsiness), and three nonspecific (thirst, nausea, and headache) (31). The sum of each of these constituted the total symptom score.

In addition, at baseline, before inducing hypoglycemia, and at the hypoglycemic plateau (indicated as time -30, time 0, and time 120), cognitive function was assessed by applying a battery of hypoglycemia-sensitive tests: Trail making A and B tests (32), verbal fluency (33), verbal memory test (33), digit vigilance test (33), forward and backward digit span (34), Stroop word, color, and color-word (interference) subtests (35), and paced auditorial serial addition test (PASAT 2 and 3 s) (36), with tests always performed in this order. The whole battery took ~30 min to complete and was, therefore, suitable for repeated administration. All tests presented were paper-based except PASAT,

which was presented on an audiocassette tape to control the rate of stimulus presentation. Each subject practiced these tasks on each study occasion, i.e., before the commencement of the glucose clamp, until stable performance was achieved; in addition, to prevent any learning/practice effects, two alternate forms of each test were prepared and used.

Analytical methods. Plasma glucose was measured by means of a Beckman glucose analyzer (Glucose Analyzer II; Beckman Instruments, Fullerton, CA). Plasma insulin, C-peptide, pancreatic polypeptide, counterregulatory hormone, glucagon, adrenaline, norepinephrine, glycerol, β -OH-butyrate, lactate, and alanine were measured by previously described assays (37). Serum ghrelin concentrations were measured using a commercial radioimmunoassay kit (Phoenix Pharmaceuticals, Phoenix, AZ) that uses ¹²⁵I-labeled bioactive ghrelin as tracer and a rabbit polyclonal antibody against full-length octanoylated human ghrelin. The assay detects both ghrelin and des-octanoyl-ghrelin. The sensitivity of the assay is 30 pg/ml. The intra- and interassay CVs are <5 and <14%, respectively. Plasma free fatty acid (FFA) concentration was measured using a commercial kit (Wako NEFA C test kit; Wako Chemicals, Neuss, Germany).

Statistical analysis. Study conditions (placebo vs. glucose), time, and study by time interactions for values measured repeatedly over time were assessed by repeated-measures ANOVA with Huynh-Feldt adjustment for nonsphericity when appropriate (38). Post hoc comparisons (Newman-Keuls test) were carried out to pinpoint specific differences on significant interaction terms.

A modified Bonferroni procedure (39) for multiple cognitive test adjustments was used to maintain an overall type I error rate of 5% ($\alpha = 0.05$).

The sample size calculations were based on the hypothesis of a 30% difference in adrenaline response (primary endpoint) between treatments. A sample size of 10 subjects achieves 80% power to detect a difference of 30% between treatments with an estimated SD of 30% and with a significance level (α) of 0.05 using a two-sided paired *t* test.

Data are given as means \pm SE, except where SD is specified. We considered differences to be statistically significant if the *P* value was 0.05 or less. We conducted the statistical analyses using NCSS 2007 and PASS 2005 software (NCSS, Kaysville, UT).

RESULTS

Plasma glucose, C-peptide, and insulin concentrations and rates of glucose infusion. In both study conditions, plasma glucose was maintained at euglycemia for 30 min by variable infusion of glucose. Thereafter, similar increments were produced from 30 to 60 min in both study conditions, and then the rate of glucose infusion was decreased to reduce plasma glucose concentration to the target hypoglycemic plateau of 47 ± 1.2 mg/dl at 120 min. Subsequently, plasma glucose concentration was maintained at the nominal plateau of 47 mg/dl for 40 min until the end of the studies with no differences between study conditions ($P > 0.2$). The calculated rate of fall of plasma glucose from euglycemia (60 min) to first hypoglycemic plateau point (120 min) was $0.76 \pm 0.05 \text{ mg} \cdot \text{dl}^{-1} \cdot \text{min}^{-1}$ in subjects given glucose and 0.65 ± 0.05 in those given placebo ($P = 0.106$) (Fig. 1). Plasma C-peptide concentrations decreased at 30 min in both studies. However, at 60 min, plasma C-peptide concentration increased in the glucose study ($1.3 \pm 0.4 \text{ nmol/l}$), whereas it continued to decrease in the placebo study ($0.5 \pm 0.1 \text{ nmol/l}$, $P = 0.035$). Thereafter, plasma C-peptide concentrations decreased in both studies, although, on average, they were higher in subjects given glucose than in those given placebo (0.66 ± 0.2 and $0.34 \pm 0.1 \text{ nmol/l}$, respectively, $P < 0.04$). Plasma insulin concentrations was similar in placebo and glucose studies (188 ± 4 and $181 \pm 6 \text{ } \mu\text{U/ml}$, respectively, $P > 0.2$). The rates of glucose infusion were lower in the glucose compared with the placebo study (5.7 ± 0.4 vs. $2.8 \pm 0.4 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$, respectively, $P < 0.001$).

Plasma glucagon, norepinephrine, adrenaline, pancreatic polypeptide, cortisol, and growth hormone concentrations. After an initial decrease at 60 min in both studies, plasma glucagon concentrations increased to

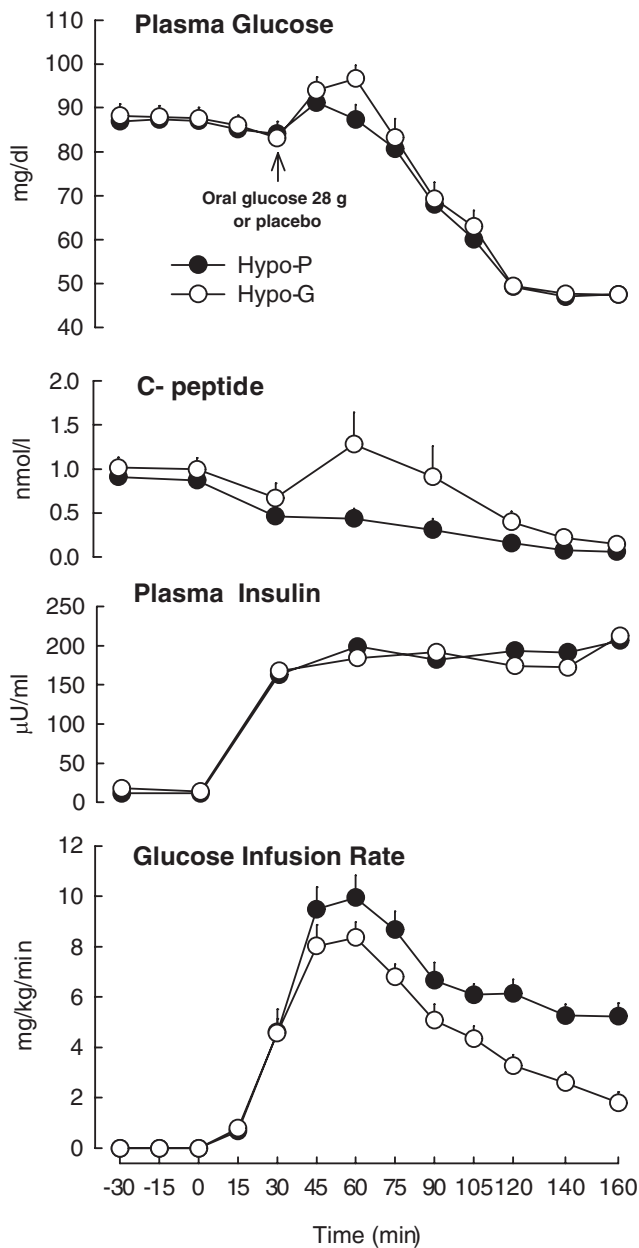


FIG. 1. Plasma glucose, C-peptide, insulin concentrations, and rates of glucose infusion during clamped hypoglycemia both with placebo (●) and oral glucose (○).

a peak of 156 ± 20 pg/ml (vs. baseline 68 ± 3 pg/ml, $P = 0.001$) in the placebo and to 143 ± 17 pg/ml (vs. baseline 66 ± 4.6 pg/ml, $P = 0.001$) in the glucose study (Fig. 2). These differences were not statistically significant ($P > 0.02$). Plasma pancreatic polypeptide concentration increased both during placebo and glucose studies with no difference between studies (peak response 155 ± 20 and 166 ± 18 pmol/l, respectively, $P > 0.2$). Plasma adrenaline levels were not different between glucose and placebo studies (peak response 4 ± 0.9 and 3.7 ± 1.0 nmol/l, respectively, $P = 0.271$). Similarly, plasma norepinephrine concentrations increased in both placebo and glucose studies with no difference between studies. Responses of plasma cortisol were not different between studies (peak response 16.2 ± 3.7 and 15.5 ± 2.6 µg/dl, respectively, $P > 0.2$). Plasma growth hormone increased more in response to glucose than to placebo, although the difference was not

statistically significant ($P = 0.107$). Plasma ghrelin concentrations (data not shown) decreased over time from basal to nadir values during the hypoglycemic plateau (from 268 ± 32 to 172 ± 19 pmol/l, $P = 0.018$, and from 252 ± 16 to 185 ± 19 pmol/l, $P = 0.034$, placebo and glucose studies, respectively) with no difference between study conditions ($P > 0.2$).

Plasma nonglucose substrate. Plasma FFA levels decreased with no difference between studies (68 ± 5 and 84 ± 12 µmol/l in placebo and glucose studies, respectively, $P > 0.2$). Similarly, plasma glycerol and β-OH-butyrate concentrations decreased from baseline with no difference between placebo and glucose studies (Fig. 3). Plasma lactate concentrations increased in both studies. The first similar increment was observed at 60 min, and then a further increase was detected at 140 min with no difference between studies ($P = 0.075$). Plasma alanine concentrations were similar during placebo and glucose studies (366 ± 45 and 369 ± 29 µmol/l, respectively, $P > 0.2$).

Symptoms. Symptom scores increased during hypoglycemia during both placebo and glucose studies. However, mean and peak values for total, autonomic, neuroglycopenic, adrenergic, and cholinergic symptom scores were not different between studies ($P > 0.2$ for all comparisons) (Fig. 4).

Cognitive function. With the exception of digit vigilance test, all cognitive tests deteriorated significantly during hypoglycemia both in placebo and glucose studies (Table 1). The Stroop color and colored words subtest deteriorated less in the glucose study than in the placebo study ($P < 0.05$).

DISCUSSION

The present study was undertaken to examine the effects of portal glucose on the counterregulatory, symptomatic, and cognitive responses to slow-fall hypoglycemia in humans after concomitant ingestion of either glucose to prevent portal hypoglycemia while maintaining systemic hypoglycemia or placebo. The results indicate that prevention of portal hypoglycemia by oral glucose did not have any impact on counterregulatory and symptomatic response to hypoglycemia. Thus, this study supports the view that in nondiabetic subjects, it is systemic glucose sensing (i.e., the brain) that plays the key counterregulatory role when plasma glucose decreases (16). However, oral glucose affected responses of β-cells of pancreatic islets, as shown by the greater response of C-peptide. In addition, oral glucose preserved some aspects of cognitive function during hypoglycemia, such as mental flexibility and attention.

It has been suggested that glucose sensors localized in the portal vein modulate sympathoadrenal responses to hypoglycemia in rats (14) and dogs (15). In these animals, the increase in portal glucose levels, by infusing glucose directly in the portal vein, in the face of systemic hypoglycemia, causes a net suppression of the sympathoadrenal response, which is, instead, normally observed when hypoglycemia is allowed to occur in the portal vein (14,15).

Interestingly, recent studies in rats indicate that there are glucose sensors in the superior mesenteric vein (25), in addition to the portal vein, and that they play an essential role in sensing slow, but not fast, fall in blood glucose defined as rate of decrease of 1.6 and 3.78 mg · dl⁻¹ · min⁻¹, respectively (26). In humans, it has been shown

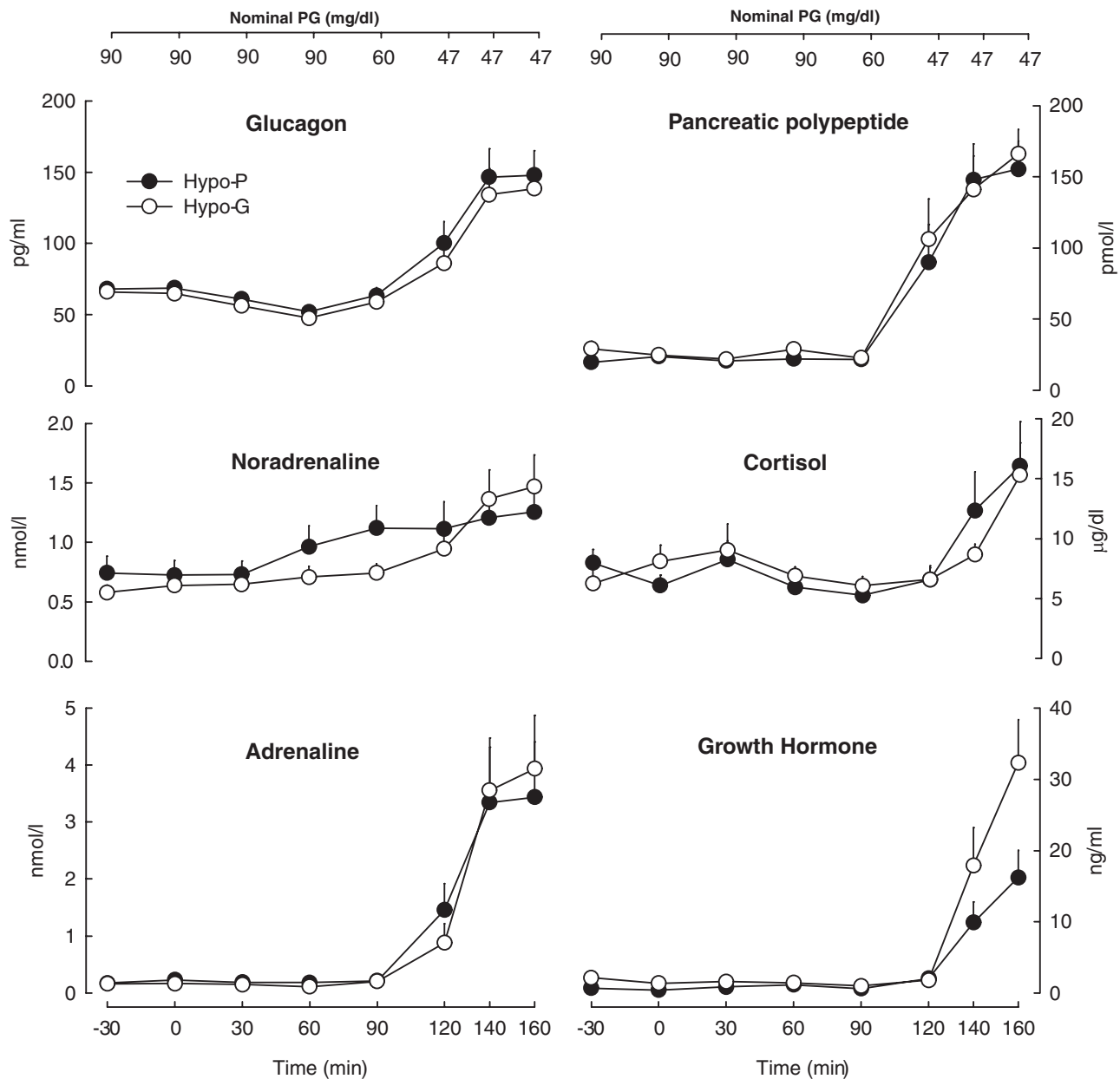


FIG. 2. Plasma glucagon, norepinephrine, adrenaline, pancreatic polypeptide, cortisol, and growth hormone concentrations during clamped hypoglycemia both with placebo (●) and oral glucose (○). PG, plasma glucose.

that in postprandial conditions (27), when portal glucose levels are higher than those in systemic circulation, responses to hypoglycemia are affected by the rate of fall of glucose. Specifically, the sympathoadrenergic response was greater in slow compared with fast fall of blood glucose, whereas cognitive function deteriorated more during fast fall of blood glucose. Notably in humans, slow and fast fall are usually at rates of 0.6 and $1.8 \text{ mg} \cdot \text{dl}^{-1} \cdot \text{min}^{-1}$, respectively (27). However, under those conditions, the relative contribution of glucose and of other nutrients cannot be separated. Therefore, we carried out the present study by inducing hypoglycemia (47 mg/dl) slowly, over 60 min at the rate of $0.75 \text{ mg} \cdot \text{dl}^{-1} \cdot \text{min}^{-1}$ (slow fall) and maintaining the hypoglycemic plateau for an additional 40 min . Because the rate of fall in our study was slower compared with that achieved in rats ($\sim 1.6 \text{ mg} \cdot \text{dl}^{-1} \cdot \text{min}^{-1}$) (26) in which slow-fall but not fast-fall hypoglycemia had effects, it is concluded that in humans

the portal glucose sensors do not play a major counter-regulatory role in the clinical situation in contrast to rats.

The oral load (28 g) was given before hypoglycemia, which was allowed to occur slowly. As a consequence, from the beginning of hypoglycemia the portal vein was exposed to high glucose levels arriving from the intestine. Most likely, elevation of portal plasma glucose prevented activation of portal sensors during systemic hypoglycemia. If portal glucose sensors had a prominent role in modulating the sympathoadrenal response, we should have observed a suppression of this response compared with placebo, as described in animals (14,15). However, that was not the case. Likely, species differences account for the different findings in the present study in humans compared with previous study in rats (26).

In the present study, sympathoadrenal responses, adrenaline and norepinephrine, were not different after oral glucose compared with placebo. In addition, we did not

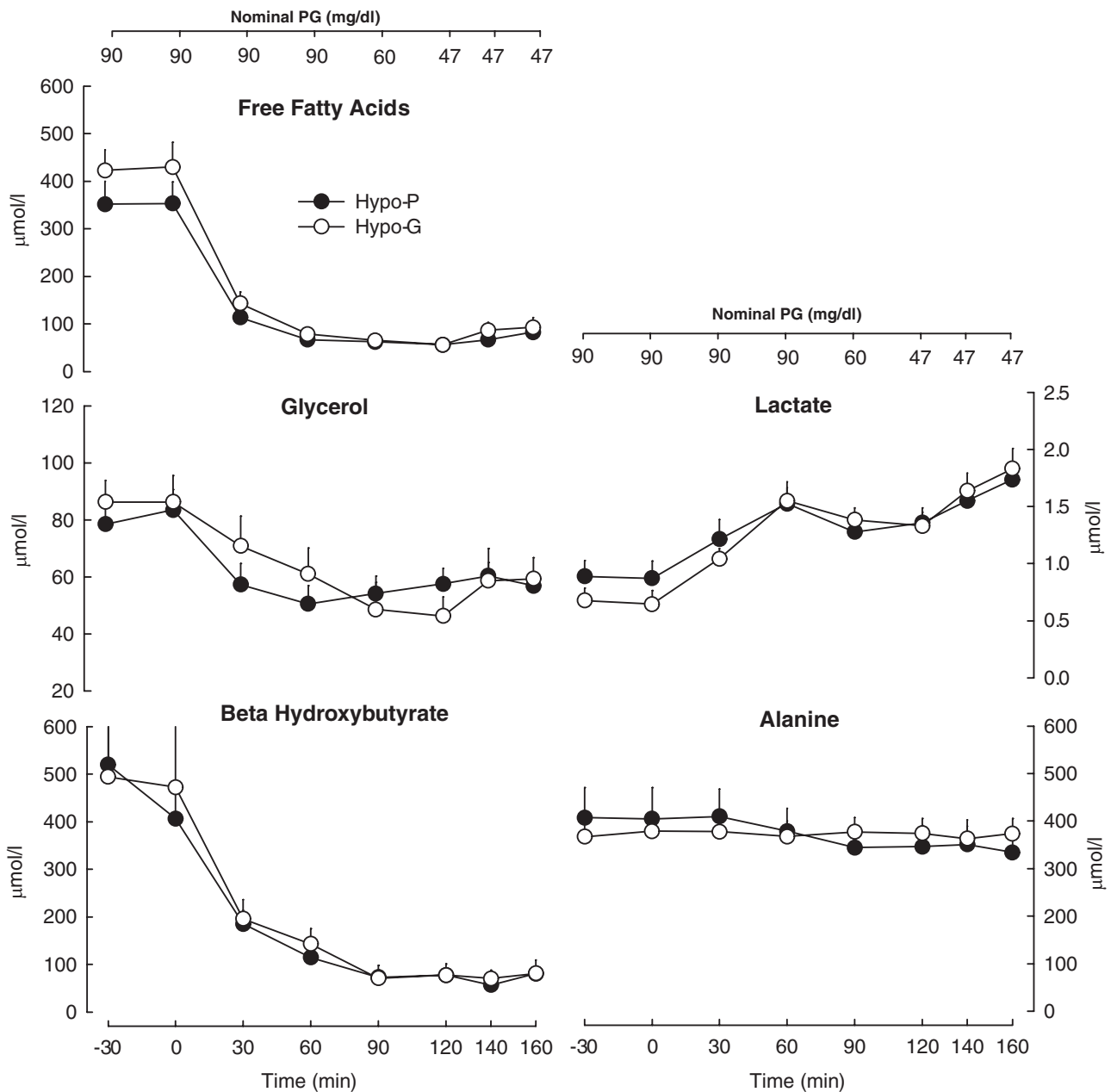


FIG. 3. Plasma concentrations of FFAs, glycerol, β -hydroxybutyrate, lactate, and alanine during clamped hypoglycemia both with placebo (●) and oral glucose (○). PG, plasma glucose.

observe any effects of oral glucose compared with placebo on the other counterregulatory hormones, such as glucagon, cortisol, and growth hormone. Plasma concentrations of ghrelin, a potent stimulator of growth hormone secretion (40,41), were similarly suppressed during insulin-induced hypoglycemia in both study conditions. Ghrelin decreases during insulin-induced hypoglycemia, being inhibited by hyperinsulinemia (42). Although oral glucose is a further mechanism of ghrelin inhibition (43), likely hyperinsulinemia was the main determinant of its suppression. Finally, it is interesting to note that, according to plasma ghrelin levels, the hunger symptom, which has been shown to increase after ghrelin administration (44), in our study was not different under both hypoglycemic conditions.

The greater plasma C-peptide levels observed after oral glucose might suggest that greater insulin levels have occurred in the portal area in the face of identical periph-

eral arterial plasma insulin concentrations. This is likely to be explained both by the slight increase in arterial plasma glucose after oral glucose and by incretin effects mediated mostly by GLP-1 release (45). Certainly GLP-1 favored insulin secretion in the portal vein. However, the relative contribution to portal hyperinsulinemia of GLP-1 and of the oral glucose per se cannot be inferred from our study. Although we did not measure GLP-1, its glucose-independent stimulation of insulin secretion after ingestion of glucose is well known (45).

GLP-1 has been found to suppress glucagon in healthy subjects under euglycemic (46) but not during hypoglycemic conditions (47). Because in our study glucagon levels were similar with oral glucose and placebo, it is likely that any inhibitory effect of GLP-1 on glucagon secretion was offset by the predominant stimulatory effects of systemic hypoglycemia achieved during the clamp procedure.

In line with counterregulatory hormone responses,

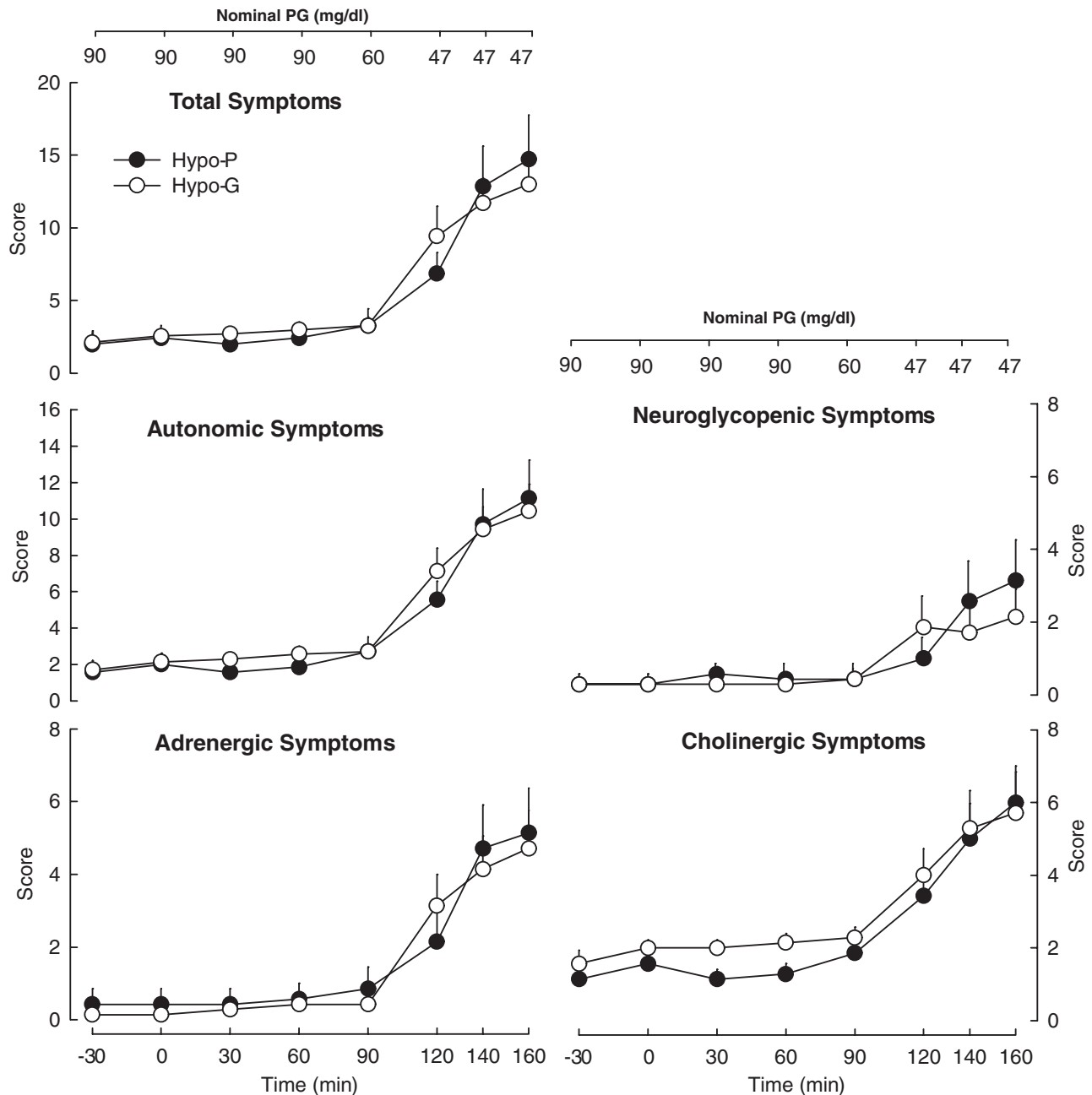


FIG. 4. Total, autonomic, and neuroglycopenic symptom and adrenergic and cholinergic symptom scores during clamped hypoglycemia both with placebo (●) and oral glucose (○). PG, plasma glucose.

symptoms of hypoglycemia were not affected by oral glucose, in contrast to results previously reported by Smith et al. (6). However, we did find better preservation in the color and color-words subtests of the Stroop test. This finding is intriguing. Speculatively, it might be related to neuroprotective effects of GLP-1 (48), although peripheral infusion of GLP-1 during stepped hypoglycemic clamps does not alter cognitive function in healthy subjects (47). However, these results do not negate a possible effect related to portal increments of GLP-1 and its involvement in the generation of neuroprotective portal signals capable of modulating aspects of cognitive function.

One important limitation of our study is that we have no direct measurement of portal vein glucose and do not know with absolute certainty the degree of portal glucose elevation achieved after oral glucose. Therefore, the conclusions of the present study that portal vein glucose

sensors do not play a role in hypoglycemia in humans rely entirely on the assumption that oral glucose prevented hypoglycemia in the portal vein in our experimental model during the slow fall of blood glucose (60–120 min) and during clamped hypoglycemia (120–160 min). We believe that this was the case for the following reasons. In fact, Smith et al. (6) have shown that the ingestion of 20 g glucose labeled with the tracer $[U-^{13}C_6]$ glucose is fully absorbed in a parabolic manner over ~2 h in humans. Based on this finding, it is conceivable that in our study, in which a larger amount of oral glucose was given (28 g), portal hypoglycemia was prevented, both during the period of time of the slow fall of blood glucose (for 60 min) and during the stable hypoglycemic plateau (additional 40 min). In addition, and most importantly, the lower amount of glucose infused during hypoglycemia in the oral glucose study compared with placebo represents a compensation

TABLE 1
Cognitive tests scores during clamped hypoglycemia both with placebo and oral glucose ingestion

	-30 min	0 min	160 min	<i>P</i>		
				Study (placebo vs. glucose)	Time	Study × time
Nominal plasma glucose (mg/dl)	90	90	47			
Trail-making A*						
Placebo	38.7 ± 2.6	33.8 ± 4.8	69.6 ± 12	0.372	0.002	0.367
Glucose	34.2 ± 3.1	36.4 ± 3.3	59.0 ± 3.8			
Trail-making B*						
Placebo	54.0 ± 2.2	56.0 ± 7.6	74.0 ± 6.6	0.304	0.001	0.327
Glucose	52.8 ± 5.2	55.4 ± 5.1	84.3 ± 7.4			
PASAT (3 s)†						
Placebo	56.2 ± 0.4	56.3 ± 0.3	51.0 ± 1.7	0.101	0.001	0.576
Glucose	57.3 ± 0.5	57.0 ± 0.4	53.0 ± 0.7			
PASAT (2 s)†						
Placebo	43.0 ± 3	43.4 ± 3.4	39.0 ± 2.9	0.168	0.001	0.737
Glucose	46.7 ± 0.8	47.5 ± 1.0	43.5 ± 0.4			
Digit span forward‡						
Placebo	4.8 ± 0.2	5.0 ± 0.3	4.3 ± 0.3	0.385	0.009	0.853
Glucose	4.6 ± 0.3	4.8 ± 0.2	4.2 ± 0.3			
Digit span back.‡						
Placebo	4.8 ± 0.1	4.7 ± 0.1	4.0 ± 0.3	0.411	0.000	0.852
Glucose	4.6 ± 0.3	4.6 ± 0.3	3.9 ± 0.2			
Digit vigilance§						
Placebo	35.7 ± 1.2	35.5 ± 1.2	36.5 ± 1.0	0.303	0.284	0.196
Glucose	35.9 ± 3.7	40.4 ± 2.4	38.6 ± 2.1			
Verbal fluency¶						
Placebo	13.5 ± 0.1	12.3 ± 1.0	10.8 ± 0.9	0.318	0.007	0.307
Glucose	13.3 ± 0.9	11.7 ± 0.8	11.6 ± 0.9			
Stroop word						
Placebo	103.5 ± 3.8	97.5 ± 3.8	95.5 ± 1.8	0.129	0.004	0.084
Glucose	103.7 ± 1.6	105.2 ± 1.3	102.3 ± 2.3			
Stroop color						
Placebo	77.5 ± 2.5	78.5 ± 2.8	59.0 ± 0.7	0.014	0.001	0.011
Glucose	77.1 ± 1.5	79.2 ± 1.3	71.5 ± 1.8**			
Stroop color-words						
Placebo	51.6 ± 1.3	51.5 ± 0.8	44.0 ± 0.3	0.862	0.001	0.003
Glucose	49.6 ± 1.9	50.7 ± 2.1	47.0 ± 1.6**			
Verbal memory test††						
Placebo	5.0 ± 0.1	5.0 ± 0.1	3.5 ± 0.4	0.318	0.007	0.307
Glucose	5.0 ± 0.1	4.3 ± 0.4	3.2 ± 0.5			

Data are means ± SE. *P* values are calculated from repeated-measures ANOVA. ***P* < 0.05 vs. placebo; *time (s) required to complete the task; †number of correct responses; ‡number of digit sequences correctly repeated; §number of correct targets crossed out in 90 s; ¶number of words named in 60 s; ||number of correct responses in 45 s; ††number of words recalled.

for the arrival of oral glucose absorbed from the intestine in the portal vein and in the systemic circulation. Thus, it is conceivable that the difference in glucose infusion rates (GIRs) between the two studies represents a reliable estimate of intestinal absorption of glucose, ultimately elevating portal glucose. Based on the Smith et al. calculation of the rate of systemic absorption of the oral glucose from the intestine, assuming absorption over 130 min and a portal blood flow of 0.8 l/min, it is possible to estimate that the administration of 28 g glucose in our study increased the venous portal glucose by a mean of ~25 mg/dl, achieving a concentration of 72 mg/dl against a systemic glucose concentration of 47 mg/dl. An alternative approach to such an estimate is based on the following considerations: subjects were hyperinsulinemic and hypoglycemic, with identical plasma insulin, glucose, and counterregulatory hormone concentrations both in the placebo and the glucose experiments of our study. Although we did not measure glucose fluxes, most likely under these circumstances, endogenous glucose production and glucose

utilization were equivalent in the placebo and the glucose experiments. As a consequence, the difference in the GIRs (GIR placebo - GIR glucose, ΔGIR_{P-G}) may represent a good estimate of the glucose appearance rate from the gut into systemic circulation. Although some of ΔGIR_{P-G} could represent glucose that was initially taken up by the liver and subsequently released, studies using oral ingestion of labeled glucose suggest that this process is quantitatively small (49–51). On these grounds, it is possible to achieve an estimate of portal glucose level by dividing the difference in ΔGIR_{P-G} by the average portal plasma flow (assumed to be ~10 ml · min⁻¹ · kg⁻¹), resulting in an estimated mean portal glucose elevation of 29 mg/dl that corresponds to 20–22 mg/dl after adjusting for the amount of glucose that might be used by other tissues (i.e., gut and erythrocytes) of ~25–30%. The above two independent calculations yield similar conclusions, i.e., the oral glucose administration in the present study successfully met the goal of prevention of portal hypoglycemia during systemic hypoglycemia.

Our results are at variance with previous studies in humans (5–7). However, only one study aimed at prevention of portal hypoglycemia after administration of 20 g oral glucose (6). In contrast to our study, Smith et al. (6) reported a slightly lower response of adrenaline early after oral glucose. However, the effect was modest and not confirmed over the entire hypoglycemic plateau (6). In the present study, greater oral glucose (28 g) given earlier than in Smith study and in a model of slow-fall hypoglycemia to prevent portal hypoglycemia had no effects.

The two other studies (5,7) do not explore the role of portal-hepatic glucose sensors during slow-fall hypoglycemia. In fact hypoglycemia was induced quickly in both studies (5,7): respectively $\sim 2\text{--}4$ and $\sim 1.3 \text{ mg} \cdot \text{dl}^{-1} \cdot \text{min}^{-1}$. In addition, in one study (5) oral glucose was given after, not before, induction of hypoglycemia; in the other study (7), a small amount of carbohydrates as orange juice (15 g), not glucose, was given, and calculations of estimated portal plasma glucose were not performed.

In conclusion, the results of the present study indicate that ingestion of oral glucose to prevent portal hypoglycemia from an early phase of slow-fall systemic hypoglycemia does not affect responses of counterregulatory hormones and symptoms to hypoglycemia. Thus, in contrast to rats (25), the putative portal glucose sensors in humans do not play an appreciable role in modulating these responses to hypoglycemia, at least in the clinically relevant condition of slow-fall hypoglycemia. In humans, portal glucose sensors likely have a role different from that in animals (14,15,23–25). Additional studies are required to explore the complex potential of portal glucose sensors in glucose homeostasis in humans.

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