Treatment of Streptozotocin-Diabetic Dogs With a Long-Acting Somatostatin Analog

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SUMMARY

A long-acting somatostatin analog, Wy-41,747, improved glucose homeostasis in steptozotocin-diabetic dogs. In insulin-withdrawn, fasted dogs, a single 100 μg/kg s.c. injection of Wy-41,747 significantly suppressed plasma glucagon levels for 2 h (a nadir of 53 ± 8% of basal being reached at 1 h) and plasma glucose for 3 h (77 ± 5% of basal); even more potent suppression of glucagon and glucose levels was observed during experiments with 500 μg/kg of Wy-41,747. During meal studies, the combined administration of Wy-41,747 (100 μg/kg) and insulin resulted in lower postprandial glucagon and glucose levels than did the administration of insulin alone. When the somatostatin analog was given alone to fed animals, a rise in glucose occurred 4–5 h after the meal, suggesting the possibility of delayed absorption under these conditions. Improved glucose tolerance profiles followed oral administration of glucose or ^{14}C-3-O-methyl glucose; a normal or near-normal time course of absorption was observed with diminished peak plasma glucose or ^{14}C levels. No major malabsorption of carbohydrate occurred in the ^{14}C-3-O-methyl glucose study, since only low levels of ^{14}C were found in the feces of peptide-treated dogs. No undesirable gastrointestinal effects were noted during these studies. In conclusion, administration of a single subcutaneous dose of the long-acting somatostatin analog Wy-41,747 to streptozotocin-diabetic dogs lowered fasting plasma glucose levels and augmented insulin action in lowering postprandial glucose levels.

DIABETES *28*:491–495, May 1979.

Diabetes mellitus is characterized by elevated plasma glucagon levels[1,2] as well as impaired insulin secretion or action. Plasma glucagon levels decrease in normals in response to an oral glucose load, whereas glucagon levels increase in both juvenile-type and adult-type diabetic patients; this α cell defect cannot be corrected by short-term normalization of insulin levels (by infusion), but if insulin infusion rates are elevated to produce hyperinsulinemia, then plasma glucagon levels can be normalized in the juvenile-type diabetic patients.[3] In contrast, aggressive insulin injection therapy for as long as 10 days does not normalize hyperglucagonemia of juvenile-type diabetic patients.[4] Infusion of the tetradecapeptide somatostatin resulted in suppression of plasma glucagon and glucose levels[5–7] in fasted, insulin-withdrawn diabetic patients and a lowering of postprandial hyperglycemia in insulin-treated diabetic patients.[5,8,9] Gerich et al.[10] established optimal control in diabetic patients treated with insulin alone for 3 days followed by 3 days of continuous somatostatin infusion with a 50% reduction in insulin dosage; somatostatin treatment lowered postprandial glucose levels, 24-h glycosuria, and mean daily serum glucose levels.

The above results indicate a potential use for somatostatin as a powerful adjunct to insulin in the treatment of the hyperglycemia of diabetes. However, its potent suppression of insulin release[11] and short biologic half-life[12] make somatostatin unacceptable as a therapeutic agent. Several somatostatin analogs, [D-Cys[14]]- and [D-Trp[8], D-Cys[14]]-somatostatin, have been reported to selectively suppress the release of glucagon relative to that of insulin in rats.[13] These analogs, although possessing the desired specificity, are not long-acting. One potent, long-acting somatostatin analog—Wy-40,793—is capable of lowering plasma growth hormone in rats for 5 h and plasma glucose and glucagon for 3 h in diabetic dogs; unfortunately this analog is a potent suppressor of insulin in arginine-stimulated rats[14] and thus may inhibit residual β-cell function found in many juvenile diabetic patients.

A long-acting somatostatin analog—des-Ala[1],Gly[2][His[4,5], D-Trp[8]]-somatostatin (Wy-41,747)—has recently been synthesized; it was found to be a potent suppressor of glucagon and growth hormone release in pentobarbital- and arginine-stimulated rats with little insulin-suppressing activity in short-term experiments (15 min from peptide administration to blood sampling) and to inhibit growth hormone release for 2 h.[15] The present investigation was undertaken to determine the effects of Wy-41,747 on streptozotocin-

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Accepted for publication 6 February 1979.

TABLE 1
Basal plasma glucose and glucagon levels in groups of diabetic dogs*

Experiment	Group treatment	Glucose (mg/dl)†	Glucagon (pg/ml)†
Fasting studies	Saline	279 ± 19	495 ± 202
	Wy-41,747 (100 μg/kg)	306 ± 39	359 ± 123
	Wy-41,747 (500 μg/kg)	276 ± 28	443 ± 197
Meal studies	Insulin	356 ± 60	427 ± 81
	Insulin + Wy-41,747	318 ± 30	381 ± 50
	Saline	323 ± 51	359 ± 85
	Wy-41,747	359 ± 59	358 ± 99
Oral glucose tolerance test	Saline	333 ± 51	327 ± 68
	Wy-41,747	309 ± 56	264 ± 68

* Six dogs per group.
† Mean ± SEM.

diabetic dogs in the fasting state and after a meal. Oral glucose tolerance tests (OGTTs) and absorption of ^{14}C-3-O-methyl glucose were also studied.

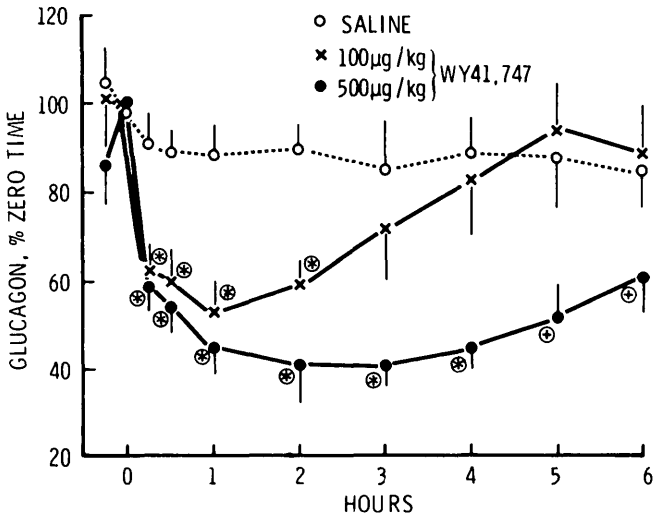
MATERIALS AND METHODS

Experiments were carried out on unanesthetized streptozotocin-diabetic dogs. Each dog served as its own control in fasting, meal, and oral glucose tolerance studies; results of all experiments were calculated as percent of 0 time for both glucose and glucagon values because of large differences between animals. The absolute means (±SEM) of all groups are listed in Table 1; no significant differences were noted between treatment groups in any of the three types of experiments. In fasting experiments, dogs did not receive food or insulin for 18 h; after a pretreatment blood sample was obtained, animals were injected s.c. with 100 or 500 μg/kg of Wy-41,747 and blood collected for 6 h. All blood samples were collected in Trasylol-EDTA (3 mg EDTA and 1500 U Trasylol/1.5 ml blood). In meal studies, a meal consisting of 200 g of canned dog food (12% protein and 7% fat) mixed with 70 g pulverized dog food (25% protein and 8% fat) plus 100 ml of cow's milk mixed with 400 ml of water was given to the dogs, and in all cases the meal was consumed immediately. Insulin dosage in the meal studies was based on each dog's daily insulin requirements, and once determined remained the same for all experiments.

OGTTs were performed by administering 2.4 g/kg of glucose to six diabetic dogs via stomach tube; blood was sampled via the jugular vein for the next 6 h. Three dogs received a mixture of 2.4 g/kg of 3-O-methyl glucose and 4 μCi/kg of ^{14}C-3-O-methyl glucose (New England Nuclear) per os. Plasma radioactivity was determined by counting 0.2 ml plasma in 10 ml Biofluor (New England Nuclear). Feces were collected for 7 days after administration of the radioactive glucose analog. Radioactivity in the feces was determined by homogenizing feces with 3 vol of water (w/v) and oxidizing 0.5 ml of the resultant fluid in a Packard Tri-Carb Oxidizer.

Glucagon was determined by the radioimmunoassay method of Faloona and Unger[16] utilizing Unger 30K glucagon antiserum. Glucose was determined by the glucose oxidase method by the Technicon Autoanalyzer. Wy-41,747 was synthesized as described previously.[15] All statistical analyses were performed using paired *t* tests.

FIGURE 1. Effect of Wy-41,747 on plasma glucagon levels in six fasting, insulin-withdrawn diabetic dogs. *P < 0.01; †P < 0.05 by paired *t* test comparing peptide treatment and saline treatment.



RESULTS

Fasting studies. Fasting, insulin-withdrawn dogs were treated with 500 μg/kg and 100 μg/kg s.c. doses of Wy-41,747 and plasma glucagon and glucose levels determined for the subsequent 6 h. Glucagon levels in saline-treated animals remained stable after an initial drop of approximately 10% (Figure 1). Glucagon levels dropped 40% in Wy-41,747–treated dogs 15 min after peptide administration. In dogs treated with 100 μg/kg of the peptide, these levels reached a nadir, 53 ± 8% of basal, 1 h after injection, then returned to control levels; no rebound was observed. In dogs treated with 500 μg/kg of Wy-41,747, glucagon levels reached a nadir, 41 ± 5% of basal, 3 h after administration of the peptide and slowly increased during the duration of the experiment, with significant differences noted when compared with control values at all time points. Glucose levels were consistently lowered in peptide-treated animals during these experiments (Figure 2), the nadir being reached at 3 h in dogs treated with both 500 and 100 μg/kg of Wy-41,747 (71 ± 6% and 77 ± 5% of basal, respectively). Animals treated with the high dose of peptide had glucose levels that remained significantly suppressed for 5 h, whereas glucose in dogs receiving the low dose of peptide returned more rapidly toward control values.

Meal studies. Postprandial glucose and glucagon profiles were determined after administration of a standard meal plus one of the following: (a) insulin treatment, 3–6 U; (b) 100 μg/kg of Wy-41,747; (c) insulin plus 100 μg/kg of Wy-41,747; or (d) saline. Figure 3 shows that the glucose response to the combination of the meal, insulin, and Wy-41,747 was significantly lower than the meal plus insulin alone, the nadir in both cases being reached at 2 h postmeal. Although the glucose levels in peptide and insulin-treated dogs returned to the values in dogs treated with insulin alone, no rebound was observed. Postprandial hyperglycemia was observed in saline-treated dogs, with peak values reached 1 h after ingestion of the meal. Glucose levels in Wy-41,747–treated dogs declined transiently, then rose steadily, reaching a peak 5 h postmeal.

Although mild hyperglycemia was observed, glucose levels were never significantly higher than glucose values during the meal alone.

Postprandial glucagon levels in dogs treated with a combination of Wy-41,747 and insulin decreased rapidly and remained significantly lower than in animals treated with insulin alone for 2 h (Figure 4). Glucagon profiles were qualitatively similar to the glucose profiles for control dogs and dogs receiving Wy-41,747 alone. A fourfold increase in glucagon levels was observed 1 h postprandially in dogs treated with saline, whereas a glucagon spike was not noted in Wy-41,747–treated dogs until 4 h after the meal.

Glucose tolerance tests. OGTTs (2.4 g/kg) were run on fasted dogs withdrawn from insulin. In control studies, plasma glucose concentration increased rapidly during the first hour, reaching a peak of 185 ± 20% of basal (Figure 5). Although glucose levels followed the same time course in dogs treated with Wy-41,747, glucose levels increased to only 120 ± 10% of basal; no glucose rebound was observed. Surprisingly, plasma glucagon levels were not lowered in dogs treated with Wy-41,747; no explanation for this observation is apparent.

In order to study the possibility of delayed absorption or malabsorption of carbohydrates induced by Wy-41,747, ^{14}C-3-O-methyl glucose (2.4 g/kg, 4 μCi/kg) was administered orally to three dogs with and without Wy-41,747. Plasma ^{14}C levels were measured for 6 h and fecal ^{14}C levels determined for 7 days. The plasma ^{14}C appearance in control dogs was rapid, reaching a peak 1 h after administration of the label; subsequent clearance of the label was also rapid (Figure 6). In dogs treated with Wy-41,747, plasma ^{14}C levels peaked at 90 min then slowly declined; the peak radioactivity was significantly less than in control experiments (4615 ± 1301 cpm vs. 10,003 ± 1304 cpm, $P < 0.05$). The total amount of radioactivity appearing in the plasma of Wy-41,747–treated dogs was not significantly different from controls (mean area ± SEM of plasma ^{14}C curves in dogs receiving Wy-41,747 = 93 ± 7% of controls). Radioactivity appeared in the feces during only the first and second days of the experiment. The amount of ^{14}C in the feces was consistently higher in dogs treated with Wy-41,747 than in controls, but in no case did a major loss of carbohydrate occur (percent of administered ^{14}C in feces, mean ± SEM; control: 0.2 ± 0.1; + Wy-41,747: 2.8 ± 1.7, the difference between groups is not significant).

DISCUSSION

The present study and previous studies in both fasting diabetic man[5–7] and diabetic dogs[17] have shown that suppression of glucagon release results in a 25–30% reduction in plasma glucose levels (intestinal glucose absorption cannot be a factor in these studies). The present study achieved glucagon deficiency in diabetic dogs by administration of a long-acting somatostatin analog, Wy-41,747, capable of suppressing glucagon release for 6 h by an injection of 500 μg/kg s.c., whereas previous studies achieved a similar effect by intravenous infusion of somatostatin. The central role of glucagon in glucose homeostasis has been demonstrated by tracer studies in dogs: somatostatin-induced glucagon deficiency resulted in an overall decrease in hepatic glucose production[18,19] and a decrease in gluconeogenesis.[20] The effects of somatostatin (and

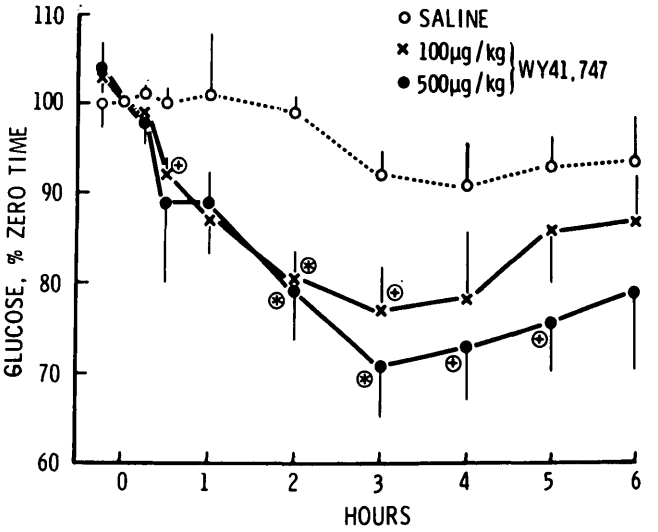
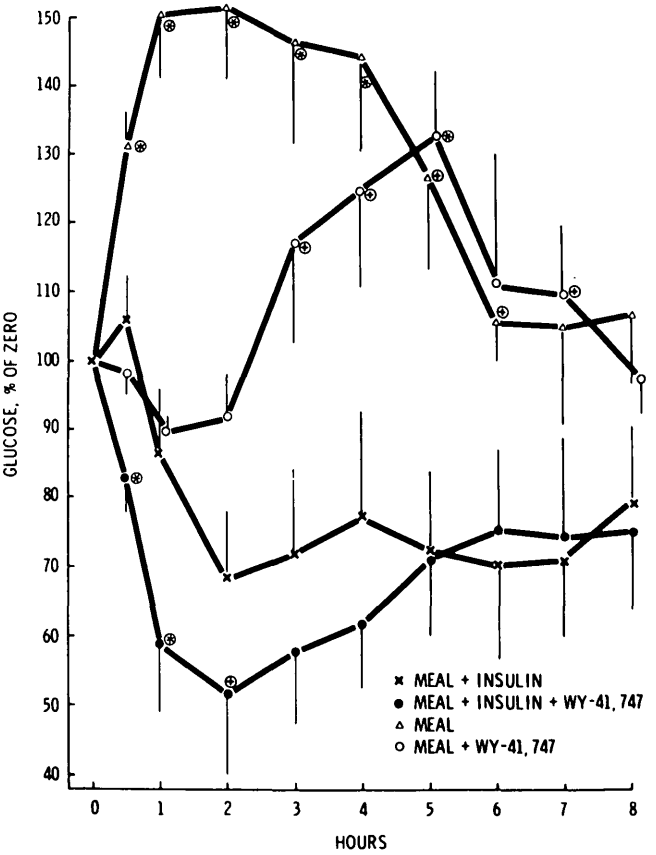


FIGURE 2. Effect of Wy-41,747 on plasma glucose levels in six fasting, insulin-withdrawn diabetic dogs. *P < 0.01; +P < 0.05 by paired *t* test comparing peptide treatment and saline treatment.

Wy-41,747) on glucose homeostasis in the fasting state are most likely due to these hormonal changes, although a direct hepatic effect cannot be ruled out since somatostatin itself may alter hepatic glucose production in response to glucagon.[19,21,22] Additionally, changes in other

FIGURE 3. Effect of Wy-41,747 ± insulin (3–6 U) on plasma glucose in six diabetic dogs after a standard meal. Insulin dosage was determined by each dogs' individual daily insulin requirement. Statistical significance determined by comparing insulin-treated animals with other groups by paired *t* test. *P < 0.01; +P < 0.05.



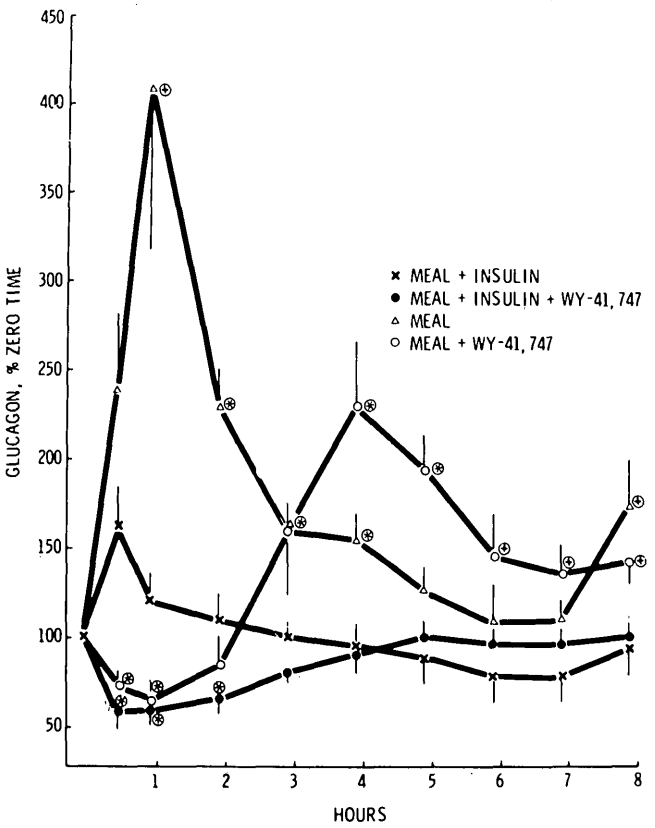


FIGURE 4. Effect of Wy-41,747 ± insulin (3–6 U) on plasma glucagon in six diabetic dogs after a standard meal. Insulin dosage was determined by each dog's individual daily insulin requirement. Statistical significance determined by comparing insulin treated animals with other groups by paired *t* test. *P < 0.01; +P < 0.05.

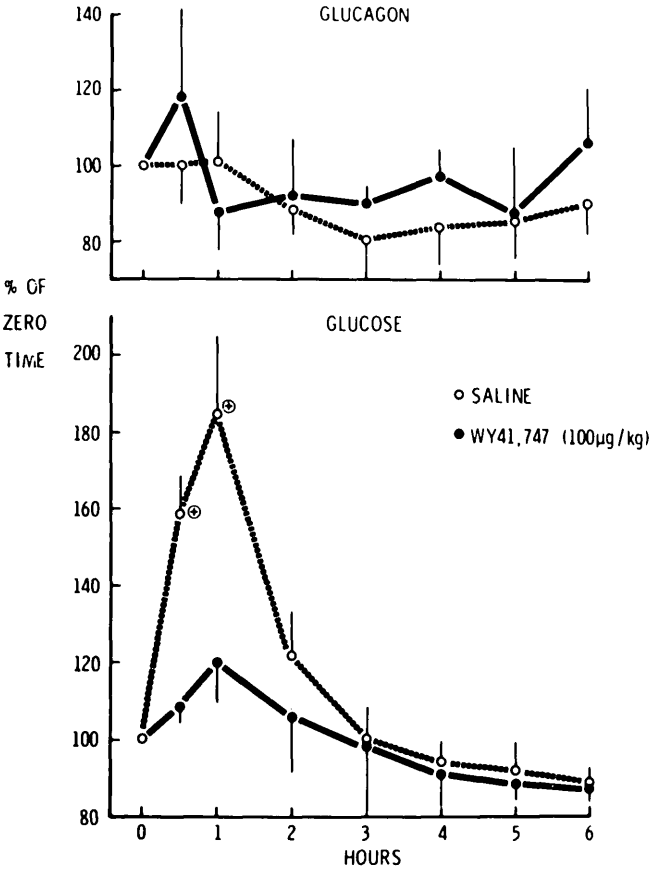
hormones not measured in this study, such as growth hormone, may contribute to changes in glucose homeostasis.

The combined administration of Wy-41,747 and insulin before feeding of a standard meal lowered postprandial glucose and glucagon levels significantly more than administration of insulin alone. Infusion of somatostatin in fed diabetic man improved glucose homeostasis during both short-term (one-meal) studies[6–8] and long-term (1-3–day) studies.[9,10] Similarly, a 24-h infusion of somatostatin to insulin-treated alloxan-diabetic dogs resulted in a consistent lowering of plasma glucose when compared with control dogs.[17] Improvement of glucose control in these studies has been attributed at least in part to suppression of plasma glucagon levels. The present results conform with this conclusion, since Wy-41,747 administration lowered glucagon and glucose in parallel during the meal studies with insulin. Somatostatin itself has previously been reported to produce delayed gastric emptying,[23] delayed xylose absorption,[24] decreased splanchnic blood flow,[24] diminished gastric acid secretion,[25] and diminished exocrine pancreatic function.[26] Delayed gastric emptying or intestinal absorption are possibly responsible for the retarded increase of plasma glucose and glucagon observed when the meal and Wy-41,747 were given without insulin; if Wy-41,747 altered intestinal absorption of nutrients in the combination experiments with insulin, then a potentiation of insulin action might occur and contribute to the reduced plasma glucose and glucagon levels. Preliminary studies for 2 wk in diabetic dogs receiving two injections of insulin and Wy-41,747 daily produced lower postprandial glucose levels than did injections of insulin alone, and no severe gastrointestinal signs or weight loss were observed (E. Lien, unpublished results).

OGTTs with Wy-41,747 followed the same time course as control (peak at 1 h), suggesting that gastric emptying was not altered by the peptide during a liquid load (as opposed to the meal studies). The amount of glucose entering the peripheral circulation was reduced by Wy-41,747, possibly caused by a decreased rate of absorption or improved clearance by the liver before reaching the periphery. In order to further examine the possibility of malabsorption of glucose, ^{14}C-3-O-methyl glucose absorption was studied. This sugar is absorbed via the glucose transport mechanism but is not further metabolized.[27] Only minor amounts of radioactivity were found in the feces; blood levels in peptide-treated animals peaked 30 min later than controls, suggesting a retardation of absorption.

Although somatostatin infusions lower the hypersecretion of glucagon and growth hormone and improve glucose control under both fed and fasted conditions in diabetes mellitus, the short biologic half-life[12] precludes the use of this peptide as a practical adjunct to insulin therapy in the treatment of the hyperglycemia of diabetes. Previous attempts to synthesize somatostatin analogs with prolonged duration of action have resulted in various peptides capable of suppressing growth hormone for prolonged periods of time with (a) no insulin or glucagon-suppressing

FIGURE 5. Effect of Wy-41,747 on plasma glucose and glucagon during an OGTT in six diabetic dogs (2.4 g glucose/kg). Statistical significance determined by comparing saline and Wy-41,747–treated dogs (six per group) by paired *t* test. +P < 0.05.



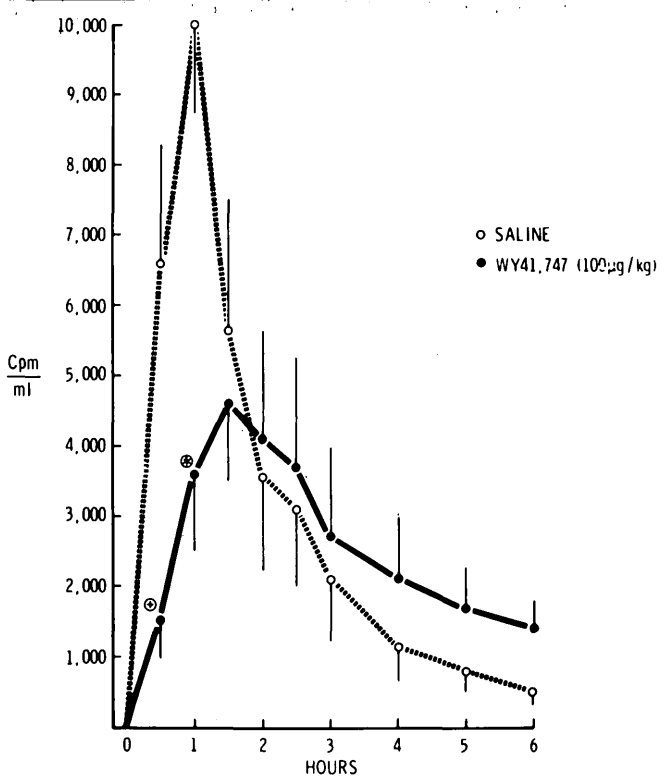


FIGURE 6. Plasma appearance of radioactivity after oral administration of ^{14}C-3-O-methyl glucose in three diabetic dogs. *P < 0.01; $^{+}$P < 0.05.

activity,[28] (b) insulin-suppressing but no glucagon-suppressing activity,[29] (c) insulin- and glucagon-suppressing activity.[14] Des-Ala^{1},Gly^{2}[His^{4,5},D-Trp^{8}]-somatostatin (Wy-41,747) has been reported by Sarantakis et al.[15] to selectively lower growth hormone and glucagon release and to be long-acting in rats. The present study in dogs has further demonstrated the potential for Wy-41,747 as an adjunct to insulin in the treatment of diabetes mellitus.

ACKNOWLEDGMENTS

The authors wish to thank Gerald Deitch and Robert Whitley for their excellent technical assistance.

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