The Role of Autoregulation of the Hepatic Glucose Production in Man

Response to a Physiologic Decrement in Plasma Glucose

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SUMMARY

In man, a decrease in plasma glucose concentration results in a compensatory increase in hepatic glucose release. Studies in vitro have suggested that a low glucose concentration per se may directly stimulate hepatic glucose release, an effect often referred to as autoregulation. Whether autoregulation occurs in man in response to a physiologic decrement in blood glucose is not known. Therefore, seven healthy, nonobese subjects were studied on two occasions to determine the role of autoregulation in mediating the increase in glucose production that accompanies a physiologic decrement in plasma glucose concentration. On both occasions, plasma glucose concentrations were clamped successively at 95, 65, and 95 mg/dl for 2 h each. Insulin (~14 μU/ml) and glucagon (~70 pg/ml) were maintained constant on both occasions by an infusion of somatostatin and insulin. Phentolamine and propranolol also were infused on one occasion to produce combined alpha- and beta-adrenergic blockade. In the absence of adrenergic blockade, glucose production increased by approximately 1.3 mg/kg·min when the plasma glucose concentration was decreased from 95 to 65 mg/dl and decreased by approximately 1.5 mg/kg·min when glucose was increased from 65 to 95 mg/dl. In the presence of adrenergic blockade, the increase and decrease in glucose production averaged 0 and 0.5 mg/kg·min, respectively, representing 70–100% inhibition. We conclude that, in the presence of low physiologic insulin concentrations, autoregulation is not a major contributor to the hepatic response to a physiologic decrement in plasma glucose concentration in man. DIABETES 1986; 35:186–91.

Following the classic work of Soskin and Levine, the concept that hepatic glucose uptake and release are regulated by the prevailing glucose concentration has become well established.[1] This process, frequently referred to as autoregulation, has received support from many in vitro studies. These studies have demonstrated that the activity of the enzymes involved in glycogen synthesis and breakdown (glycogen synthase and phosphorylase) is modulated by the glucose concentration, thus providing an enzymatic basis for autoregulation.[2–4] Furthermore, increased glucose concentrations inhibited glucose release from hepatocytes, isolated perfused liver, and cross-perfused puppy livers.[5–7] On the other hand, the effects of hypoglycemia on hepatic glucose release are not as clear. Lowering the glucose concentration in the perfusion medium resulted in an increase in glucose release from isolated rat livers in some but not all studies;[8,9] glucose was totally absent from the perfusate in the former[8] but not the latter experiments.[9] The applicability of these in vitro observations to the changes in hepatic production that occur in man in response to physiologic decrements in plasma glucose concentrations is unknown.

The concept of autoregulation of hepatic glucose production in response to hypoglycemia, i.e., that glucose production is an inverse function of the ambient plasma glucose concentration independent of hormonal and neural glucoregulatory factors, has been invoked in humans. Hypoglycemia normally results in a prompt compensatory increase in hepatic glucose release.[10,11] Recent studies[11] have emphasized the roles of glucagon and epinephrine in mediating this increase. Glucagon is the primary stimulus; epinephrine becomes critical when glucagon secretion is deficient, although it may also play a role during severe hypoglycemia even when glucagon secretion is intact.[12] Cortisol and growth hormone appear to have no substantial role in rapid glucose counterregulation. However, a persistent, albeit blunted, increase in glucose production was observed in studies when glucagon secretion and catecholamine action both were inhibited, suggesting that hypoglycemia per se influenced glucose production.[11] This suggestion was strengthened by the report of Sacca et al. that the initial increase in glucose production observed during a continuous low-dose insulin in-

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fusion preceded detectable changes in circulating counterregulatory hormone concentrations by approximately 30–45 min.[13] Although both of these reports are consistent with a role of autoregulation in the response to hypoglycemia, neither directly examined this question, since portal venous insulin and glucagon concentrations were not maintained constant in either study, and intrasynaptic catecholamine concentrations may have increased in the latter study without changes being detected in the peripheral circulation. In addition, steady-state differences in glucose concentration were not present in either instance; reliance was placed on temporal patterns of change rather than on persistent differences in glucose production rates.

The current studies were therefore undertaken to determine the role of autoregulation in modulating the hepatic response to a physiologic decrement in plasma glucose from 95 to 65 mg/dl, such as is observed after carbohydrate ingestion.[14] To avoid the confounding effect of changing hormonal stimuli, plasma insulin and glucagon concentrations were maintained constant in all experiments by means of a combined insulin and somatostatin infusion. Catecholamine action was inhibited by infusion of the alpha- and beta-adrenergic antagonists phentolamine and propranolol. Plasma glucose concentrations were clamped at steady-state concentrations of either 95 or 65 mg/dl during determination of glucose production rates.

MATERIALS AND METHODS

Informed, written consent was obtained from seven volunteers (three F, four M) aged 23–34 yr. All were nonobese (BMI = 20–25 kg/m^2) and had no immediate family history of diabetes mellitus. Subjects were admitted to the outpatient facility of the Mayo Clinic Research Center between 7:00 a.m. and 8:00 a.m. on the morning of each experiment after an overnight fast (10–12 h).

All subjects were studied on two occasions separated by at least 1 wk. Women were studied at 1-mo intervals during the same phase of their menses. On each occasion, a primed (17 μCi)-continuous (0.17 μCi/min) infusion of 3-^{3}H-glucose (New England Nuclear, Boston, Massachusetts) was begun for isotopic determination of glucose appearance and disappearance rates; 2 h were allowed for isotopic equilibration before other infusions were begun. On one occasion, insulin (Iletin II pork insulin, Eli Lilly and Company, Indianapolis, Indiana; 0.2 mU/kg·min), somatostatin (Beckman Instruments, Palo Alto, California; 250 μg/h), and saline (30 cm^3/h) were infused from 0 to 360 min. On the other occasion, propranolol (Inderal, Ayerst, New York, New York; 5 mg over 2 min followed by 80 μg/min) and phentolamine (Ciba-Geigy Corp, Summit, New Jersey; 5 mg over 2 min followed by 50 μg/min) were infused from 0 to 360 min along with the somatostatin and insulin infusions.

Plasma glucose concentration on all occasions was maintained at a concentration of 95 mg/dl from 0 to 120 min by means of a glucose infusion as previously described.[15] The glucose infusion was stopped at 120 min and the plasma glucose concentration was allowed to decrease to 65 mg/dl, where it was maintained by a glucose infusion until 240 min. The glucose infusion rate then was increased rapidly until the glucose concentration reached 95 mg/dl, where it was clamped until 360 min.

Plasma insulin,[16] C-peptide,[17] glucagon,[18] and growth hormone[19] concentrations were determined by radioimmunoassay. Plasma cortisol was determined using a Serono Kit (Randolph, Massachusetts). Plasma free fatty acids were determined colorimetrically.[20] Plasma catecholamines were assayed using a single-isotope derivative method.[21] Glucose specific activity was measured as previously described.[15]

Rates of glucose utilization and endogenous glucose production were calculated during the final 30 min of each insulin infusion.[15]

Means of the hormone and free fatty acid concentrations during the 30 min before initiation of the clamps and during each 120-min clamp period (i.e., first 95-mg/dl, 65-mg/dl, and second 95-mg/dl glucose clamp) were used for statistical analysis. Similarly, mean glucose production rates during the final 30 min before the initial clamp and during each clamp were used for analysis. Data in the text are presented as mean ± SEM. Statistical comparison was performed using Student's paired and nonpaired *t*-tests where appropriate. A P-value of <0.05 was considered statistically significant.

RESULTS

Plasma glucose, insulin, and C-peptide concentrations (Figure 1). Plasma glucose concentration before the somatostatin and insulin infusion (basal) did not differ significantly on the saline and adrenergic blockade days (97 ± 1 versus 95 ± 3 mg/dl) nor did plasma glucose concentration during the final 30 min of each period (95 ± 1 versus 96 ± 1, 65 ± 1 versus 62 ± 2, and 96 ± 1 versus 97 ± 1 mg/dl at 90–120, 210–240, and 330–360 min, respectively, hereafter referred to as the first 95-mg/dl clamp, 65-mg/dl clamp, and second 95-mg/dl clamp, respectively). Plasma insulin concentrations were similar on the saline and adrenergic blockade days, averaging 7 ± 1 versus 6 ± 1 μU/ml at baseline, 12 ± 1 versus 12 ± 1 μU/ml during the first 95-mg/dl clamp, 12 ± 1 versus 14 ± 1 μU/ml during the 65-mg/dl clamp, and 12 ± 1 versus 14 ± 1 μU/ml during the second 95-mg/dl clamp on the saline and adrenergic blockade days, respectively. Basal plasma C-peptide concentrations did not differ significantly on the saline and adrenergic blockade days (1.1 ± 0.1 versus 1.2 ± 0.3 ng/ml). After initiation of the somatostatin and insulin infusion, C-peptide concentrations de-

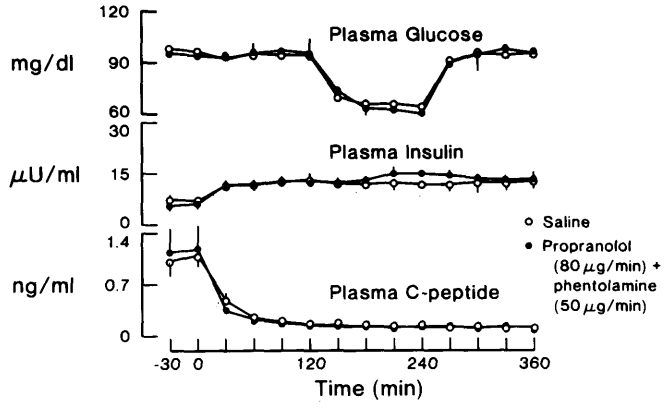

FIGURE 1. Plasma glucose, insulin, and C-peptide concentrations. An infusion of somatostatin (250 μg/min) and insulin (0.2 mU/kg·min) was initiated at time 0 on each occasion. Either saline (open circles) or propranolol and phentolamine (closed circles) was also infused.

creased to equivalent levels during both saline and adrenergic blockade.

Counterregulatory hormones and free fatty acid concentrations (Table 1). Basal glucagon, growth hormone, cortisol, epinephrine, and norepinephrine concentrations did not differ significantly on the saline and adrenergic blockade days. After somatostatin and insulin infusion, plasma glucagon, growth hormone, and cortisol concentrations were comparable during saline and adrenergic blockade. Plasma epinephrine concentrations were significantly lower during saline than during adrenergic blockade (first 95-mg/dl clamp, 14 ± 5 versus 41 ± 26 pg/ml, $P < 0.03$; 65-mg/dl clamp, 107 ± 32 versus 611 ± 216 pg/ml, $P < 0.03$; and second 95-mg/dl clamp, 49 ± 8 versus 110 ± 22 pg/ml, $P < 0.01$). Plasma norepinephrine concentration increased progressively during adrenergic blockade, being significantly greater than saline throughout the study ($P < 0.01$). Free fatty acid concentrations did not differ significantly at baseline or during any of the clamps on the saline or adrenergic blockade days.

Glucose production rates (Figure 2). Basal glucose production rates did not differ significantly on the saline and adrenergic blockade days (2.1 ± 0.1 versus 2.0 ± 0.1 mg/kg·min). After somatostatin and insulin infusion, glucose production rates during the first 95-mg/dl clamp period were slightly but not significantly lower with saline than with adrenergic blockade (0.6 ± 0.1 versus 0.9 ± 0.1 mg/kg·min). When the plasma glucose concentration was allowed to decrease to 65 mg/dl, the glucose production rate increased to 1.9 ± 0.2 mg/kg·min on the saline day but did not change on the adrenergic blockade day (0.9 ± 0.2 mg/kg·min, $P < 0.007$). The mean increment in glucose production when the glucose concentration was decreased from 95 to 65 mg/dl was significantly greater during saline than during adrenergic blockade (1.3 ± 0.2 versus 0.0 ± 0.2 mg/kg·min, $P < 0.003$). Glucose production rates during the second 95-mg/dl clamp did not differ with saline and adrenergic blockade (0.4 ± 0.1 versus 0.4 ± 0.1 mg/kg·min). The mean decrement in glucose production when glucose was increased from 65 to 95 mg/dl was significantly greater during saline than during adrenergic blockade (1.5 ± 0.2 versus 0.5 ± 0.1 mg/kg·min, $P < 0.001$).

Assuming a linear decrease in production with time, the increment at 65 mg/dl determined by subtracting the observed value from the calculated value averaged 1.4 ± 0.1 mg/kg·min during saline compared with 0.3 ± 0.1 mg/kg·min during adrenergic blockade ($P < 0.001$).

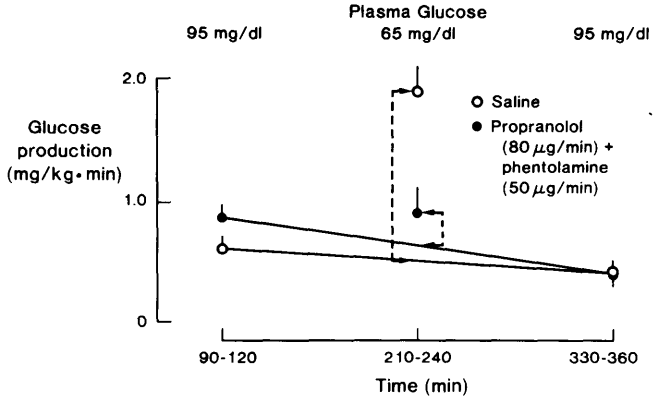

FIGURE 2. Glucose production rate during the final 30 min of each clamp period. The target plasma glucose concentration during each clamp period is indicated at the top of the figure as a reference. The dotted line represents the increment in glucose production at 65 mg/dl calculated assuming a linear decrease in production with time. Infusions are as in Figure 1: adrenergic blockade (closed circles) and saline (open circles).

DISCUSSION

Under the current experimental conditions (constant plasma insulin, glucagon, and growth hormone concentrations), glucose production increased by approximately 1.3 mg/kg·min when glucose was lowered from 95 to 65 mg/dl and decreased by approximately 1.5 mg/kg·min when glucose was then raised to 95 mg/dl. Seventy to one hundred percent of these changes were blocked by the adrenergic antagonists propranolol and phentolamine. Thus, at most only 0–30% of the increase in glucose release could be accounted for by autoregulation. Since the plasma glucose concentration was only lowered by approximately 30 mg/dl (i.e., from 95 to 65 mg/dl), the current studies do not preclude a more important role of autoregulation in man during severe hypoglycemia.

There are several theoretical reasons why the current experimental design may have resulted in either an underestimate or overestimate of hepatic glucose autoregulation. First, autoregulation may have been underestimated if portal venous insulin concentrations were sufficiently high to obscure the stimulatory effect of hypoglycemia per se. Since pancreatic insulin release was blocked by somatostatin, portal venous insulin concentrations presumably were the same as the peripheral circulating levels. Assuming a 2:1 portal:peripheral insulin gradient, the portal insulin concentrations present during the somatostatin and insulin infusion (12–14 μU/ml) were similar to those present in the postabsorptive state when the peripheral insulin concentrations averaged 6–7 μU/ml. Thus, portal hyperinsulinemia was unlikely. On the other hand, since glucagon secretion was inhibited by the somatostatin infusion, the insulin:glucagon ratio increased. This allowed a decrease in glucose concentration in the absence of hyperinsulinemia. If glucagon is required for the liver to respond to a decrement in glucose concentration, then autoregulation may have been underestimated using the current experimental design. One could argue, however, that such a response does not truly reflect autoregulation but rather the modulation by the prevailing glucose concentration of the hepatic response to counterregulatory hormones.[22] Furthermore, in vitro, a hepatic response to low glucose occurs in the absence of glucagon.[8] In addition, since the degree of hypoglucagonemia was equivalent on all study days, the brisk increase in glucose production in the absence of adrenergic blockade, despite a minimal increase in circulating epinephrine and norepinephrine concentration, argues against hepatic refractoriness.

It is also possible that somatostatin interfered with the hepatic response to glucose. Although in vitro high concentration of somatostatin has been reported to alter hepatocyte function by some[23,24] but not all investigators,[25] its effects are mediated by inhibition of pancreatic and pituitary hormonal secretion in vivo.[22] Furthermore, the infusion rate of somatostatin, which was the same on all study days, allowed a

TABLE 1
Counterregulatory hormone and free fatty acid concentrations during combined adrenergic blockage using propranolol (80 μg/min) and phentolamine (50 μg/min)

Hormone	Time (min)													
	−30	0	30	60	90	120	150	180	210	240	270	300	330	360
Glucagon (pg/ml)														
Saline	113 ± 26	109 ± 20	72 ± 18	72 ± 16	75 ± 16	72 ± 15	69 ± 17	71 ± 15	69 ± 17	69 ± 18	69 ± 16	67 ± 16	63 ± 15	67 ± 16
Propranolol and phentolamine	110 ± 24	113 ± 23	71 ± 18	70 ± 16	67 ± 19	69 ± 18	70 ± 17	70 ± 16	69 ± 19	70 ± 16	70 ± 16	70 ± 16	64 ± 16	64 ± 14
Growth hormone (ng/ml)														
Saline	1.0 ± 0.3	0.8 ± 0.1	0.7 ± 0.1	0.7 ± 0.1	0.6 ± 0.1	0.6 ± 0.1	0.6 ± 0.1	0.6 ± 0.1	0.7 ± 0.1	0.6 ± 0.1	0.6 ± 0.1	0.6 ± 0.1	0.6 ± 0.1	0.6 ± 0.1
Propranolol and phentolamine	1.5 ± 0.8	0.8 ± 0.2	0.8 ± 0.2	0.7 ± 0.1	0.6 ± 0.1	0.6 ± 0.1	0.6 ± 0.1	0.7 ± 0.1	1.1 ± 0.2	1.1 ± 0.2	1.0 ± 0.2	0.8 ± 0.2	0.7 ± 0.1	0.6 ± 0.1
Cortisol (μg/dl)														
Saline	11 ± 1	10 ± 1	9 ± 1	8 ± 1	9 ± 1	9 ± 1	8 ± 1	9 ± 1	10 ± 1	13 ± 1	10 ± 1	9 ± 1	8 ± 1	8 ± 1
Propranolol and phentolamine	10 ± 1	9 ± 1	8 ± 1	9 ± 1	10 ± 2	11 ± 3	12 ± 2	13 ± 2	16 ± 2	16 ± 2	15 ± 2	13 ± 2	12 ± 2	12 ± 2
Epinephrine (pg/ml)														
Saline	27 ± 7	34 ± 11	18 ± 4	12 ± 0.8	12 ± 1.2	16 ± 3	27 ± 7	106 ± 32	150 ± 30	148 ± 24	57 ± 14	36 ± 8	49 ± 14	42 ± 9
Propranolol and phentolamine	32 ± 9	34 ± 13	37 ± 13	30 ± 6	43 ± 14	53 ± 16	94 ± 33	463 ± 178	951 ± 260	1126 ± 290	620 ± 448	150 ± 54	111 ± 34	112 ± 29
Norepinephrine (pg/ml)														
Saline	196 ± 22	189 ± 24	177 ± 25	174 ± 19	174 ± 27	194 ± 27	210 ± 28	217 ± 23	225 ± 23	228 ± 21	225 ± 22	215 ± 21	225 ± 22	220 ± 31
Propranolol and phentolamine	180 ± 19	185 ± 14	279 ± 30	312 ± 49	329 ± 58	341 ± 48	368 ± 44	444 ± 102	435 ± 70	465 ± 85	484 ± 75	515 ± 75	553 ± 125	543 ± 111
Free fatty acid (μM)														
Saline	500 ± 80	502 ± 71	271 ± 41	163 ± 24	126 ± 30	99 ± 23	105 ± 22	140 ± 37	131 ± 27	160 ± 27	149 ± 33	128 ± 32	140 ± 36	113 ± 24
Propranolol and phentolamine	557 ± 88	588 ± 95	342 ± 56	203 ± 58	179 ± 44	123 ± 30	103 ± 33	110 ± 23	116 ± 32	98 ± 22	98 ± 13	112 ± 19	140 ± 30	163 ± 34

clearly demonstrable increase in glucose production when the plasma glucose concentration was lowered in the absence of adrenergic blockade.

On the other hand, several aspects of the experimental design may have led to an overestimation of autoregulation. First, the method of calculation of autoregulation may have been incorrect. Adrenergic blockade completely abolished the increase in glucose production that was observed when glucose was allowed to decrease from 95 to 65 mg/dl. In themselves, these data imply that the hepatic response observed in the control experiment could be totally ascribed to adrenergic stimulation without evidence of any autoregulation. However, if autoregulation was calculated as the decrement in glucose production when glucose was increased from 65 to 95 mg/dl, then the 0.5-mg/kg·min decrease in glucose production during adrenergic blockade could represent 30% of the response observed during saline alone. If the increment in glucose production at 65 mg/dl was calculated assuming a linear decrease in glucose production with time, a 10–20% increment in glucose production persisted after adrenergic blockade. However, both of these latter calculations may have overestimated the potential role of autoregulation if the glucose production during the second 95-mg/dl clamp was influenced by the increase that occurred during the 65-mg/dl clamp. Whatever method was used in the calculation, the physiologic decrement in glucose concentration had a minor effect on glucose production.

Second, incomplete adrenergic blockade could also result in an overestimate of autoregulation. Plasma epinephrine and norepinephrine concentrations were greater during the 65-mg/dl clamp in the presence of adrenergic blockade than in the presence of saline. The results are consistent with the ability of phentolamine to decrease norepinephrine release and propranolol to decrease catecholamine clearance.[26] The dose of the beta-adrenergic antagonist propranolol used in the present experiment (5-mg loading dose followed by 80 μg/min) has previously been shown to be adequate to inhibit the effects of catecholamines on plasma glucose, beta-hydroxybutyrate, glycerol, lactate, free fatty acid concentrations, and pulse and blood pressure.[27–29] On the other hand, the dose of phentolamine necessary to block the alpha-adrenergic action of catecholamines is not as well established. The recent report by Tse et al. that adrenergic blockade produced by phentolamine and propranolol does not have the same metabolic effect as epinephrine deficiency produced by adrenalectomy casts doubt on the ability of competitive adrenergic antagonists to ever achieve total adrenergic blockade in man.[14]

Finally, the increase in glucose production attributed to autoregulation may have been overestimated if stimulatory effects of the other counterregulatory hormones (glucagon, cortisol, and growth hormone) persisted. Although somatostatin prevented any change in circulating glucagon concentrations, a small increase in portal-venous glucagon concentrations cannot be excluded. The 6–8-μg/dl increase in circulating cortisol concentration and the 1-ng/ml increase in growth hormone conceivably could have increased hepatic glucose release. However, the small magnitude of these changes and the fact that the onset of stimulation of glucose production by these hormones is generally slow militates against a role of these hormones in the current experiments. Nevertheless, the possibility cannot be excluded that the combined effects of the small increases in each of these hormones account for the residual increase in glucose production without invoking any role for autoregulation.[30]

Of interest, glucose production rates tended to be higher during the first 95-mg/dl glucose clamp in the presence of adrenergic blockade than in the presence of saline. A similar observation has been reported by Wolfe et al. in the conscious dog.[31,32] These investigators noted that infusion of propranolol and phentolamine resulted in an increase in glucose production when portal insulin concentrations of insulin and glucagon were held constant by a concomitant infusion of somatostatin, insulin, and glucagon. They proposed that decreased free fatty acid availability produced by adrenergic blockade caused the increase in hepatic glucose production. They subsequently supported this postulate by demonstrating that the increase in glucose release produced by adrenergic blockade did not occur if the fall in circulating free fatty acid concentrations was prevented by infusion of heparin and Intralipid.[32] That lack of difference in free fatty acid concentrations during the 95-mg/dl clamp in the presence of saline and adrenergic blockade in the current experiments argues against a role of free fatty acids in mediating basal glucose production in man. The mechanism and significance of this finding awaits further study.

In summary, we have demonstrated that the increase in glucose production that accompanies a physiologic decrement in plasma glucose is mediated primarily by changes in counterregulatory hormone concentration. Although the prevailing glucose concentration may modulate the hepatic response to counterregulatory hormones, the current studies indicate that, in man, a physiologic decrease in glucose concentration per se does not have a major influence on hepatic glucose production.

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