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### Research Article

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# Endogenous Somatostatin-28 Modulates Postprandial Insulin Secretion

## Immunoneutralization Studies in Baboons

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### Abstract

Somatostatin-28 (S-28), secreted into the circulation from enterocytes after food, and S-14, released mainly from gastric and pancreatic D cells and enteric neurons, inhibit peripheral cellular functions. We hypothesized that S-28 is a humoral regulator of pancreatic B cell function during nutrient absorption. Consistent with this postulate, we observed in baboons a two to threefold increase in portal and peripheral levels of S-28 after meals, with minimal changes in S-14. We attempted to demonstrate a hormonal effect of these peptides by measuring their concentrations before and after infusing a somatostatin-specific monoclonal antibody (mAb) into baboons and comparing glucose, insulin, and glucagon-like peptide-1 levels before and for 4 h after intragastric nutrients during a control study and on 2 d after mAb administration (days 1 and 2). Basal growth hormone (GH) and glucagon levels and parameters of insulin and glucose kinetics were also measured. During immunoneutralization, we found that (a) postprandial insulin levels were elevated on days 1 and 2; (b) GH levels rose immediately and were sustained for 28 h, while glucagon fell; (c) basal insulin levels were unchanged on day 1 but were increased two to threefold on day 2, coincident with decreased insulin sensitivity; and (d) plasma glucose concentrations were similar to control values. We attribute the eventual rise in fasting levels of insulin to its enhanced secretion in compensation for the heightened insulin resistance from increased GH action. Based on the elevated postmeal insulin levels after mAb administration, we conclude that S-28 participates in the enteroinsular axis as a dectin to regulate postprandial insulin secretion. (*J. Clin. Invest.* 1997. 100: 2295–2302.) Key words: somatostatin-28 • gastrointestinal tract • pancreatic islet • insulin • growth hormone

### Introduction

Somatostatin-14 (S-14)<sup>1</sup> and somatostatin-28 (S-28) are secreted into the blood stream and share the ability to inhibit the release of a variety of peptides and neurotransmitters (1–3). It

has been held generally that S-14, which is processed from prosomatostatin (Pro-S) in D cells of the stomach and pancreas and in peripheral neurons, is a paracrine regulator of the release of several hormones and neuropeptides (1, 2, 4–7). Although it has also been postulated that S-14 acts as a hormone (8, 9), this notion is problematic, since S-14 enters the circulation from diverse sources, its putative target organ is not obvious, and its peripheral plasma levels show little, if any, change after physiologic events such as nutrient intake (3). Further, studies by Stagner, Samols, and colleagues do not support an endocrine role for S-14 in pancreatic islets based on the lack of effect of antisomatostatin serum on insulin or glucagon levels when it was perfused through isolated pancreata from several species (10–13). However, these results have been challenged recently by Kleinman et al., who observed a rise in insulin levels after perfusion of a monoclonal somatostatin antiserum and its Fab fragment in isolated human pancreata (14, 15).

By contrast, in addition to its ubiquitous distribution in the central nervous system (CNS), S-28 is synthesized selectively in endocrine cells of the gastric pylorus and upper gut (16), and plasma concentrations of this peptide are increased consistently and significantly after intake of nutrients (3, 17). Thus, we hypothesized that S-28 is a regulator of glucose metabolism during the postprandial state. In earlier studies, we showed that infusion of S-28 in humans inhibited both the secretion of insulin from pancreatic B cells and the products of pancreatic acinar cells (18, 19). Although these results support a physiologic role for S-28, a more compelling approach is to prevent the peptide from accessing its receptors and, thereafter, evaluate any changes in the release of products from the putative target cell (20–22). Thus, we performed two studies in baboons, an animal selected because of its close phylogenetic relationship to humans. In the first study, we confirmed the time course of S-14 and S-28 in response to meal ingestion. In the second, we infused a monoclonal Ig into the animals. Measurements included basal hormone levels, hormone responses to meal intake, and insulin sensitivity on 3 d. The first was a control study, the second was performed 30 min after antibody infusion (day 1), and the third was carried out 24 h later (day 2). The studies on day 2 were performed because of our expectation that the effect of the circulating Ig would persist, and that during this time frame, sufficient Ig would have diffused into the interstitial space to allow assessment of the paracrine effects of S-14. Since postnutrient insulin perturbations are also influenced by incretins from the gut, we evaluated the influence of immunoneutralization of the somatostatins on the re-

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1. *Abbreviations used in this paper:* AIR<sub>g</sub>, acute insulin response to glucose; AUC, area under curve; GH, growth hormone; GLP-1, glucagon-like peptide-1; IVGTT, intravenous glucose tolerance test; K<sub>g</sub>, glucose disappearance rate; Pro-S, prosomatostatin; S-13, -14, -28, somatostatin-13, -14, and -28; S<sub>g</sub>, glucose effectiveness; S<sub>i</sub>, insulin sensitivity; TFA, trifluoroacetic acid.

lease of the incretin, glucagon-like peptide-1 (GLP-1) (23, 24). Growth hormone (GH) was also measured, since it is regulated by somatostatin from the hypothalamus (1, 25). Insulin sensitivity was derived from the results of intravenous glucose tolerance tests (IVGTTs) to facilitate interpretation of changes in insulin secretion.

## Methods

**Animals.** 12 male baboons, 4–8 yr of age, weighing between 7.6 and 12.9 kg, were studied. Six of the animals were used to determine the changes in S-14, S-13 (derived from aminopeptidase-mediated cleavage of S-14 in serum and tissue), and S-28 in plasma after a meal. The other six baboons were used to examine the effects of the antisomatostatin Ig. Under anesthesia, each animal had vascular catheters and a gastrostomy tube inserted. After recovery from surgery, all animals were placed in separate cages. They wore jackets with a tether attached to a swivel in the ceiling through which the vascular and intragastric catheters were led to the exterior. Vascular lines were kept patent by a slow infusion of 150 mM NaCl containing heparin. The health of the animals was monitored by skilled technologists and a veterinarian. Because of frequent blood withdrawal, red cells were separated from plasma by centrifugation and reinfused into the donor animal to maintain a hematocrit > 30%.

**Protocols.** The following protocols were approved by the Animal Welfare Committee at the University of Washington. The animals ate standardized monkey chow ad libitum between protocols. They were studied while awake at intervals over 3 mo. All experiments were performed in the morning after food had been withheld for 16–18 h. To examine levels of Pro-S-related peptides in response to meal ingestion, catheters were implanted chronically in the femoral artery and portal vein of each animal, and they were trained to drink Ensure® (Ross Laboratories, Columbus, OH) as their sole source of nutrient. The animals ingested Ensure® containing 200 mg carbohydrate, 40 mg protein, and 50 mg fat/ml. Each animal consumed a volume providing 25–35 kcal/kg, usually within 3–5 min. Blood was collected simultaneously from the arterial and portal venous catheters into heparinized tubes at intervals before and up to 240 min after food intake.

For the immunoneutralization studies, catheters were placed in their femoral arteries and inferior vena cavae, and gastrostomy tubes were introduced into their gastric antra and fixed in place by subcutaneous tunneling. Blood was sampled for 60 min before and 270 min after the intragastric meal. Subsequently, a 90-min tolbutamide-modified IVGTT was performed to measure insulin sensitivity. During control experiments, intravenous NaCl (150 mM) was infused through the femoral arterial catheter. In the before meal period, blood was withdrawn every 2 min for 30 min to determine basal insulin levels. Thereafter, Ensure® was infused through the gastrostomy tube to deliver 35 kcal/kg within 5 min. Blood was sampled every 15 min for 240 min. At 270 min, each animal received an intravenous bolus of glucose (0.3 g/kg) within 20 s. Blood was obtained at 2, 3, 4, 5, 6, 8, 10, 12, 14, 16, and 19 min. At 20 min, tolbutamide (2 mg/kg) was given intravenously over 30 s, followed by further blood sampling at 22, 23, 24, 25, 27, and 30 min, and then at 10-min intervals for an additional 60 min. At least 1 wk after completing the control studies, the animals were studied on consecutive days. On day 1, baseline samples were collected over 30 min, followed by an intravenous bolus of antisomatostatin Ig (mAb MS-12, 40 mg/kg). Blood was taken for an additional 30 min, followed by intragastric instillation of Ensure®, and an IVGTT as described for the control study then ensued. 24 h later, this protocol was repeated without infusion of the mAb (day 2).

**Generation of mAb.** An mAb designated MS-12, which cross-reacts with both S-14 and S-28, was used for immunoneutralization. Details of its genesis have been published previously (16). In brief, BALB/c mice were immunized against S-14, and their spleen cells were fused with NS-1 myeloma cells and cultured according to the methods of Kohler and Milstein (26) as modified by Nowinski (27).

After intraperitoneal injection of the transformed cells into BALB/c mice, ascites fluid was harvested, and the Ig was purified by precipitation with 30% ammonium sulfate and subsequently dialyzed and lyophilized. The antibody was typed as IgG<sub>1</sub> by a mouse monoclonal isotyping kit (Amersham International, Little Chalfont, UK). Affinity and specificity were measured using S-14, S-28, and analogs of S-14 as reported previously (3). The mAb was found to interact with Phe<sup>7</sup>, Trp<sup>8</sup>, and Lys<sup>9</sup> residues of S-14. By Scatchard analysis, MS-12 had affinities of  $1.8 \times 10^{12}$  and  $2.8 \times 10^{11}$  mol/liter for S-14 and S-28, respectively.

**Plasma analyses.** Blood was collected in tubes containing 500 U heparin and centrifuged immediately, the red cells were returned to the animal, and 100 mM EDTA and 500 U/ml aprotinin were added to plasma which was stored at  $-20^{\circ}\text{C}$ . Analyses of glucose were carried out by a glucose oxidase method and of insulin and glucagon by previously described RIA methods (28, 29). GLP-1 levels were analyzed in ethanol extracts of plasma by a minor modification of the method of Ørskov and Holst (30), using antibody 89390 (kindly donated by Dr. Jens Juul Holst, Panum Institute, Copenhagen). Growth hormone levels were measured by RIA in the General Clinical Research Center Core Laboratory at the University of Virginia, with the kind assistance of Dr. Alan Rogol. For analyses of Pro-S-related peptides, aliquots of plasma were adjusted to pH 3 with 1 N HCl (100  $\mu\text{l}$ /ml plasma) and stored at  $-20^{\circ}\text{C}$  to prevent enzyme-mediated destruction. Plasma was thawed and passed through cartridges of octadecylsilyl silica (SepPak C-18; Waters Chromatography, Milford, MA), prepared by washing with absolute methanol and distilled water. Plasma proteins were removed by washing sequentially with 5 ml of water and 5 ml of 0.1% trifluoroacetic acid (TFA) in water. Adsorbed peptides were eluted with 5 ml of a solution of 80% methanol and 1% TFA in water. The eluates were air-dried and reconstituted in 130 mM borate buffer, pH 8.5.

Details of the separation of S-28 from Pro-S, S-14, and S-13 by immunoadsorption and their measurements by RIA in plasma have been published previously (3). In brief, eluates from SepPak were applied to a column of agarose coupled with partially purified Ig binding selectively to the Asn<sup>5</sup>-Pro<sup>6</sup>-Ala<sup>7</sup> sequence in the NH<sub>2</sub> region of S-28. The column was washed with 130 mM borate buffer, pH 8.5, in which S-14, S-13, and Pro-S were eluted. S-28, which adhered to the immunoadsorbent, was removed with 200 mM acetic acid and 0.2% BSA (Miles, Inc., Kankakee, IL), pH 3.5, and lyophilized. Pro-S, S-14, and S-13 from the first wash, and S-28 that was eluted from the immunoadsorbent were assayed separately with antiserum AS-10. Antiserum AS-10 interacts with the Phe<sup>7</sup>-Trp<sup>8</sup>-Lys<sup>9</sup> residues shared by S-14 and S-28. Assays were carried out in 130 mM borate buffer, pH 8.5 in BSA, with AS-10 antiserum diluted to 1:100,000 using <sup>125</sup>I-Tyr<sup>11</sup> S-14 as tracer. Free and antibody-bound iodopeptides were separated by the addition of 1 ml 1% activated charcoal (Norita; Eastman Kodak Co., Rochester, NY). The values for Pro-S, S-14, and S-13, collectively, were read against synthetic S-14 as standard and expressed as picogram equivalents per milliliter. Measurements were corrected for an average recovery of S-14/S-13 of 78%. The values for S-28, corrected for average recoveries of 50% from the immunoadsorbent, were expressed as picograms per milliliter using synthetic S-28 (Peninsula Laboratories Inc., Belmont, CA) as standard.

After infusion of Igs, titers of mAb in baboon plasma were determined by incubation of plasma and <sup>125</sup>I-Tyr<sup>11</sup> S-14 for 48 h at  $4^{\circ}\text{C}$  in the presence of protease inhibitors and standard amounts of authentic S-14 and S-28. Bound and free tracers were separated using charcoal. To measure free and bound peptides in plasma, aliquots of plasma were passed through SepPak as described above to separate the MS-12-peptide complex in the fall-through fraction from the eluates that contain the adsorbed Pro-S-derived peptides. The fall-through fraction was boiled for 10 min to remove bound peptides, centrifuged, and the supernatant was lyophilized. The fraction eluted with 80% methanol and 1% TFA, and water was lyophilized. Both fractions were then analyzed for S-28 and S-14/S-13/Pro-S.

**Data analyses.** Plasma glucose and hormone levels before and 4 h after stimulation by intragastric Ensure®, when values had returned

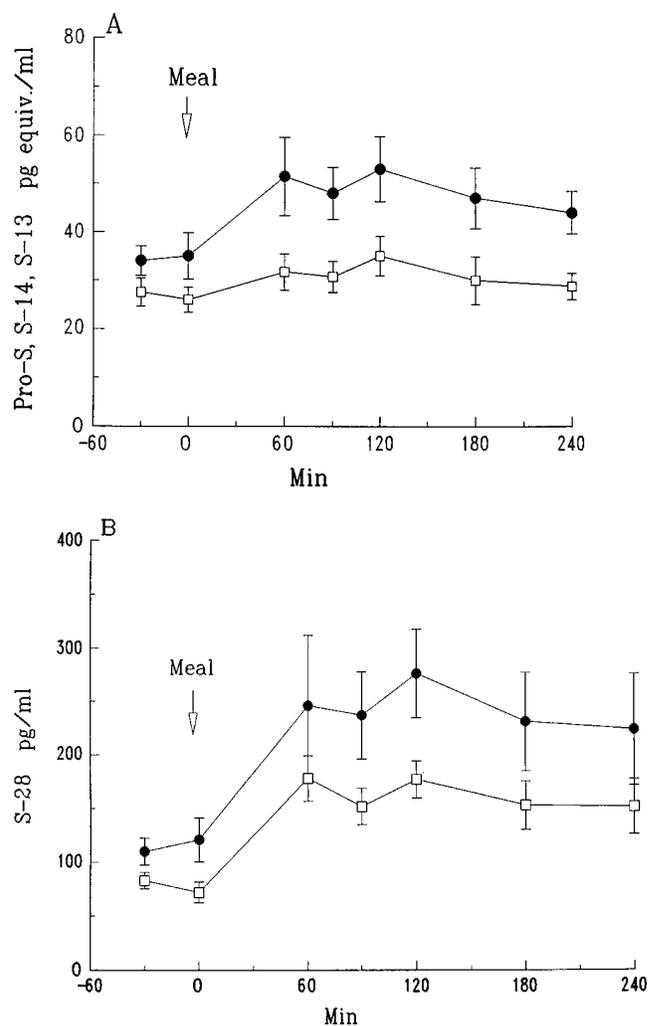
to a steady state, were considered basal. Basal levels for insulin were the mean of the 16 before meal samples, five samples obtained before the IVGTT, and six samples at 90 min after the IVGTT. The amplitude and periodicity of insulin secretion were measured by the adaptive threshold pulse detection method of Clifton (31) in the 16 samples for insulin drawn at 2-min intervals before intragastric feeding. The responses of glucose and insulin during the intragastrically administered meal were defined by the incremental areas under the curve (AUC) of glucose, insulin, and GLP-1 as calculated by the trapezoidal method. The acute insulin response to intravenous glucose ( $AIR_g$ ) was taken as the average rise in insulin in the samples obtained 2–10 min after the intravenous glucose bolus. The glucose disappearance constant ( $K_g$ ) was determined as the slope of the least square linear regression of the natural logarithm of the glucose values at 10, 12, 14, 16, and 19 min after intravenous glucose. Insulin sensitivity ( $S_i$ ) and glucose effectiveness ( $S_g$ ) were calculated using the minimal model of glucose kinetics as described by Bergman et al. (32), and reliability of our results was evaluated using Monte Carlo methods (33).

Data from each animal were compared among the three conditions (a) control, (b) within 6 h after mAb (day 1), and (c) within 24–30.5 h after mAb (day 2). Basal, postprandial, and IVGTT values for the six animals were analyzed using two-tailed *t* tests for paired samples. Data are presented as mean  $\pm$  SE, and a *P* value of 0.05 or less was considered significant.

## Results

**Circulating basal and postprandial levels of Pro-S-related peptides in baboons.** Levels of Pro-S/S-14/S-13 measured in the plasma from blood samples obtained simultaneously in the hepatic portal vein and femoral artery of six baboons before and after the ingestion of Ensure<sup>®</sup> are shown in Fig. 1 A. In the portal vein, mean levels at baseline were  $35 \pm 4$  pg equiv S-14/ml, reached a maximum of  $53 \pm 6.7$  pg equiv S-14/ml by 120 min ( $P < 0.01$ ), and slowly declined. By contrast, in the arterial circulation, basal values were  $27 \pm 2.8$  pg equiv S-14/ml, and were not statistically ( $P = 0.3$ ) altered throughout the ensuing 240 min. Thus, although food intake resulted in a modest increment in these peptides in the prehepatic circulation, they were cleared rapidly and efficiently by the liver such that no significant perturbations could be discerned in the peripheral blood. In Fig. 1 B, S-28 levels are shown in the same portal vein and femoral arterial samples before and after nutrients. Mean basal levels of S-28 in the prehepatic circulation were  $115 \pm 16$  pg/ml, rose to a maximum of  $275 \pm 41$  pg/ml at 180 min postcibum ( $P < 0.001$ ), and remained elevated thereafter. Coincident mean basal arterial levels of S-28 were  $79 \pm 5$  pg/ml, increased significantly by 60 min to attain a maximum of  $177 \pm 17$  pg/ml at 120 min, and did not subside by 240 min ( $P < 0.01$  for all time points). By comparison of AUC at each site, S-28 was cleared partially by the liver; however, unlike Pro-S/S-14/S-13, a two to threefold rise in arterial levels of S-28 was also observed.

**Effect of antisomatostatin Ig on basal glucose and hormonal levels.** When examined at 0.5, 4.5, 24.5, and 28.5 h after the administration of the mAb MS-12, antibody titers in the baboon plasma that bound 80% of radioiodinated S-14 without displacement by cold somatostatin were detected at a plasma dilution of 1:10,000. Separation of the somatostatin species complexed with the mAb from free peptide demonstrated that 99.9% of circulating Pro-S-related peptides were bound to the mAb, and no consistent detectable basal levels of Pro-S-related species were measured in eluates of the plasma samples that were passed through SepPak to separate antibody-bound IgS from free peptide. Therefore, we conclude that for



**Figure 1.** Levels of Pro-S/S-14/S-13 (A) and S-28 (B) obtained simultaneously from the hepatic portal veins (●) and femoral arteries (□) from conscious baboons ( $n = 6$ ) for 30 min before and 240 min after intragastric infusion of Ensure<sup>®</sup> (30 kcal/kg). Values represent mean levels, and vertical bars represent SE.

up to 28.5 h after the bolus of mAb, the Ig was present in excess, and most, if not all, circulating Pro-S-related peptides were bound to the Ig.

Table I shows the basal plasma concentrations of glucose, insulin, glucagon, and GH before and between 4.5 and 4.75 h after intragastric administration of Ensure<sup>®</sup> to the baboons in the control setting, and on days 1 and 2 after the injection of mAb. Fasting and 4-h postabsorptive glucose concentrations ranged from  $66 \pm 2$  to  $74 \pm 1$  mg/dl. By contrast, distinctive patterns in hormonal levels were observed. Within 30 min after the antibody bolus, GH levels in the six animals rose from pre-mAb values by 10-fold and remained elevated significantly at 24 h. On the other hand, basal insulin levels were unchanged throughout the first 4.75 h after mAb infusion. However, on day 2, coincident with continued high antibody titers, mean fasting insulin levels at 24 and 28.5 h had risen 2.5–3-fold ( $P < 0.001$ ). Five of six animals displayed a pulsatile secretory profile during the control period. The amplitude of the oscilla-

Table I. Mean Basal Levels of Glucose and Hormones in Six Baboons before and after Infusion of Antisomatostatin Ig

Condition	Time	Glucose	Insulin	GH	Glucagon*	GLP-1*
	<i>h</i>	<i>mg/dl</i>	$\mu\text{U/ml}$	<i>ng/ml</i>	<i>pg/ml</i>	<i>pg/ml</i>
Control basal	0–0.5	70±1.3	18±1.9	—	22±1.9	23±1.5
Control postabsorption	4.5–4.75	67±1.1	19±1.8	—	—	—
Day 1 basal, pre-mAb	0–0.5	69±1.8	17±1.8	1.9±0.8	—	—
Day 1 basal, post-mAb	0–0.5	66±2.3	14±1.5	20±7.7 <sup>‡</sup>	23±1.7	28±1.4
Day 1 postabsorption, post-mAb	4.5–4.75	71±2.3	21±2.8	—	—	—
Day 2 basal, post-mAb	24–24.5	74±1.0	52±5 <sup>§</sup>	7.7±1.7 <sup>‡</sup>	17±1.2 <sup>  </sup>	24±3.2
Day 2 postabsorption, post-mAb	28.5–28.75	74±1.5	41±4.3 <sup>§</sup>	—	—	—

Values represent mean±SE of 10 samples in 6 animals for each condition. \*Mean±SE of 8 samples in 4 animals for each condition. <sup>‡</sup>vs. mean values for pre-mAb MS-12;  $P < 0.05$ . <sup>§</sup>vs. mean values for control and day 1;  $P = 0.001$ . <sup>||</sup>vs. mean values at 4.5 h post-mAb MS-12;  $P < 0.001$ .

tions, when calculated as percent deviation from the mean, was  $12.4 \pm 10\%$  during the control period vs.  $34 \pm 10\%$  at 24 h post-mAb ( $P = 0.04$ ). However, the pulse interval was not influenced by the presence of the circulating IgG. Mean basal glucagon concentrations during the control and on day 1 post-mAb MS-12 were not different from each other, whereas on day 2, they declined significantly. By contrast, GLP-1 levels at baseline were not altered significantly from control values on days 1 and 2. Thus, as a result of the administration of the antisomatostatin Ig, basal GH levels were increased immediately, remained elevated, and were followed 24 h later by hyperinsulinemia and hypoglucagonemia without perceptible changes in GLP-1 concentrations.

*Effect of antisomatostatin Ig on postprandial glucose and hormonal levels.* Fig. 2 displays the plasma glucose levels throughout the 240 min after the intragastric instillation of En-

sure<sup>®</sup> in six animals before (control), immediately after (day 1), and 24 h (day 2) after infusion of mAb. When the three sets of observations were compared by inspection, the postprandial glucose profile for day 1 appeared greater than that of the control; however, only the mean value at 60 min was significantly higher ( $P < 0.02$ ), and there were no significant differences among the three conditions when AUC was calculated (control AUC:  $19,486 \pm 804$  vs. day 1 AUC:  $20,325 \pm 897$  vs. day 2 AUC:  $19,198 \pm 275$  mg/dl/240 min;  $P > 0.19$ ).

The effects of the mAb infusions on plasma insulin levels in the animals within the 4 h after nutrient administration during the control, day 1, and day 2 experiments are shown in Figs. 3 and 4. Compared with controls, postprandial insulin concentrations on day 1 immediately after mAb infusion were increased significantly (control AUC [0–240 min]:  $17,390 \pm 5,796$  vs. day 1 [0–240 min]:  $26,984 \pm 5,671$   $\mu\text{U/ml}/240$  min;  $P = 0.001$ ). Similarly, on day 2, 24 h after the infusion of the Igs (Fig. 4), insulin levels after the meal were also elevated compared with

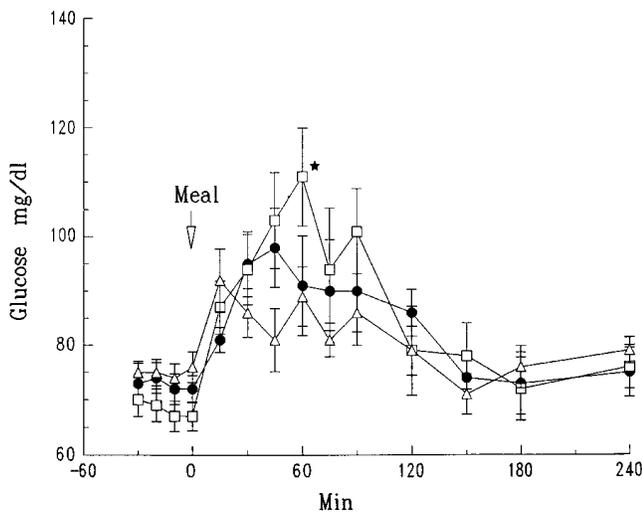


Figure 2. Venous plasma glucose levels in conscious baboons ( $n = 6$ ) 30 min before and 240 min after intragastric instillation of Ensure<sup>®</sup> (30 kcal/kg). Values represent mean levels±SE during the control condition (●), and from 0.5–5 h (day 1, □) and 24–28.5 h (day 2, △) after intravenous bolus administration of mAb MS-12. \*Significant difference ( $P < 0.02$ ) between values for controls and for day 1.

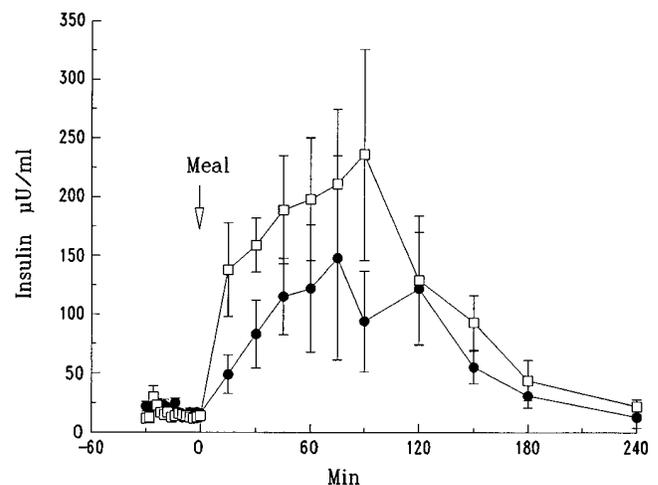


Figure 3. Venous plasma insulin levels in conscious baboons ( $n = 6$ ) from 30 min before until 240 min after intragastric instillation of Ensure<sup>®</sup> (30 kcal/kg). Values represent mean levels±SE during the control condition (●) and 0.5–5 h after intravenous bolus administration of mAb MS-12 (day 1, □).

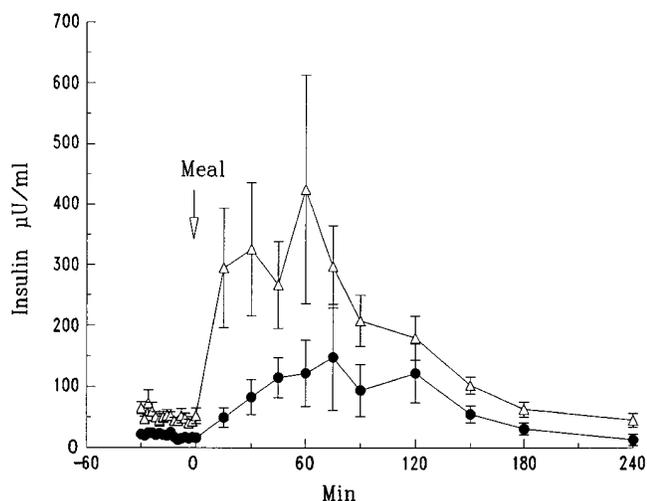


Figure 4. Venous plasma insulin levels in conscious baboons ( $n = 6$ ) from 30 min before until 240 min after intragastric instillation of Ensure® (35 kcal/kg). Values represent mean levels  $\pm$  SE during the control condition (●) and 24–28.5 h after intravenous bolus administration of mAb MS-12 (day 2,  $\Delta$ ).

the controls (control AUC [0–240 min]:  $17,390 \pm 5,796$  vs. day 2 AUC:  $39,236 \pm 6,583$   $\mu\text{U}/\text{ml}/240$  min;  $P = 0.03$ ). Thus, in the presence of the circulating Igs, the insulin release evoked by intragastric nutrient instillation on two consecutive days was enhanced in these animals. That this was not related to changes in circulating GLP-1 levels is shown in Fig. 5, in which postprandial concentrations of GLP-1 were similar in the control condition and on days 1 and 2 (control AUC [0–240 min]:  $9,438 \pm 1,456$  vs. day 1 AUC:  $9,352 \pm 1,182$  vs. day 2 AUC:  $8,407 \pm 962$   $\text{pg}/\text{ml}/240$  min;  $P > 0.5$  for all comparisons).

*Effect of antisomatostatin Igs on insulin levels and glucose disposition during intravenous glucose and tolbutamide toler-*

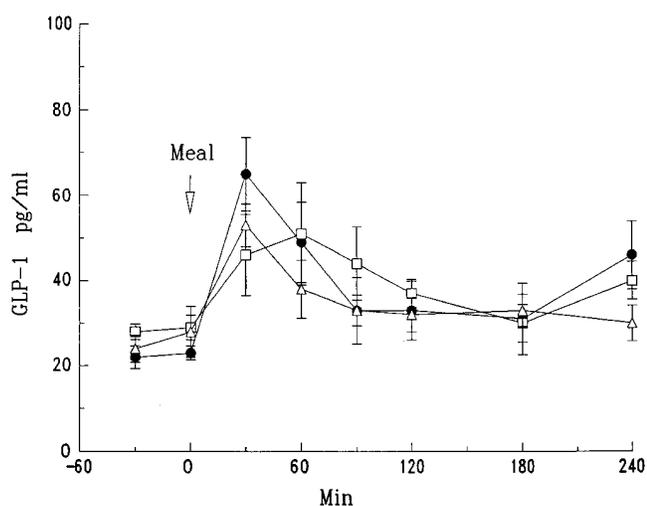


Figure 5. Venous plasma GLP-1 levels in conscious baboons ( $n = 4$ ) from 30 min before until 240 min after intragastric instillation of Ensure® (35 kcal/kg) values. Values represent mean levels  $\pm$  SE during the control condition (●), and from 0.5–5 h (day 1,  $\square$ ) and 24–28.5 h (day 2,  $\Delta$ ) after intravenous bolus administration of mAb MS-12.

*ance tests.* The tolbutamide-modified IVGTT was performed between 5 and 6.5 h after initiation of blood sampling in the control condition, and on days 1 and 2 after administration of mAb. These intervals were selected because the postprandial glucose and insulin concentrations had declined to basal after intragastric nutrient instillation, and were presumed to represent steady state conditions (Figs. 2–4). Measurement of insulin secretion and parameters of glucose disposition were calculated, the latter using the minimal model of glucose kinetics (Table II). Two sets of relevant information are apparent from these data. First, when compared with controls, insulin secretion in response to either glucose ( $\text{AIR}_g$ ) or tolbutamide (not shown) was not perturbed significantly on day 1 compared with controls. As noted previously (Table I), baseline insulin levels between 4.5 and 4.75 h in the two conditions were also not different. Taken together, these findings suggest that circulating S-28 is unlikely to influence insulin secretion in the basal state. Second, by contrast, the acute insulin responses to glucose and tolbutamide (data not shown) were augmented significantly on day 2 in comparison with controls, which corresponded to increased basal insulin levels (Table I). Although reliable parameters for  $S_i$  on day 1 at 5–6.5 h after the IgG could not be calculated, this measurement on day 2 was decreased significantly compared with the control experiments. No differences in  $K_g$  and  $S_g$  were observed at any time after administration of the antibody. From these data, we infer that in order to compensate for the increased insulin resistance, basal insulin secretion was enhanced, and the B cells became hyper-responsive to parenteral secretagogues.

## Discussion

The salient observations from our study in which murine monoclonal IgG<sub>1</sub> cross-reacting with S-14 and S-28 was given intravenously to conscious baboons and evaluated over 30 h include: (a) undetectable levels of S-28 after mAb, compared with a two to threefold increase during nutrient absorption in controls; (b) inordinate rises in postprandial insulin levels without significant differences in glycemia, when tested within 4 and 24–28 h after mAb administration; (c) immediate and persistent elevations of GH; (d) marked increase in basal insulin levels with heightened oscillatory excursions and augmented pancreatic B cell secretory responses to parenteral stimuli first observed 24 h after infusion of mAb; and (e) development of tissue insulin resistance.

There are long-standing precedents for use of passive immunoneutralization with polyclonal and, more recently, monoclonal antibodies that have been administered to humans and

Table II. Effects of Antisomatostatin Ig on Parameters of Glucose and Insulin Kinetics Derived from a Tolbutamide-modified IVGTT

Condition	Time	$K_g$	$\text{AIR}_g$	$S_i$	$S_g$
	h	%/min	$\mu\text{U}/\text{ml}$	$\times 10^{-5} \text{ min}^{-1}/\text{pM}$	$\times 10^{-2} \text{ min}^{-1}$
Control	5–6.5	$3.94 \pm 0.77$	$408 \pm 61$	$14.4 \pm 3.2$	$4.9 \pm 0.1$
Day 1	5–6.5	$3.73 \pm 0.32$	$603 \pm 83$	—	—
Day 2	28.5–30	$3.98 \pm 0.31$	$906 \pm 112^*$	$5.6 \pm 0.8^\ddagger$	$5.0 \pm 0.01$

\* $P = 0.008$  vs. control.  $^\ddagger P = 0.03$  vs. control.

animals as well as perfused through isolated organ systems to search for regulatory factors (20–22). In principle, this approach offers a specific and selective means of eliminating the endogenous factor of interest, permitting assessment of its action in a physiologic context. In this study, the mAb bound virtually all of the circulating bioactive Pro-S–related peptides, including S-28, which rises in the arterial circulation after meals, thereby preventing signaling by these factors. This is apparent from both the measurement of bound and free peptides in the plasma of the baboons and the rise in GH, a hormone that has been shown to increase with immunoneutralization of S-28 in rats (34, 35). The increase in postprandial insulin concentrations after immunoneutralization is explained best by the removal of the inhibitory effects of S-28 on the B cell. This interpretation is based on the selective rise of S-28 but not S-14 in the peripheral blood stream after food intake reported previously by us in humans (3, 17), and confirmed here in baboons. Most of the S-28 in the circulation is derived from enterocytes within the proximal gut (3, 16), with a small contribution from hypothalamic neurons (our unpublished results). By contrast, S-14, secreted into the hepatic portal blood stream from D cells of the stomach, pancreas, and enteric neurons, is cleared rapidly by the liver (36), and arterial levels do not change perceptibly after nutrient intake (Fig. 1) (3, 16, 37). Thus, neither S-14 nor its conversion product S-13 is likely to serve as a hormone outside the splanchnic bed. However, based on inhibition of insulin release by S-28 infused to achieve postprandial levels in humans (18, 19), coupled with the present findings, a creditable case can be made for S-28 as a humoral signal to islet B cells.

S-28 is a potent inhibitor of insulin release, both *in vivo* and *in vitro* (38–41), with a high binding affinity for B cells (42, 43). Because arterial access of S-28 to the islet is key to its function, neutralization with a specific antibody should be an effective means of ablating its action. It is possible, however, that a direct effect of S-28 is not the complete explanation of these results, and that modification of the release of other regulatory factors after immunoneutralization might have contributed to heightened insulinemia. In considering this possibility, we measured plasma levels of GLP-1, one of the two major incretins, which, if increased, would be expected to augment insulin release (23, 24). The lack of difference in the GLP-1 levels in the control and post-mAb studies excluded this option. While we cannot rule out that immunoneutralization affected other insulin secretagogues regulated by S-28, we believe that it is most likely that S-28 is exerting its effect directly on B cells. As a component of the enteroinsular axis, we envision S-28 as a incretin that constrains the enhanced release of insulin in response to absorbed nutrients and synchronously secreted incretins during the early phases of alimentation, and leads to a regulated waning of insulin to avoid unwanted hypoglycemia.

These results suggest that pancreatic S-14 does not have a hormonal role in control of insulin secretion. Schusdziarra et al. were the first to report augmentation of nutrient-stimulated insulin levels after administration of an antisomatostatin serum to dogs (8). This was attributed to blockade of circulating S-14, as they were unaware that S-28 is the major Pro-S–related peptide released during meal ingestion. However, it has been proposed that S-14 secreted into the islet vasculature exerts a tonic inhibitory influence on B cell function (14, 15). That this is unlikely is suggested by our results, in which no differences in basal and intravenous glucose- or tolbutamide-stimulated insulin secretion were detected 4.5 h after mAb administration

when presumably most of the S-14 in islet blood vessels was bound to the IgG. This interpretation is consistent with the work of Sorenson and Elde (44) and Samols, Stagner, and colleagues (10–13), who were unable to modify insulin secretion in isolated pancreata perfused arterially with antisomatostatin serum, leading them to conclude that S-14 from D cells in the islet mantle does not access the centrally located B cells by intraislet vascular channels.

An intriguing finding in these studies was the increase in basal insulin levels that first became apparent 24 h after mAb administration. This was not due to removal of S-28 and other Pro-S–related peptides from the circulation, since (a) baseline levels of insulin within 30 min after antibody injection were not elevated; (b) postprandial insulin levels were restored to pre-immunoneutralization levels by 4.5 h on day 1; and (c) B cells were not hyperresponsive to intravenous glucose or tolbutamide at 5–6.5 h, which would be an expected consequence of increased insulin production. We were unable to discern when sustained hypersecretion of insulin began, as insulin concentrations were not measured between 6 and 24 h. There was no consistent pattern of increased glucose levels that might have contributed to increased insulin secretion, and for the reasons cited above, it seems reasonable to infer that basal hyperinsulinemia was related to the presence of the mAb.

Of the possible explanations for heightened basal insulin secretion, insulin resistance secondary to elevated circulating GH would appear to be the most plausible. That GH is regulated by somatostatins via the hypothalamus has been firmly established (1, 25). After injection into rats of an antiserum recognizing both S-14 and S-28 (34), or S-28 specifically (35), plasma GH levels increased immediately and profoundly. Our findings of prompt and sustained rises in GH levels in baboons after immunoneutralization corroborate the observations in rats. In healthy humans, infusions of GH achieving plasma levels 2–10-fold above baseline resulted in a decline in insulin-mediated glucose disposal within 12 h, accompanied by a two to threefold increment in insulin levels (45, 46). These observations are mirrored by the 4–10-fold increase in GH levels maintained in our baboons during the course of the 2 d of study after immunoneutralization. That the higher levels of GH may have caused some degree of insulin resistance during the early phase of the experiments may be inferred from the normal postmeal glucose profile, despite the greatly heightened insulin secretion during day 1. However, basal hyperinsulinemia was not observed until the second day, when GH had been elevated for 24 h. Thus, the well-known attribution of increased insulin resistance and concomitant hyperinsulinemia to elevated GH levels provides the most compelling explanation for the findings in our baboons.

An alternate but less likely cause of fasting hyperinsulinemia on day 2 is interference with paracrine regulation of the B cell by interstitial S-14. Since mAb-12 is an IgG<sub>1</sub> with an approximate molecular mass of 160 kD, it is confined initially to the intravascular space. In humans and in other mammals, homologous IgG<sub>1</sub> has an estimated plasma half-life of 31–35 d (47, 48), and it has been theorized that 50% extravasates into the extravascular space within 24 h (49, 50). In our study, the mAb was present in high concentrations in the intravascular pool for at least 30 h, so it is plausible that a significant fraction had diffused gradually into the interstitial space of the islets, which contain fenestrated membranes that are permeable to macromolecules (51). It has long been held that S-14 plays a

paracrine role in insulin secretion (1, 4–7), a concept based on experiments in isolated islets in which insulin secretion was increased by incubation with either somatostatin antibody (6) or cysteamine, a compound that depletes S-14 selectively from D cells (52). Nonetheless, this interpretation did not consider that the islet preparations were subject to random access of peptides to all cells, and not through vascular channels as found in the intact pancreas (53, 54). Recently, Kleinman and colleagues lent credence to a paracrine effect for S-14 by demonstrating augmented insulin secretion in isolated human cadaveric pancreas perfused with intact antisomatostatin IgG or its Fab fragment, which may have percolated into the interstitial space (14, 15). It has been estimated that interstitial islet S-14 concentrations may reach  $10^{-9}$  M (13). Thus, in our baboons, it is possible that insufficient antibody had accumulated in the interstitium by 4.5 h after mAb administration to bind enough S-14 to alter insulin release, but that over 24 h of continued extravasation of antibody enabled eventual immunoneutralization. Nevertheless, this mechanism for the basal hyperinsulinemia documented in our baboons was not tested directly and remains conjectural.

In summary, after parenteral administration to conscious baboons of a monoclonal IgG<sub>1</sub> with an epitope shared by S-28 and S-14, both postprandial and fasting insulin levels were increased; however, they were dissociated temporally. The former was observed within 4 h, whereas the latter was not apparent until 24 h. The fasting hyperinsulinemia is explained best as a compensatory response to insulin resistance induced by GH. Our data are not in keeping with a significant role for intraislet S-14 in B cell regulation. By contrast, we believe that they provide compelling evidence that during the postprandial period, S-28 released from the gut into the circulation in response to luminal nutrients inhibits the islet B cell and, as a participant in the enteroinsular axis, provides a regulated attenuation of insulin release.

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