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THE EFFECT OF SOMATOSTATIN ON BODY WEIGHT
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THE EFFECT OF SOMATOSTATIN ON BODY WEIGHT AND FOOD INTAKE IN RATS

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ABSTRACT

THE EFFECT OF SOMATOSTATIN ON BODY WEIGHT AND FOOD INTAKE IN RATS

By

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Chronic protamine zinc somatostatin (PZ-SRIF) treatment will decrease body weight in rats. Whether this weight loss is due to decreased food intake or absorption is unknown. Male Sprague-Dawley rats (250 g) treated with subcutaneous injections of PZ-SRIF (200 μg/kg) for seven days after pre-treatment with PZ-SRIF (100 μg/kg) for sixteen days showed a significant decrease in body weight gain. This effect on weight gain was coupled with a significant decrease in food intake but no change in fecal lipids or xylose absorption. Following PZ-SRIF treatment, serum concentrations of glucose, immunoreactive insulin (IRI) and immunoreactive pancreatic glucagon (IRG_a) were unchanged but serum immunoreactive total glucagon (IRG_t) was decreased. Pancreatic islets showed diminished content and secretion of both IRI and IRG_a. It is suggested that the observed weight loss in PZ-SRIF treated rats was due to a decrease in food intake rather than nutrient absorption. Chronic PZ-SRIF treatment also appeared to selectively decrease circulating levels of enteroglucagon.

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INTRODUCTION

Both intake and absorption of food must exist to provide cellular energy. If a sufficient energy deficit occurs because of either decreased consumption and/or impaired absorption, the ultimate consequence may be loss of body weight.

Regulation of food intake is a complex process. Many theories, both centrally and peripherally oriented, have been proposed to explain what signals hunger or satiety. After food is consumed, normal absorption of nutrients is dependent on the integrity of digestive processes in the gastrointestinal tract. Malabsorption of nutrients, especially fat, can occur if digestion is diminished.

In addition to its primary function in decreasing growth hormone, the newly-discovered peptide somatostatin has been shown to have metabolic and central nervous system effects that may alter food intake. Somatostatin may be implicated in food intake control because of its central hypothalamic location, neurotransmitter actions or its effects on circulating metabolites and hormones that may act as peripheral hunger/satiety signals. Somatostatin may be involved in digestive events by inhibiting secretory processes of the stomach and pancreas as well as by altering

gall bladder and gastrointestinal motility.

In previous studies, chronic somatostatin treatment has been shown to produce a significant loss of body weight in growing and mature rats. Because of the potential effect of somatostatin on food intake and nutrient utilization it is not surprising to see a weight loss in somatostatin treated animals. Whether these reported weight losses are due to an effect of somatostatin on food intake or digestion and absorption of nutrients is presently unknown.

REVIEW OF LITERATURE

Regulation of Food Intake

Classical studies of the role of the central nervous system have shown that the principal neural components which integrate the system for food intake regulation are located in the hypothalamus. The ventromedial hypothalamus (VMH) is the center for control of satiety (Hetherington, 1943) and the lateral hypothalamus (LH) the center for control of hunger (Anand and Brobeck, 1951). By lesion of the VMH it is possible to produce hyperphagia and by injury to the LH, aphagia. Conversely, stimulation of the VMH inhibits feeding and the LH initiates feeding even in satiated animals.

Current neuroscience research has shifted the focus from the hypothalamus as an integrative center for hunger signals to the brain monoamines and their roles in hunger and satiety. Catecholamine pathways implicated in feeding are the dopamine nigrostriatal and the dorsal norepinephrine pathways. These central dopamine and norepinephrine neurons are required for initiation of ingestive behavior. Ungerstedt (1971), Striker and Zigmond (1976) suggest that the syndrome of aphagia and adipsia following lesions of

the lateral hypothalamus are caused by damage to the catecholamine pathways that project rostrally through this area and in particular, damage to the dopamine nigrostriatal pathway. Rowland and Antelman (1976) suggest that chronic activation of this dopaminergic system could underlie stress related hyperphagia. It has been further suggested that the activity of neurons containing the indoleamine, 5-hydroxytryptamine, is reciprocally related to the activity of the catecholamine neurons so that serotonergic neurons might be expected to be involved in the cessation of ingestive behavior (Saller and Striker, 1976). Loss or destruction of brain serotonin could then lead to hyperphagia (Breisch et al., 1976). However, this hyperphagia might be secondary to alteration in the secretion, metabolism or effectiveness of pituitary hormones. Knife cuts through areas where serotonergic neurons are expected to ascend have been reported not only to increase food intake but also to increase longitudinal growth and circulating levels of growth hormone (Palka et al., 1971; Mitchell et al., 1973). Traversing the same pathway as part of the serotonin system is the ventral bundle of noradrenergic ascending fibers. The ventral bundle carries epinephrine as well as norepinephrine neurons as it travels from the midbrain to the lateral hypothalamus (Hoebel, 1977). Hyperphagia and increased body weight have also been reported to occur following selective damage to the ventral bundle (Hoebel,

1976; Gold, 1973). This hyperphagia following adrenergic depletion is also dependent on pituitary function (Ahlskog et al., 1975). Norepinephrine and epinephrine pathways ascending from the midbrain are, therefore, postulated to serve a satiety function (Ahlskog and Hoebel, 1973).

On the contrary, Leibowitz (1976) using chemical stimulus data has theorized an alpha-adrenergic (norepinephrine or epinephrine) receptor mechanism located in the medial hypothalamus with a role in hunger stimulation, and beta-adrenergic and dopaminergic receptor mechanisms located in the lateral hypothalamus with a role in hunger suppression. This new theory can be correlated with the classical VMH/LH theory of satiety and feeding if the alpha-adrenergic system excites feeding by inhibiting part of the classical VMH satiety system and the beta-adrenergic system causes satiety by inhibiting part of the LH feeding system (Hoebel, 1977). Regardless of proposed pathways, if mechanisms in the brain centrally control food intake, they must respond to nutrient related chemical changes in the extracellular environment, neural inputs or external receptors which monitor environmental events such as temperature fluctuations of the periphery.

Metabolic factors such as glucose, fat breakdown products, amino acids or peptide hormones such as insulin, glucagon, growth hormone or cholecystokinin-pancreozymin (CCK-PZ) are postulated to be peripheral sensors (Bray,

1976). The blood levels of these substances fluctuate according to the metabolic state and alteration in their concentrations could evoke changes in feeding behavior.

The hypothesis that blood glucose controls feeding has maintained interest for two decades. Mayer (1953) postulated that the VMH might respond to changes in the rate of glucose utilization and modify food intake. This hypothesis has been supported by studies demonstrating that injection of glucose into the ventricles reduces food intake (Herberg, 1960) and injection of phloridzin, a drug which blocks glucose uptake, increases food intake (Glick and Mayer, 1968). Injections of the cytotoxin gold thioglucose (GTG) in mice selectively destroys the VMH. This lesion will cause hyperphagia and obesity. The glucose moiety is necessary for the production of the lesion which suggests that gold is accumulated by cells that can transport glucose (Debons et al., 1962). Using electrophysiological recording techniques, glucosensitive neurons have been identified in the LH as well as the VMH. These neurons are believed to be the functional units of the hypothalamic control system (Oomura et al., 1969). These neurons change their rate of firing in response to modifications in blood glucose. When glucose utilization is enhanced, neurons of the LH have a decreased discharge frequency whereas VMH neuron firing is increased above control levels (Oomura,

1976). This increase is followed by a post-excitatory depression below resting levels. The biphasic nature of the VMH neuronal response suggests that perhaps there is one type of glucoreceptor with a range of thresholds (Marrazzi, 1976). Exactly how these VMH neurons are subsequently involved in the regulation of metabolic homeostasis, including food intake, is presently unknown. In addition to central glucoreceptors, liver and intestinal receptors which produce afferent discharges on changes in blood glucose are proposed to exist (Bell, 1976).

The lipostatic hypothesis of Kennedy (1953) proposed that some metabolite of fat, plasma free fatty acids or glycerol, serves as a feedback signal for food intake regulation. Glycerol, released into the circulation in proportion to the rate of hydrolysis of triglycerides, was viewed as the most likely candidate. Glycerol then may be an indirect messenger through glucose formation in the liver or a direct messenger via possible glycerol receptors in the hypothalamus.

The rapid change in circulating concentrations of free fatty acids and their response to variations in glucose and insulin concentrations make them an unlikely feedback element. Fatty acids and glycerol are elevated in states of both deprivation and obesity; therefore it is difficult to imagine how these substrates could serve, by themselves, as reliable metabolic indices. However, hunger seems to be

correlated with fatty acid release and satiety correlated with fatty acid uptake by adipose tissue (Brobeck, 1974).

Studies of hypothalamic neurons demonstrate that electro-osmotic application of free fatty acids to the glucosensitive neurons increases the activity of the feeding center (LH) and decreases activity of the satiety center (VMH). The neuronal monitoring of increased free fatty acids causes activation of the LH and disinhibition of LH suppression by VMH and these changes may motivate eating (Oomura, 1976). Although free fatty acids and glycerol have received the most attention as possible lipostatic regulators of food intake, other possible regulatory substances coming from adipose tissue are the steroids (Hervey, 1969) or prostaglandins (Baile et al., 1973).

Alterations in blood amino acid concentration seem to decrease food intake when the protein content of the diet is very low (Peng et al., 1974) or high (Mellinkoff et al., 1956); the proportions of amino acids in the diet deviate from requirements (Rogers and Leung, 1973); or when the diet is deficient in an essential amino acid (Harper, 1976). The receptor system for detecting amino acid deficiency appears to be in the brain although not localized in the VMH (Bray, 1976).

Besides nutrient metabolites, hormonal factors play a role in modifying food intake. Although insulin is secreted continuously in basal amounts, a major stimulus

for increases from basal secretions is the presence of the products of digestion. One of the major actions of insulin is to enable the tissues of the body to utilize and/or store products of digestion. If rats are regularly injected with insulin, food intake increases and body weight rises (MacKay et al., 1940; Hoebel and Teitelbaum, 1966). This increased food intake could result from peripheral hypoglycemia. However, there is increasing evidence for a direct involvement of insulin with food intake mechanisms. Several researchers (Bagdade, 1968; Decker and Hagerup, 1967; Bernstein et al., 1975) have shown that basal insulin levels positively correlate with body weight in humans. VMH lesioned hyperphagic animals show increased insulin levels independent of overeating (Woods and Porte, 1976). In experiments in which animals are given large alimentary doses of glucose, there is a paradoxical stimulation of food intake. Prolonged and exaggerated insulin response is believed to be responsible for the increased food intake (Rezek et al., 1979). Insulin-sensitive receptors in the central nervous system have also been demonstrated. Debons et al. (1977) showed that insulin is necessary for gold thioglucose destruction of the VMH glucoreceptors. Szabo and Szabo (1972), by carotid artery insulin injections and jugular vein anti-insulin serum injections, demonstrated a decrease in systemic blood glucose. This reduction in glucose, evidence for insulin-sensitive receptors in the

brain, was later shown to be due to a direct neural effect on hepatic metabolism and not mediated by pituitary (Szabo and Szabo, 1975b) or pancreatic hormones (Szabo and Szabo, 1975a). Injection of insulin directly into the VMH followed by the same decrease in systemic blood glucose has localized these central nervous system insulin-sensitive receptors in the VMH (Storlein et al., 1975). Electroosmotic application of insulin to neurons of the VMH will decrease firing frequency and LH insulin exposure will cause increased neuronal firing; whereas if glucose is applied with the insulin, VMH neurons will increase firing (Oomura, 1976). More recently, Havrankova et al. (1978) have demonstrated insulin receptor binding throughout the nervous system. However, Goodner and Berrie (1977) showed only median eminence and not ventral medial or lateral hypothalamic tissue insulin receptor binding. These authors suggest that insulin communicates with deeper brain centers by neuronal transmission.

deCastro et al. (1978) have suggested that glucagon together with insulin are important in maintaining body weight. Manipulation of glucagon together with insulin, affecting glucose availability and utilization, will alter intake in a predictable manner according to the glucostatic mechanism of food intake. Injections of glucagon into humans (Shulman et al., 1957) and rats (Sudsaneh and Mayer, 1959) will reduce food intake. As in the case of insulin

it is not known whether this effect is strictly related to the glycemic state or also to the central nervous system.

Growth hormone has also been implicated in regulation of food intake. In humans and experimental animals, injections of growth hormone are accompanied by an increase in food intake and growth. Growth hormone is not essential for obesity to develop because rats without pituitaries will still become obese after VMH injury (Bray, 1974). However, in hypophysectomized, intact VMH animals a decrease in food intake has been observed (Kennedy and Parrott, 1958).

Gastrointestinal hormones, by acting as satiety signals, may have a role in regulating food intake. Smith et al. (1974) have suggested that cholecystokinin-pancreozymin (CCK-PZ) may be one of these hormones. In rats with open gastric fistulas, satiety did not occur if food failed to enter the small intestine. Intake of food was inhibited by injections of physiological doses of CCK-PZ or by closing the gastric cannula and allowing entry of food into the intestines. A more direct role for CCK-PZ as a neuroregulator of food intake has been suggested by findings that CCK peptides are found in the brain and appear to be localized in cortical neurons (Muller et al., 1977). Extracts of the cerebral cortex of genetically obese mice with hyperphagia contain diminished brain immunoreactive CCK compared with non-obese littermates. This finding

suggests that a lower amount of CCK in the brain may be causally related to the unrestrained appetite of these mice (Straus and Yalow, 1979). Enterogastrone, a preparation now known to be rich in CCK, has also been reported to inhibit intake in mice (Schally et al., 1967). In contrast, the gastrointestinal hormones secretin and gastrin will not induce satiety in rats (Smith et al., 1974).

Rather than signals coming from the duodenum or jejunum, Deutsch et al. (1978) believe that the stomach signals satiety. They showed that when the stomach is isolated from the duodenum by means of an inflatable cuff, compensatory feeding occurs when liquid nutrient is withdrawn from the stomach. This phenomena could be related to neural inputs to the hypothalamus as opposed to hormonal regulation of food intake. Gastric distension is known to diminish food intake and gastric fistulas which allow food to leave the stomach immediately prevent satiety. When food enters into and distends the stomach, stretch receptors in the stomach wall are activated and stimulate the vagus nerve (Paintal, 1954). This stimulation of vagal afferents from gastric distention increases neuronal activity of the VMH (Sharma et al., 1961). These findings suggest that gastric distention stimulates the vagus nerve which in turn affects the satiety center.

Another type of signal that might influence hypothalamic feeding systems is heat production as reflected in changes

of body temperature (Brobeck, 1960). Body temperature is regulated in the preoptic region of the anterior hypothalamus. Interaction within the hypothalamus between the temperature regulation and food intake system may cause an animal to overeat when exposed to the cold and undereat when exposed to heat. Animals with lesions in the anterior hypothalamus overeat in the heat and eat too little in the cold (Hamilton and Brobeck, 1966). Warming or cooling the preoptic region leads to suppression or stimulation of feeding (Andersson and Larsson, 1961).

There is also a rapid increase in heat production following food ingestion. This added heat could be utilized as a signal that feeding has occured (Brobeck, 1974). Thyroid hormones can modulate the basal metabolic rate and cause an increase in heat production by accelerated catabolism of fats, proteins and carbohydrates (Bray and Campfield, 1975). In response to a thyroxine induced increase in thermogenesis, lean mice will adjust their food intake so that increased heat production will not result in body weight loss whereas obese mice cannot adjust and lose weight (Vander tuig et al., 1979).

From the previous discussion it is apparent that regulation of food intake is a complex physiological process involving the recognition and integration of many different types of signals. Much effort has been devoted to identifying signals and receptors that initiate hunger and satiety

and hypothalamic integrative mechanisms for these signals. No single physiological mechanism can fully explain appetite or satiety. Food intake is suppressed when glucose supply is abundant, fat stores become large, protein intake is imbalanced, insulin or growth hormone levels are low or glucagon and cholecystokinin are high. A variety of chemical signals exist with one common result on food intake. In addition to or perhaps interrelated with these signals are more generalized factors influencing food intake - gastric distention or environmental temperature and body heat content. Although each mechanism is proposed to exist, how they all interact to exert a common control is not fully understood.

Malabsorption

After food intake has been initiated in response to the signals discussed previously, the ingestion of foodstuffs does not necessarily ensure a sufficient supply of energy to maintain body weight. Nutrients from the food must be delivered by processes of digestion and absorption to the cells where they can be utilized as energy substrates. The gastrointestinal tract serves to digest and absorb foods and nutrients supplied to it exogenously by the diet as well as endogenously from luminal secretions and sloughed cells. Failure to absorb ingested nutrients or to reabsorb endogenous biologically useful substrates results in malabsorption

(Holt, 1977).

The cardinal feature of malabsorption is steatorrhea followed by weight loss (Borgstrom, 1969). In humans ingesting a typical diet supplying 50-100 g fat per day, steatorrhea is defined as an excretion of greater than 5 g of fat as fatty acids in the feces per day (Frazer, 1969). This quantity signifies fecal fat in excess of 10-20% of ingested dietary fat. Excessive fat excretion is believed to be the most sensitive index of intestinal tract malfunction.

The most important cause of faulty fat digestion and absorption can be ascribed to lack of pancreatic enzymes or effective bile salts activity. Disturbances in various aspects of motility including contraction, segmentation, mixing and rate of transit previously have been considered to be important in the etiology of steatorrhea. However, the effects of alterations in motility on intestinal absorption are little understood. Increases in the forward propulsion of intestinal contents and possibly decreased time for absorption can be compensated for by increased mixing of the food with digestive secretions and exposure to absorptive surfaces. Absorption could possibly increase, decrease or remain the same (Losowsky et al., 1974).

Pancreatic digestive enzymes play an essential role in normal gastrointestinal functioning. Deficiency of pancreatic enzymes may be brought about by atrophy of the

pancreatic alveolar cells with consequent lack of production of the enzymes, by obstruction of the pancreatic ducts or by ineffective stimulation for release of the enzymes into the lumen of the intestine. The gastrointestinal hormones, cholecystokinin-pancreozymin and secretin are major stimuli to the pancreas for release of enzymes, bicarbonate and water. If these hormones are deficient, the pancreatic digestive enzymes, amylase, lipase and a variety of proteases may be insufficient for complete digestion of the energy nutrients. Except in cases of very severe pancreatic insufficiency, a significant malabsorption of carbohydrates is not a severe problem. Pancreatic secretions normally contain excess amylase and in addition, a large portion of carbohydrates are mainly dependent on intestinal brush border enzymes. As in the case of carbohydrates, malabsorption of nitrogen often fails to develop in spite of diminished secretion of pancreatic enzymes. According to Crane (1969), increased proteolytic activity by pepsin may compensate for lack of pancreatic secretions. The decrease in bicarbonate leads to a lowered upper intestinal pH and subsequent continuation of peptic activity beyond the stomach. In contrast to carbohydrate and protein digestive enzymes, human and rat pancreatic juice contain three distinct lipolytic enzymes. Therefore, a pancreatic enzyme deficiency is most effective in decreasing fat digestion and absorption.

Bile salts released from the gall bladder by CCK-PZ stimulation also play an important part in the digestion and absorption of fats. If a bile deficiency exists or bile is completely lacking, fat absorption declines but does not disappear completely. In rats with external biliary fistulas, fat digestion was still 40-70% of normal levels (Krondl et al., 1971). However, in rats deficient in both pancreatic juice and bile, the fat level after absorption in the lymph was 8 mg whereas in normal rats on the same diet the level was 120 mg of fat (Wiseman, 1964).

These results would seem to emphasize the importance of pancreatic lipase for normal fat digestion. The entire digestive and absorptive processes follow a cascade of events: food signals the gastrointestinal hormones, these hormones then signal the pancreas and the pancreas then releases digestive secretions. A block at any point in this chain of events could compromise the ability to handle nutrients and malnutrition and weight loss could result.

Somatostatin in Relation to Food Intake and Malabsorption

Somatostatin, or somatotrophin release inhibiting factor (SRIF), is a small peptide composed of fourteen amino acids. It is produced in the lateral hypothalamus which, as discussed previously, is considered the feeding center in the hypothalamic theory of food intake regulation. Somatostatin is carried through neurons to the median

eminence of the hypothalamus where it is stored. From the nerve endings, it is delivered into the hypopheseal portal system and subsequently carried to the anterior pituitary where it exerts one of its functions, inhibition of growth hormone (GH) or thyroid stimulating hormone (TSH) (Brazeau et al., 1974; Guillemin and Gerich, 1976; Hansen and Lundbaek, 1976).

In addition to being considered a hypothalamic hormone, a neuromodulator or neurotransmitter role for somatostatin has been suggested. Because of its presence in numerous neurons, its release by depolarizing stimuli (Lee et al., 1978), its direct effects on central and peripheral nerves, and its behavioral effects in animals, somatostatin may have a significant role in the regulation of nervous system functions, possibly including neural regulation of food intake.

By light and electron microscope studies and immunofluorescent techniques, somatostatin has been demonstrated in many areas of the nervous system: nerve cell bodies in the thalamus; neo and limbic cortical areas, the area of the brain in charge of emotions; the subcommissural organ; and the periventricular region in the anterior hypothalamus (Hokfelt et al., 1975; Pelletier, 1976). Somatostatin is also located in secretory granules of neuronal fibers localized in the arcuate, suprachiasmatic, ventromedial and ventral premammillary nuclei, organum vasculosum of the

lamina terminalis, external zone of the median eminence, spinal ganglia and dorsal horns of the spinal cord, the lamina propria of the gut and Auerbach's plexus (Luft et al., 1978). In the same SRIF positive neurons of the spinal ganglia, the presence of dopamine-β-hydroxylase, the enzyme catalyzing the conversion of dopamine to norepinephrine, has been demonstrated (Elde et al., 1978). This finding suggests the presence of somatostatin and a known neurotransmitter, norepinephrine, in a single neuron.

Somatostatin also exerts a direct depressant activity on central and peripheral neurons. It has been shown to depress spike discharge frequency and amplitude when applied microiontophoretically to neurons of the cortex, brain stem and hypothalamus (Renaud et al., 1975). Given intravenously to rats, somatostatin increased pentobarbital sedation and the lethal dose for strychnine (Kastin et al., 1978). In addition somatostatin will inhibit the electrically induced release of acetylcholine in guinea pig myenteric plexus (Guillemin, 1976). In contrast to these central depressant effects already referred to, evidence for stimulatory activities of somatostatin is provided by behavioral observations. Somatostatin will potentiate the motor effects of L-dopa (Kastin et al., 1978). After direct cerebral administration to rats, it will reduce REM sleep, cause paraplegia in extension, and produce circular running evolving into catatonia (Rezeck et al., 1976; Havlicek et al., 1976).

Opposing effects of somatostatin on the nervous system - depression or stimulation - are believed to be dose related. Another hypothalamic peptide, melanocyte stimulating hormone release inhibiting factor, has also been shown to have biphasic dose-related effects (Kastin et al., 1978).

In addition to its presence in the nervous system, somatostatin has been found in similar concentrations in a discrete population of cells, the D cells (Orci et al., 1975). These cells are located in the pancreatic islets and in the mucosa of the gastrointestinal tract, mainly in the pylorus and fundus of the stomach and in smaller amounts in the duodenum, jejunum and ileum (Arimura et al., 1975). Somatostatin is also located in a small number of parafollicular cells in the thyroid (Hokfelt et al., 1975).

Insulin and glucagon, also produced by pancreatic islet cells, are important hormones for nutrient utilization and may be involved in food intake regulation. In humans, fasting insulin and glucagon plasma levels as well as insulin and glucagon responses to various stimuli are diminished by somatostatin infusion (Christensen et al., 1974; Efendic and Lins, 1978). Virtually all known stimuli of insulin secretion are blocked by somatostatin - glucose, isoproterenol, tolbutamide, glucagon, arginine and secretin (Gerich, 1976). Similarly, glucagon responses to meals, intravenous arginine, insulin induced hypoglycemia, epinephrine and insulin deprivation are suppressed by somatostatin

(Guillemin and Gerich, 1976). Since the inhibition of insulin and glucagon produced by somatostatin can be reduced by phentolamine, an alpha-adrenergic blocker, it is believed that somatostatin inhibits both these hormones by interaction with alpha-adrenergic pathways (Taborsky et al., 1978). Despite concomitant lowering of both plasma insulin and glucagon levels, short term infusion of somatostatin will induce hypoglycemia in rats due to indirect suppression of glycogenolysis and gluconeogenesis (Byrne et al., 1977). Prolonged somatostatin infusion, however, has been reported to cause hyperglycemia due to decreased glucose utilization (Lins and Efendic, 1976). Somatostatin will also cause a decrease in plasma free fatty acids in normal (Byrne et al., 1977) or diabetic rats (Micossi et al., 1976). This effect results from either increased tissue utilization of these lipid moieties (Byrne et al., 1977) or, more likely, decreased lipolysis due to glucagon inhibition (Micossi et al., 1976).

The presence of somatostatin in the D cells, which are situated in the pancreas between the glucagon secreting alpha cells and the central mass of insulin secreting beta cells, raises the possibility that it may function as a local regulator of insulin and glucagon release (Unger, 1977). The islet cells would receive information from one another via the interstitial spaces and exert a paracrine influence in which insulin inhibits glucagon and may

decrease somatostatin, glucagon stimulates insulin and somatostatin and somatostatin inhibits insulin and glucagon (Unger et al., 1978).

In addition to these actions within the islets, there is evidence that somatostatin may function in nutrient homeostasis. By inhibition of various digestive events in response to signals from enteric hormones and rising nutrient concentrations, somatostatin may restrain nutrient entry from the gut. With somatostatin functioning in this capacity, the pancreas would have control over exogenous nutrient flux as well as control, by means of insulin and glucagon, over endogenous nutrient flux (Unger et al., 1978).

Somatostatin has been shown to cause a circulation-dependent delay in carbohydrate absorption. During somatostatin infusion, Wahren and Felig observed a 30% reduction in splanchnic blood flow coupled with reduced reactive hyperglycemia in diabetic patients after oral but not intravenous glucose tolerance tests (Wahren and Felig, 1976). Somatostatin infusion will also inhibit galactose absorption in humans and rats. The decrease in blood galactose is proposed to result from delayed gastric emptying, diminished gastrointestinal blood flow or direct inhibition of galactose uptake by somatostatin (Wagner et al., 1978). In rhesus monkeys, it was shown that somatostatin inhibited triglyceride absorption after ingestion of

a mixed meal (Koerker et al., 1978). Sakurai et al. (1975) also reported a failure of blood triglycerides to rise during a fat load in somatostatin treated dogs.

Somatostatin has been shown to suppress various gastrointestinal hormones: secretin, cholecystokinin-pancreozymin (CCK-PZ), gut glucagon, vasoactive intestinal peptide (VIP), gastric inhibitory polypeptide (GIP), gastrin, and motilin. Many of the digestive functions signaled by these hormones are consequently reduced. Alcohol or HCl-induced release of secretin and olive oil induced secretion of CCK-PZ from the duodenal mucosa in humans was inhibited by somatostatin (Raptis et al., 1978). Basal, non-stimulated pancreatic exocrine secretion (Boden et al., 1975) and release of pancreatic fluid and bicarbonate in response to CCK-PZ and secretin in dogs (Boden et al., 1975; Konturek et al., 1976b) and man (Dollinger et al., 1976; Konturek, 1976) were inhibited by somatostatin. Chariot et al. (1978) also showed that infusions of somatostatin resulted in a decrease in basal flow and vagal and acetylcholine stimulated bicarbonate and protein secretion from the rat pancreas. In a study by Folsch et al. (1978), somatostatin inhibited basal and CCK-PZ stimulated enzyme and volume secretion in the rat. However, it did not influence secretin stimulated bicarbonate concentration or rate of secretion at the dose used for CCK-PZ-stimulated enzyme inhibition. It has been suggested that somatostatin

infusion inhibits pancreatic secretion by a decrease of acetylcholine release at nerve endings and a direct inhibition at pancreatic effector cells (Chariot et al., 1978). In addition to decreasing CCK-PZ and pancreatic exocrine secretions, somatostatin reduces gall bladder emptying elicited by exogenous CCK-PZ or intraluminal digestive products. This inhibition could lead to bile stasis (Holtermuller et al., 1977).

Gut glucagon, or glucagon-like-immunoreactivity (GLI), released during glucose, long chain triglycerides or amino acid absorption has been demonstrated to be inhibited by somatostatin infusions (Sakurai et al., 1975). Somatostatin will suppress both VIP production from vipomas, with a reduction in small intestinal juice production (Lennon et al., 1975), and endogenous GIP release with a reduction in insulinotropic action (Pederson et al., 1975).

Somatostatin has also been shown to reduce the endocrine and exocrine activity of the stomach. In humans, it inhibits gastrin release (Konturek, 1976; Konturek et al., 1976a; Raptis et al., 1975; Vatn et al., 1977) both after a test meal and during insulin hypoglycemia (Barros D'Sa et al., 1978). In cats, vagally-induced gastrin release is suppressed by somatostatin (Uvnas-Wallensten et al., 1977). Somatostatin has a direct effect on the parietal and peptic cells to suppress the secretion of gastric acid and pepsin in cats (Albinus et al., 1976; Gomez-Pan et al.,

1975), dogs (Barros D'Sa et al., 1975; Hummelt et al., 1977) and humans (Raptis et al., 1975; Vatn et al., 1977). The inhibitory potency of somatostatin is most effective on gastric acid secretion induced by pentagastrin, urecholin or a peptone meal and less pronounced on histamine stimulated secretion (Creutzfeldt and Arnold, 1978). Vagal integrity is not mandatory for inhibition by somatostatin. Reduction of acid and pepsin was observed in vagally innervated and denervated stomach pouches (Konturek et al., 1976c). The gastric blood flow also does not change during somatostatin infusion (Konturek et al., 1976a). The mechanism for this somatostatin induced inhibition of gastric secretion has not been fully elucidated.

By interfering with normal gastroduodenal motility and serum motilin levels, somatostatin will decrease gastric emptying. Bloom et al. (1975) described retardation of gastric emptying and motilin levels during somatostatin infusion in humans. In dogs, it was found that somatostatin has a distinct motor effect on the duodenum but not on gastric antral contractions. The differential effect could diminish the antral-duodenal pressure gradient and interfere with gastric motility (Boden et al., 1976). Tansy et al. (1978) also showed the anatomical dependence of the motor effects of somatostatin. Intravenous somatostatin depressed the stomach tonus and contractile activity and increased the excitation of the small intestine segmental

motor activity. It was suggested that relaxation of the stomach could be the result of either endogenous catecholamine release, direct action of somatostatin on adrenergic receptors, or inhibition of acetylcholine release in the myenteric plexus. Since somatostatin-induced small intestine contractile events were antagonized by atropine, it was believed that these events were mediated by effects of somatostatin on acetylcholine-like receptors (Tansy et al., 1978).

In summary, from the brain to the pancreas, somatostatin has ubiquitous domains with numerous and varied effects. Many of the actions of somatostatin are related to the biological processes of food intake, digestion and absorption. Impairment of any of these processes could lead to a decreased energy supply and weight loss.

In two previous studies, a decrease in body weight was reported in rats treated with somatostatin. Brazeau and coworkers (1974) demonstrated a decreased rate of body weight gain ($P<0.01$) in young (125 g) male rats receiving three daily subcutaneous injections of somatostatin for eight days. Micossi and collaborators (1976) reported a significant ($P<0.05$) weight loss in normal and diabetic adult (470-570 g) rats treated with somatostatin for two weeks. Both of these studies, however, focused on other results of chronic somatostatin treatment such as growth hormone inhibition or decrease in serum glucose and lipid

levels. Weight loss was merely observed as a secondary result. The present experiment was designed to determine whether these reported weight losses were due to an effect of somatostatin on food intake, digestion and absorption of ingested nutrients, or a combination of these parameters.

MATERIALS AND METHODS

Animals and Treatments

Male Sprague-Dawley rats weighing approximately 250 g were randomly divided into three groups of eight each and individually housed in metabolic cages in a temperature controlled room (22°C).

Linear somatostatin[1] to be used for treatment was attached to protamine zinc according to the method of Brazeau et al. (1974) to increase its biological half life from two to four minutes to six hours.

In the first sixteen day treatment period, rats were injected subcutaneously twice daily at 9:00 a.m. and 3:00 p.m. with equal volumes of the following: Group 1, saline; Group 2, protamine zinc; Group 3, 100 μg/kg body weight protamine zinc somatostatin (Table 1). Both saline and protamine zinc were used as controls to negate the possibility of protamine zinc affecting the parameters studied.

After sixteen days of Treatment period I it appeared somatostatin had exerted no effects at the dosage level used. In the previous studies of Brazeau et al. (1974) and Micossi et al. (1976), effects on body weight had been

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[1]Biodata, Rome, Italy; Ayerst Laboratories, Montreal, Canada (Treatment II, Group I rats only).

Table 1. Design of experimental treatments[1]

Group	Treatment period[2]	
	I	II
1	saline	PZ-SRIF (100 µg/kg)
2	PZ	PZ
3	PZ-SRIF (100 µg/kg)	PZ-SRIF (200 µg/kg)

[1]Treatments of saline, protamine zinc (PZ) and protamine zinc somatostatin (PZ-SRIF) were administered as two daily subcutaneous injections.

[2]Treatment period I lasted sixteen days; treatment period II lasted fourteen days for groups 1 and 2 and seven days for group 3.

observed after seven and fourteen days, respectively, of protamine zinc somatostatin therapy. In Treatment period II, therefore, animals from Group 1 were begun on protamine zinc somatostatin injections and animals of Group 2 were maintained as a protamine zinc control for fourteen additional days of treatment. Animals of Group 3 were continued on protamine zinc somatostatin injections at an increased dosage of 200 µg/kg body weight for seven days.

Diet

All rats were fed a high fat, semi-purified diet (Table 2) and allowed water ad libitum. Rats were given a nine day acclimation period to the diet. Throughout the study, light/dark cycles were adjusted to match treatment with feeding time. Food cups were presented after the 9:00 a.m. injection and animals left undisturbed in a darkened room. At 4:00 p.m. food was taken away, weighed and daily consumption recorded. Animal body weights were measured and recorded every third day.

Fecal Lipids

A three-day accumulation of feces was collected for each rat throughout both treatment periods and maintained as a separate specimen for analysis. Following the experimental periods, these fecal collections were dried to a constant weight in a 60^{o}C oven and then finely ground with a mortar and pestle. A 0.5 g aliquot of each specimen was

Table 2. Composition of diet

Ingredient	Percentage
Sodium caseinate	20.0
DL-methionine	0.3
Cornstarch	37.1
Sucrose	10.0
Corn oil/lard (1:1)	25.0
Vitamin mix[1]	0.4
Mineral mix[2]	4.0
Choline chloride	0.2
Cellulose[3]	3.0

[1]Composed of: (in mg/kg diet): thiamin HCl, 22; pyridoxine, 22; riboflavin, 22; Ca pantothenate, 66; P-amino benzoic acid, 110; menadione, 50; inositol, 100; ascorbic acid, 200; niacin, 100; vitamin B_{12}, 0.3; biotin, 0.6; folic acid, 4; (in IU/kg diet) vitamin A acetate, 20,000; alpha tocopherol acetate, 100; vitamin D_3, 2,200 IU and cerelose to 4.0 g.

[2]Salt mixture, Draper 4164, United States Biochemical, Cleveland, Ohio 44128 (Draper et al., 1964).

[3]Avicel Food Prototype 174-2, FMC Corporation, Marcus Hook, Pennsylvania 19061.

acidified with concentrated hydrochloric acid, extracted with petroleum ether and analyzed by a gravimetric method for total lipid content (Henry et al., 1974).

Xylose Tolerance Test

Xylose tolerance tests are commonly used clinically as tests for intestinal malabsorption. Since xylose is a non-metabolizable pentose, a high percentage recovered in the urine after a test dose is usually indicative of normal intestinal absorption. To test for normal intestinal absorption in experimental animals a xylose tolerance test was administered to all animals. Prior to using this test in experimental rats, a dose of 0.5 g/kg body weight xylose, the amount usually used clinically for children, was tried on normal, untreated rats. No vomiting was observed and approximately 50% was routinely recovered in a twenty-four urine specimen. On the basis of these results, at the end of treatment period II animals were fasted overnight and a 0.5 g/kg body weight dose of a 15% solution of xylose[1] was delivered into the stomach by means of a stainless steel cannula. Rats were allowed only water throughout the test period, and a 24-hour urine specimen was collected, volume measured and frozen for analysis the following week. Injections continued as scheduled during the xylose test period and

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[1]Pfanstiehl Laboratories, Waukegan, Illinois.

for two subsequent days.

Xylose concentration is the urine samples was analyzed by the spectrophotometric method of Roe and Rice (1948). This method is based on the principle that xylose in the presence of heat and acid will form furfurals which react with the reagent p-bromoaniline to form a pink color.

Blood Sampling

Prior to Treatment period I each rat was fasted overnight, anesthetized with ether and a blood sample was collected from the intra-orbital sinus by means of a heparinized capillary pipet. At the end of Treatment period II, on the morning of the third day following the xylose tolerance tests and after an overnight fast, the animals were injected according to treatment group, anesthetized with ether and a post-treatment blood sample was collected. After both pre- and post-treatment collections, blood was placed in a chilled tube containing proteolytic enzyme inhibitor[1] allowed to clot and then centrifuged. The serum samples were frozen for later determinations of glucose, insulin and glucagon. Animals from Groups 1 and 2 were then killed by ether inhalation.

Islet Isolation and Incubation

After collection of post-treatment blood samples, the pancreas was removed from one half of the rats in Group 3

[1]Trasylol; FBA Pharmaceuticals, Inc., New York, New York.

and the islet cells were isolated by the collagenase digestion technique of Lacy and Kostianovsky (1967). Duplicate samples of five islets, selected for uniform size with the aid of a dissecting microscope, were incubated in 2 ml of Krebs bicarbonate buffer containing 0.2% bovine serum plus either glucose (50 or 300 mg/100 ml) or glucose (50 or 300 mg/100 ml) and arginine (200 mg/100 ml). The islets were incubated for 2 hours in a Dubnoff metabolic shaker at 37°C in an atmosphere of 95% oxygen and 5% carbon dioxide. Following incubation, the islets were removed from the media by centrifugation (500 r.p.m. for 2 minutes at 4°C) and the buffer frozen for later hormone analysis.

Triplicate samples of five islets were also homogenized in 3 ml of Krebs buffer with added bovine serum albumin (0.2%). This homogenate was also stored for later glucagon and insulin analyses.

After a 24 hour period following Treatment period II during which no somatostatin was given, the remaining half of the rats of Group 3 were anesthetized, their pancreas removed and then these animals were killed. The islet cells were removed, incubated, and homogenized using the same procedures as described previously. Pancreatic islets from normal untreated rats weighing approximately 250-350 g were isolated, homogenized and incubated for purposes of comparison with islets of PZ-SRIF treated rats.

Assays

Serum samples were analyzed for glucose content on a YSI Model 23A Glucose Analyzer[1] using the glucose oxidase method. Insulin was determined according to the radioimmunoassay of Hales and Randle (1963). Serum glucagon concentration was determined using the method of Foa et al. (1977) except that a dextran coated slurry was used to separate free from bound hormone. Pancreatic glucagon was determined using AGS (antiglucagon sera) 18, an antibody which binds specifically to pancreatic glucagon. AGS 10, which binds to pancreatic as well as intestinal glucagon-like materials, was used to determine total immunoreactive glucagon.

Islet incubation media and homogenized islets were measured for glucagon content as described above but only AGS 18 was used. Insulin was assayed by the method of Malaisse et al. (1967).

Statistical Analyses

All calculations were performed on a Hewlett-Packard Model 65 programmable calculator. Analysis of variance, Dunnett's t and Student's t (Gill, 1978) were used to detect mean differences ($P<0.05$) in body weights, food intake, fecal lipids and xylose absorption among treatment groups. Pre- and post-treatment serum levels of glucose, insulin and glucagon were analyzed by paired t tests.

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[1] Yellow Springs Instrument Co., Inc., Yellow Springs, Ohio 45387.

Post treatment serum levels of gut glucagon for somatostatin-treated animals were compared to levels for protamine zinc treated animals using Student's t test. Differences in insulin and glucagon content of islets and islet response to stimuli for somatostatin treated versus normal animals were analyzed by analysis of variance and Dunnett's t test.

RESULTS

Body Weight, Food Intake and Fecal Lipids

Rats treated with saline, protamine zinc (PZ) or protamine zinc somatostatin (PZ-SRIF) showed no significant differences in body weight gain, food intake or fecal lipids after sixteen days of treatment (Table 3; refer also to Appendix Tables A1 and A2 for additional data).

In Treatment period II, after fourteen days of PZ-SRIF or PZ injections, rats showed a slight but statistically non-significant decrease in body weight after PZ-SRIF treatment. There were no significant changes in food intake or fecal lipids (Table 4; Appendix Tables A1 and A2).

After an additional seven days in which they received an increased dose of PZ-SRIF (200 μg/kg body weight) Treatment II, Group 3 rats showed a significant decrease ($P<0.05$) in body weight gain. This was accompanied by a significant decrease ($P<0.05$) in food intake but no changes in fecal lipids when compared to controls (Table 5; Appendix Tables A1 and A2).

Table 3. Body weight gain, food intake and fecal lipids, treatment period I[1,2]

Group	Treatment	Body weight gain, g	Food intake g/100 g body weight/day		Fecal lipids g/day/100 g food intake	
			Initial[3]	Final[4]	Initial	Final
1	saline	82.5 ± 8.7	5.6 ± 0.4	4.6 ± 0.2	0.56 ± 0.08	0.69 ± 0.07
2	PZ	83.3 ± 8.7	5.1 ± 0.4	4.4 ± 0.2	0.69 ± 0.08	0.89 ± 0.07
3	PZ-SRIF (100 µg/kg)	86.4 ± 8.1	5.9 ± 0.4	4.1 ± 0.2	0.67 ± 0.07	0.78 ± 0.07

[1]Means ± SEM, where each mean represents values for seven rats in groups 1 and 2 and eight rats in group 3.

[2]Treatment period I lasted sixteen days.

[3]A three day average of values prior to the beginning of the treatment period.

[4]A three day average of values at the end of the treatment period.

Table 4. Body weight gain, food intake and fecal lipids, treatment period II, groups 1 and 2[1,2]

Group	Treatment	Body weight gain, g	Food intake g/100 g body weight/day		Fecal lipids g/day/100 g food intake	
			Initial[3]	Final[4]	Initial	Final
1	PZ-SRIF (100 µg/kg)	44.8 ± 5.1	4.6 ± 0.2	4.3 ± 0.2	0.69 ± 0.07	0.66 ± 0.11
2	PZ	56.6 ± 5.0	4.6 ± 0.2	4.3 ± 0.1	0.89 ± 0.11	0.78 ± 0.10

[1]Means ± SEM where each mean represents values for seven rats in group 1 and eight rats in group 2.

[2]Treatment period II lasted fourteen days.

[3]A three day average of values prior to the beginning of the treatment period.

[4]A three day average of values at the end of the treatment period.

Table 5. Body weight gain, food intake and fecal lipids, treatment period II, groups 2 and 3[1,2]

Group	Treatment	Body weight gain, g	Food intake g/100 g body weight/day		Fecal lipids g/day/100 g food intake	
			Initial[3]	Final[4]	Initial	Final
2	PZ	31.9 ± 3.0	4.6 ± 0.2	4.3 ± 0.2	0.88 ± 0.07	1.15 ± 0.09
3	PZ-SRIF (200 μg/kg)	20.6 ± 3.0[5]	4.1 ± 0.2	3.6 ± 0.2[5]	0.78 ± 0.07	1.21 ± 0.09

[1]Means ± SEM where each mean represents values for eight rats.

[2]Treatment period II lasted seven days for group 3 and fourteen days for group 2. The first seven days of Treatment period II for group 2 were analyzed for the above comparisons.

[3]A three day average of values prior to beginning treatment period II.

[4]A three day average of values at the end of treatment period II.

[5]Significantly lower ($P<0.05$) than PZ treated rats.

Xylose Tolerance Test

There was no significant difference in the percent of ingested xylose excreted in the collected 24-hour urine specimen for any animals (Table 6). The mean urine volume excreted for the 24-hour period was 14.8 ± 1.0 ml.

Serum Glucose, Insulin and Glucagon

The mean fasting serum values for glucose, immunoreactive insulin (IRI), immunoreactive pancreatic or alpha cell glucagon (IRG_a) and total glucagon (IRG_t) are listed in Table 7. All pre-treatment values were within normal limits. Serum glucose, insulin and pancreatic glucagon showed no change for PZ control and PZ-SRIF treated animals. Animals of Group 3, treated with 100 µg/kg PZ-SRIF for fourteen days followed by 200 µg/kg PZ-SRIF for an additional seven days showed a significant decrease ($P<0.05$) in immunoreactive total glucagon using paired t analysis. The animals of Group 1 treated with 100 µg/kg PZ-SRIF for fourteen days also show a decrease from pre-treatment total glucagon levels. These values were not significantly different using paired t analysis due to the large standard error in initial pre-treatment levels. Because it is not known if the effects of somatostatin are dose related, values for all animals treated with PZ-SRIF (Groups 1 and 3) were combined for statistical analyses. Using the paired t test, post treatment total glucagon concentration was

Table 6. Twenty-four hour urinary excretion of xylose following treatment period II[1,2]

Group	Treatment	Percent excretion[3]
1	PZ-SRIF (100 µg/kg)	55.6 ± 3.2
2	PZ	56.7 ± 3.0
3	PZ-SRIF (200 µg/kg)	52.5 ± 3.0

[1] Xylose administered as a 0.5 g/kg body weight dose.

[2] Treatment period II lasted fourteen days for groups 1 and 2 and seven days for group 3.

[3] Means ± SEM where each mean represents values for seven rats in group 1 and eight rats for groups 2 and 3.

Table 7. Serum glucose, immunoreactive insulin (IRI) and pancreatic (IRG_a) and total (IRG_t) immunoreactive glucagon values for protamine zinc (PZ) and protamine zinc somatostatin (PZ-SRIF) treated rats[1]

Group[2]	Treatment	Glucose mg/dl		IRI μU/ml		IRG_a pg/ml		IRG_t pg/ml	
		initial[3]	final[4]	initial	final	initial	final	initial	final
1	PZ-SRIF (100 μg/kg)	115 ± 6	104 ± 2	47 ± 5	38 ± 3	145 ± 24	125 ± 24	546 ± 79	439 ± 17
2	PZ	113 ± 6	113 ± 5	49 ± 4	38 ± 2	154 ± 28	134 ± 11	549 ± 22	526 ± 39
3	PZ-SRIF (200 μg/kg)	108 ± 3	114 ± 4	42 ± 3	39 ± 1	136 ± 13	163 ± 20	568 ± 54	456[6] ± 22
1 + 3[5]	PZ-SRIF	111 ± 3	110 ± 3	44 ± 3	39 ± 2	140 ± 13	146 ± 13	558 ± 45	448[7] ± 14

[1]Means ± SEM where each mean represents values for seven rats in groups 1 and 2 and eight rats in group 3.

[2]Treatment period II groupings.

[3]Blood samples taken prior to initiation of treatment period I.

[4]Blood samples taken after treatment period II.

[5]Values for groups treated with protamine zinc somatostatin combined and averaged for statistical analysis.

[6]Significantly lower ($P<0.05$) than initial value using paired t analysis.

[7]Significantly lower ($P<0.025$) than initial value using paired t analysis and significantly lower ($P<0.025$) than final value for PZ treated (Group 2) animals using Student's t test.

significantly lower ($P<0.025$) than pre-treatment levels. This post-treatment value was also significantly lower ($P<0.025$) than post-treatment levels for PZ treated rats using Student's t test.

Islet Studies

Chronic protamine zinc somatostatin treatment significantly diminished ($P<0.01$) the insulin content of isolated pancreatic islets to less than half of normal levels (Figure 1; individual values given in Appendix Table A3). Twenty-four hours after somatostatin treatment, islet insulin content increased slightly but still was significantly lower ($P<0.05$) than normal amounts.

Insulin secretion by isolated pancreatic islets (Figure 2; Appendix Table A3) was also suppressed by chronic somatostatin therapy. In response to a non-stimulatory concentration of glucose (50 mg/100 ml), islets of somatostatin treated rats showed decreased response when compared to islets of untreated animals ($P<0.01$). This same dose of glucose plus added arginine (200 mg/100 ml) served as a stimulation for insulin release in normal islets. However in PZ-SRIF treated islets there was little response ($P<0.01$). Twenty-four hours after PZ-SRIF treatment there was still negligible insulin secretion by the treated islets when compared to controls ($P<0.01$). In response to an insulin stimulatory concentration of glucose (300 mg/100 ml) either

Figure 1. Immunoreactive insulin (IRI) content of pancreatic islets isolated from rats treated with 100 µg/kg protamine zinc somatostatin (PZ-SRIF) for sixteen days followed by 200 µg/kg PZ-SRIF for seven days. PZ-SRIF indicates islets homogenized immediately after treatment; 24 hrs. post PZ-SRIF indicates islets homogenized 24 hours after last treatment. Values represent mean ± SEM. Asterisks denote significant difference from normal values: *$P<0.05$, **$P<0.01$.

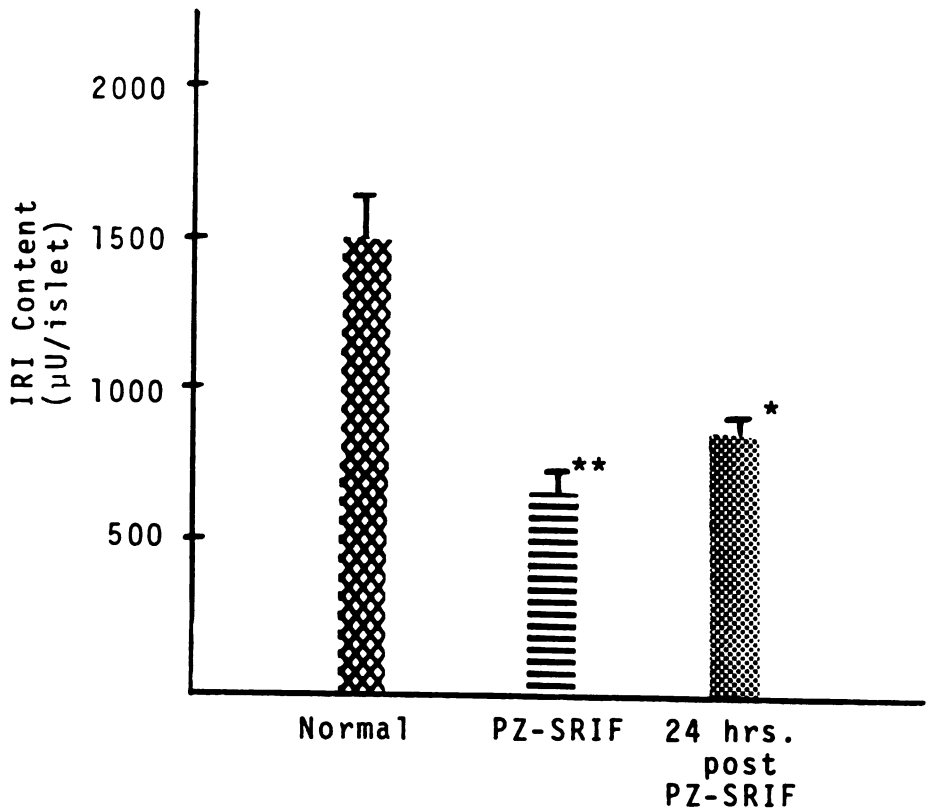
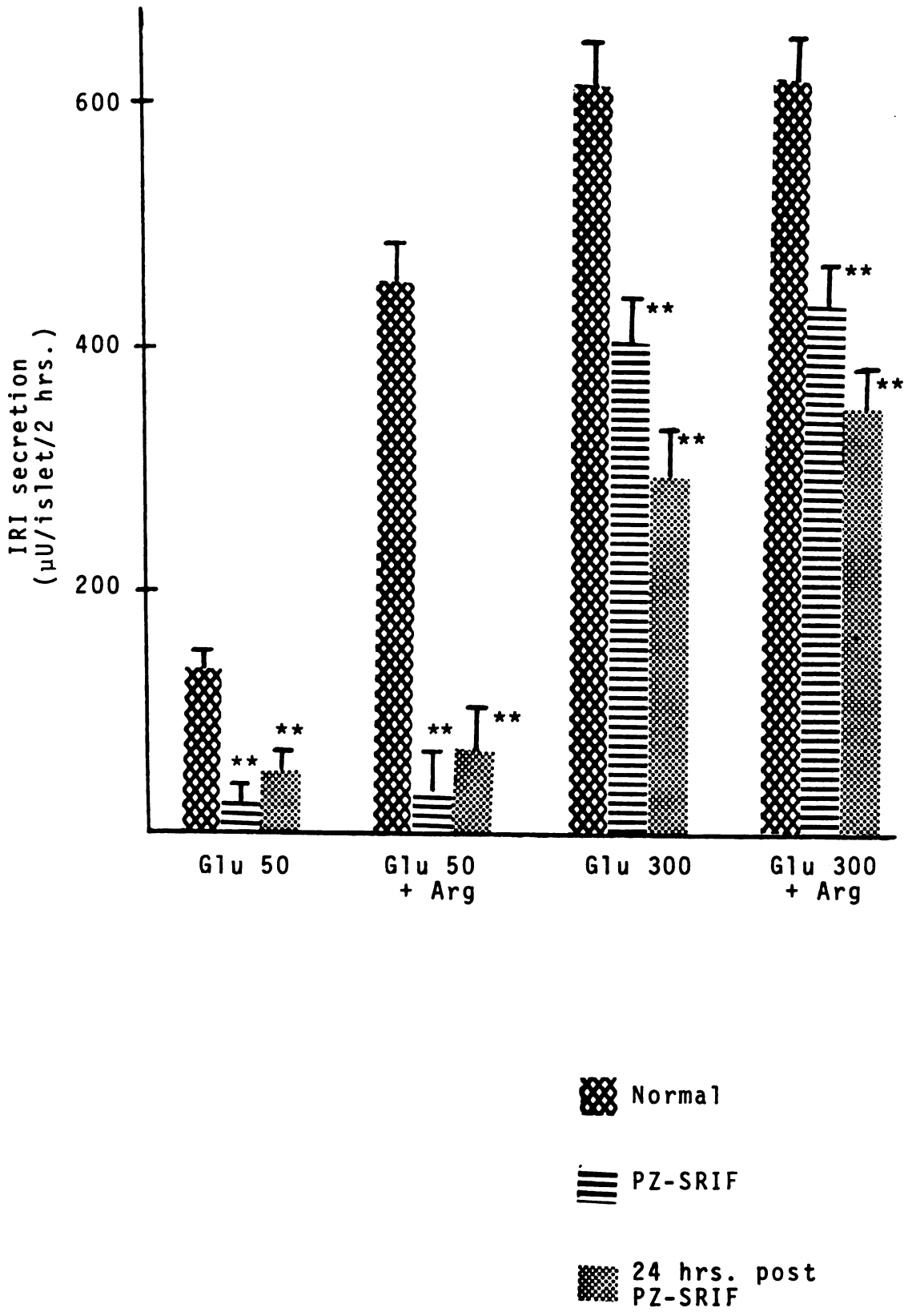

IRI Content
(μU/islet)
2000
1500
1000
500
**
*
Normal
PZ-SRIF
24 hrs.
post
PZ-SRIF

Figure 2. Immunoreactive insulin (IRI) secretion by pancreatic islets isolated from rats treated with 100 µg/kg protamine zinc somatostatin (PZ-SRIF) for sixteen days followed by 200 µg/kg PZ-SRIF for seven days. Islet cells were incubated for 2 hours in
1) low glucose, 50 mg/100 ml (50 Glu);
2) low glucose plus 200 mg/100 ml arginine (Glu 50 + Arg);
3) high glucose, 300 mg/ml (300 Glu); and
4) high glucose plus 200 mg/100 ml arginine, (Glu 300 + Arg).
Values represent mean ± SEM. Asterisks denote significant difference from normal values: **$P<0.01$.


IRI secretion
(μU/islet/2 hrs.)
600
400
200
**
**
**
**
**
**
**
**
Glu 50
Glu 50
+ Arg
Glu 300
Glu 300
+ Arg
Normal
PZ-SRIF
24 hrs. post
PZ-SRIF

alone or in combination with added arginine (200 mg/100 ml), untreated islets showed increased response and maximal secretion. Islets from PZ-SRIF treated animals in response to these stimuli secreted a considerably higher level of insulin than at non-stimulatory concentrations of glucose, but did not reach normal levels ($p<0.01$) or return to normal levels after 24-hours of non-treatment. These results, therefore, show that isolated pancreatic islets of normal rats responded to media stimuli as shown by increased insulin release. Islets of the PZ-SRIF treated rats responded to the presence of media stimuli but show an inhibited secretion of insulin.

Immunoreactive glucagon content of isolated pancreatic islets of PZ-SRIF treated rats also showed a significant depression ($P < 0.05$) from levels for normal islets (Figure 3; Appendix Table A4). Twenty-four hours after PZ-SRIF treatment, islet glucagon content had increased to levels lower but not significantly different than normal.

Secretion of glucagon by isolated pancreatic cells in response to a stimulus was lowered by chronic somatostatin treatment (Figure 4; Appendix Table A4). Arginine or low glucose levels (50 mg/100 ml) serve as stimuli for pancreatic glucagon release. Normal islets responded to these stimuli in the media by an increased glucagon secretion. PZ-SRIF treated islets showed a very low response when compared to normal levels and did not recover normal secretion

Figure 3. Immunoreactive glucagon (IRG) content of pancreatic islets isolated from rats treated with 100 µg/kg protamine zinc somatostatin (PZ-SRIF) for sixteen days followed by 200 µg/kg PZ-SRIF for seven days. PZ-SRIF indicates islets homogenized immediately after treatment; 24 hrs. post PZ-SRIF indicates islet homogenized 24 hrs. after last treatment. Values represent mean ± SEM. Asterisk denotes significant difference from normal values: *$P<0.05$.

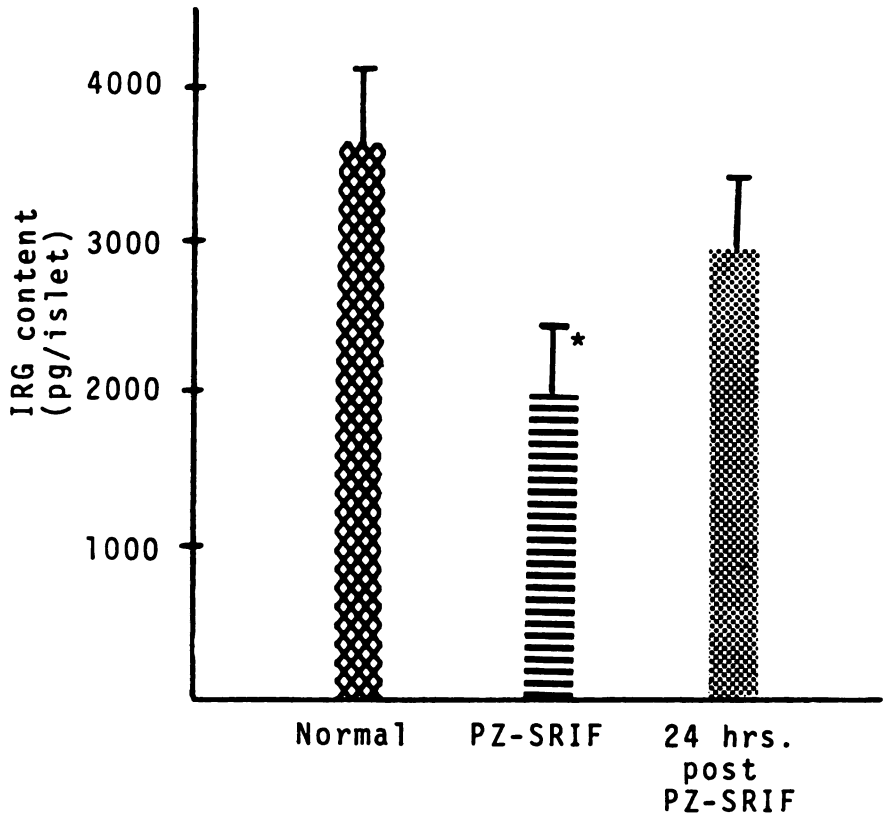

IRG content
(pg/islet)
4000
3000
2000
1000
*
Normal
PZ-SRIF
24 hrs.
post
PZ-SRIF

Figure 4. Immunoreactive glucagon (IRG) secretion by pancreatic islets isolated from rats treated with 100 µg/kg protamine zinc somatostatin (PZ-SRIF) for sixteen days followed by 200 µg/kg PZ-SRIF for seven days. Islet cells were incubated for 2 hours in
1) low glucose, 50 mg/100 ml (50 Glu);
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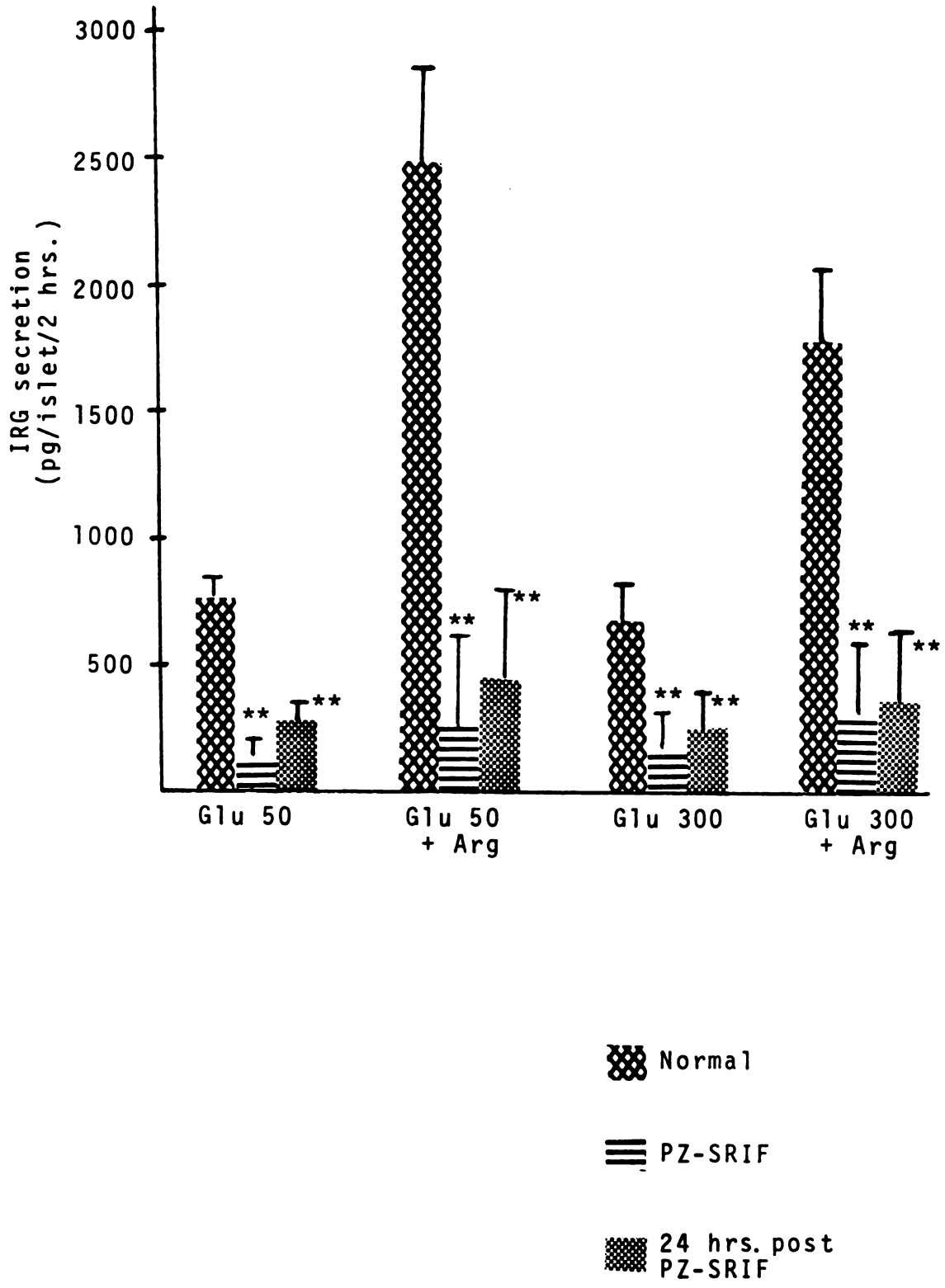
4) high glucose plus 200 mg/100 ml arginine (Glu 300 + Arg).
Values represent mean ± SEM. Asterisks denote significant difference from normal values: **P<0.01.


3000
2500
2000
1500
1000
500
IRG secretion
(pg/islet/2 hrs.)
**
**
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Glu 50
Glu 50
+ Arg
Glu 300
Glu 300
+ Arg
Normal
PZ-SRIF
24 hrs. post
PZ-SRIF

within a 24-hour period. In a high glucose media (300 mg/100 ml), glucagon secretion by untreated islets responded similarly to a 30 mg/100 ml glucose stimulus; PZ-SRIF treated islets showed a suppressed response ($P<0.01$). At this same glucose concentration the presence of added arginine acts as a further stimulus for glucagon secretion and somewhat overrides the suppression by an increased glucose concentration. Thus, in normal islets, there was an increased response to stimuli, but in PZ-SRIF treated islets there was a diminished glucagon response with minimal recovery after 24 hours.

DISCUSSION

The incubated pancreatic islets showed that chronic somatostatin treatment suppressed both the production, as measured by content, and release of insulin and glucagon. These results provide evidence that the PZ-SRIF which was administered to these rats was biologically active. The changes in islet cell hormone production and release are most likely due to a direct effect of somatostatin on the alpha and beta cells, possibly due to its presence in the pancreatic D cells and its paracrine function. It is well known that somatostatin will suppress both basal and stimulated serum insulin and glucagon levels (Christensen et al., 1974; Gerich, 1976; Guillemin and Gerich, 1976; Efendic et al., 1978). Insulin suppression could also be due to the actions of somatostatin on the gastrointestinal tract causing diminished gastrin, secretin and CCK-PZ release (Konturek, 1976). These hormones have been hypothesized to be gastrointestinal factors that are released in response to glucose and stimulate the pancreas to release insulin (McIntyre et al., 1965). Lack of these hormones could decrease pancreatic hormone secretion. Since electrical stimulation of the vagus nerve tends to increase insulin secretion (Frohman et al., 1967) somatostatin could decrease

insulin by inhibiting cholinergic activity (Chariot et al., 1978).

Although there was a definite decrease in the pancreatic islet hormones, their concentrations in the serum did not show the same decreases in response to PZ-SRIF treatment. Similarly, there was no difference in serum glucose concentration between PZ-SRIF treated or PZ treated animals. This result could be due to a concomitant suppression of both insulin and glucagon. With opposing actions on glucose metabolism, the end result of insulin and glucagon suppression by PZ-SRIF could be no change in fasted serum glucose values. Serum immunoreactive insulin and pancreatic glucagon also showed no alteration after somatostatin treatment. Since the serum values represent a fasted state, perhaps with chronic somatostatin treatment, altered hormone function may become evident only in response to a metabolic challenge. Also, with diminished islet cell hormone production a compensatory mechanism, decreasing metabolic clearance as a function of receptor-site catabolism, might exist. The serum total immunoreactive glucagon of 200 μg/kg PZ-SRIF treated rats did show a significant decrease ($P<0.05$) from normal fasting levels. In addition, if values for all PZ-SRIF treated rats are combined and Student's t as opposed to Dunnett's t test is used, there was a significant decrease ($P<0.025$) from normal rats. Whether this analysis represents a legitimate as opposed to a statistically advantageous

manipulation is debatable. However, by separating treatment effects into dose levels, a dose-response relationship is attributed to somatostatin. Whether the effects of somatostatin can be considered dose dependent is presently not known. In this study, different results for body weight gain and food intake were observed for PZ-SRIF treatment at 100 μg/kg for fourteen days followed to 200 μg/kg for seven days compared to treatment at 100 μg/kg for fourteen days. If length of treatment does not account for differences, effectiveness of somatostatin treatment could be dose-dependent, and then all results should be separated according to dosage. However, if values for all PZ-SRIF treated animals are not combined, gut glucagon was still significantly lowered by 100 μg/kg followed by 200 μg/kg PZ-SRIF treatment.

Decreased serum total immunoreactive glucagon levels by somatostatin were also reported by Matsuyama et al. (1979). Circulating levels of enteroglucagon have been shown to be elevated in hyperphagia and very low in states of starvation (Pearse et al., 1977), which is opposite to the responses observed for pancreatic glucagon. Thus, the lowered gut glucagon levels of PZ-SRIF treated rats might reflect merely a state of food deprivation as seen by decreased food intake in these animals. According to Unger et al. (1978), extra pancreatic alpha cells are more sensitive to insulin and small quantities instantly turn off the secretion of extra-pancreatic glucagon. Perhaps these

cells are also more sensitive to PZ-SRIF.

In this study, there was no significant change in body weight gain due to chronic somatostatin therapy (100 µg/kg) for sixteen or fourteen days in 250 g or 330 g rats, respectively. This dose was identical to that used by Micossi et al. (1976) in 470 g rats. These investigators reported a significant decrease in body weight within fourteen days. However, in young rats (125 g) Brazeau et al. (1974) using a dose of 100 µg, (equivalent to 800 µg/kg body weight) three times daily, showed a significant decrease in body weight gain after seven days. Perhaps in an animal that is growing rapidly, as opposed to a mature rat, an increased dosage is necessary to produce results. In view of similar weight gains for control and animals treated with 100 µg/kg PZ-SRIF for fourteen or sixteen days, it was not surprising that there was no change in food intake or fecal lipids for any of these groups.

The decrease in body weight seen in PZ-SRIF (200 µg/kg) treated rats appeared to be related to decreased food intake as opposed to malabsorption since there was no significant difference in fecal lipids or xylose tolerance from PZ treated rats. Decreased food intake was apparent after one day of 200 µg/kg PZ-SRIF treatment. Therefore, higher dosage of PZ-SRIF instead of an additional seven days of treatment may have been responsible for the decline in food intake and consequent decreased body weight gain.

This effect on food intake agrees with a study done by Rezek et al. (1978) in which repeated three-hour hepatic portal infusions of somatostatin were reported to reduce daily food intake by 29% causing a loss of body weight. In further studies, (Rezek et al., 1979) intraperitoneal administration of 100 µg/rat SRIF caused a two-hour complete anorexia followed by a decreased food intake during the following 24 hours. In a study by Lotter and Woods (1977), intraperitoneal injections of 1000 ng/kg SRIF caused a 50% decrease in food consumed in a 30-minute period.

Since somatostatin is produced in the lateral hypothalamus (Guillemin and Gerich, 1976), and may act as a neurotransmitter (Pimstone and Berelowitz, 1978) it conceivably could be involved in the complex process of food intake. Somatostatin has been shown to have a depressant effect on the neurons of the cortex and hypothalamus (Renaud et al., 1975). It could, therefore, depress the dopaminergic neurons of the lateral hypothalamus postulated to be involved in the initiation of feeding (Ungerstedt, 1971). Inhibition of these neurons by SRIF could result in apagia and adipsia.

Because somatostatin is also located in the gastrointestinal tract (Elde, 1978), it could act as peripheral hunger/satiety signal and communicate via neurotransmission directly with the central nervous system to control food intake. Somatostatin could affect food intake indirectly

by modulating peripheral signals that also may influence food intake. Gastric distention has been shown to decrease food intake (Paintal, 1954). By delaying gastric emptying (Bloom et al., 1975) and thereby promoting prolonged gastric distention somatostatin could decrease food intake. Somatostatin has been shown to depress the gastrointestinal hormones: motilin (Bloom et al., 1975), GIP (Pederson et al., 1975), VIP (Lennon et al., 1975), gut glucagon (Matsuyama et al., 1979), secretin (Raptis et al., 1978), and gastrin (Raptis et al., 1975; Abbinus et al., 1976; Konturek et al., 1976a; Vatn et al., 1977). By causing a shutdown of the gastrointestinal endocrine system, it is conceivable that food intake may be depressed. If the circulating concentrations of insulin or growth hormone are viewed as plausible peripheral signals for food intake, then somatostatin by inhibiting these hormones (Lundbaek et al., 1977; Brazeau et al., 1974) could decrease food intake.

Byrne et al. (1977) have shown that the net effect of somatostatin infusion on blood glucose is hyperglycemia due to decreased glucose utilization. This state of glycemia according to the glucostatic mechanism of food intake could serve to limit food intake. However, since somatostatin will inhibit glucagon (Lundbaek et al., 1977), hepatic glucose production by decreased glucagon-dependent glycogenolysis and consequent decreased availability of glucose may cause an increase in food intake according to the

glucostatic mechanism. Furthermore, increased food intake could be expected on the basis that SRIF inhibits cholecystokinin-pancreozymin (Raptis et al., 1978) or thyroid stimulating hormone (Hansen and Lundback, 1976). A decrease in either of these hormones could signal an increased food intake.

Since somatostatin has been shown to reduce digestive functions (Raptis et al., 1975, 1978; Albinus et al., 1976; Konturek, 1976; Holtermuller et al., 1977; Chariot et al., 1978) and cause malabsorption of carbohydrate (Wahren and Felig, 1976; Wagner et al., 1978), protein (Sakurai et al., 1975), and fats (Koerker et al., 1978; Sakurai et al., 1975) an increase in fecal lipids might be expected in somatostatin treated animals; however, increased fat excretion was not observed in this experiment. The lack of influence of PZ-SRIF on fecal lipids may have been caused by the increased fat content of the diet. It was originally believed that since PZ-SRIF is proposed to decrease pancreatic exocrine secretion (Folsch et al., 1978; Chariot et al., 1978) and consequently pancreatic lipase, any possible malabsorptive effects would be intensified on a diet containing 25% rather than the usual 4% fat. However, an increased fat content could have served as an inducer for increased lipase output since pancreatic enzymes

adapt to the type of food normally ingested (Felber et al., 1974). Hence, the dose of somatostatin may not suppress secretin, CCK-PZ and pancreatic exocrine secretion sufficiently to offset an increase in lipase caused by the high fat diet. According to Losowsky et al. (1974), over 80% of pancreatic exocrine function must be lost or enzymes in the duodenal juice must be less than 10% of normal levels before steatorrhea will result.

In the previous positive tests for malabsorptive effects of somatostatin, blood levels of the ingested or infused nutrient were monitored for 2-3 hours during somatostatin infusion. Blood levels of glucose, xylose (Wahren and Felig, 1976), galactose (Wagner et al., 1978), amino-nitrogen (Sakurai et al., 1975) and triglycerides (Sakurai et al., 1975; Koerker et al., 1978) were significantly lower than controls. By possible concomitant delay in gastrointestinal motility (Tansy et al., 1978), gastric emptying (Bloom et al., 1975), and splanchnic blood flow (Wahren and Felig, 1976) absorption could be merely delayed but not diminished. Thus, if absorption were delayed, increased fecal lipids would not necessarily be expected. Results of the xylose tolerance test showed no impaired intestinal absorption for PZ-SRIF treated rats. However, the length of collection of excreted xylose, 24-hours, would also not differentiate the possibility of delayed absorption.

SUMMARY AND CONCLUSIONS

Male Sprague-Dawley (250 g) rats treated with PZ-SRIF (200 µg/kg body weight) for seven days after an initial pre-treatment for sixteen days of PZ-SRIF (100 µg/kg body weight) showed a significant decrease in body weight gain. The level of PZ-SRIF used for this treatment was also sufficient to suppress the immunoreactive insulin and glucagon content and secretion of pancreatic islets isolated from these animals. The decreased gain in body weight in these rats was coupled with a significant decrease in food intake but no change in fecal lipids or xylose absorption. Somatostatin did not alter fasting serum levels of glucose, insulin or pancreatic glucagon in experimental rats but selectively and significantly decreased total glucagon which includes gastrointestinal as well as pancreatic glucagon.

It is suggested, therefore, that the observed weight loss in PZ-SRIF treated rats was due to a decrease in food intake rather than a decrease in nutrient absorption. Enteroglucagon, which may be involved in nutrient homeostasis, was also decreased by somatostatin. Whether lowered gut glucagon is causally related to, or merely an effect of, decreased food intake is unknown.

Although not the central focus of this study, it is suggested that somatostatin could influence food intake by its 1) central hypothalamic location and neural influence 2) peripheral gastrointestinal location and/or 3) inhibition of metabolites and hormones.

APPENDICES

Appendix Table A1. Body weight and food intake, treatment periods I and II[1].

Group	Treatment[2]	N	Body weight (g)		Food intake (g)		
					Average daily		Total
			Initial[3]	Final[4]	Initial	Final	
			Treatment period I[5]				
1	Saline	7	245±11	327±10	13.8±1.1	15.0±1.4	228.3±9.8
2	PZ	7	246±11	328±9	12.8±1.1	14.5±1.4	228.8±12.8
3	PZ-SRIF (100 µg/kg)	8	247±11	333±9	14.4±1.1	13.8±1.3	227.8±12.2
			Treatment period II[5]				
1	PZ-SRIF (100 µg/kg)	7	327±8	372±12	15.0±1.4	15.8±0.5	211.4±9.2
2	PZ	8	325±9	381±9	14.8±0.7	16.5±0.6	218.7±7.9
2	PZ[6]	8	325±9	357±10	14.8±0.8	15.1±0.8	108.6±3.8
3	PZ-SRIF (200 µg/kg)	8	333±10	353±10	13.8±0.8	12.9±0.8	94.6±4.8

[1]Means ± SEM.

[2]Treatments of saline, protamine zinc (PZ) and protamine zinc somatostatin (PZ-SRIF) were administered as two daily subcutaneous injections.

Appendix Table A1 (cont'd.).

[3]A three day average prior to beginning the treatment period.

[4]A three day average at the end of the treatment period.

[5]Treatment period I lasted sixteen days; Treatment period II lasted fourteen days for groups 1 and 2 and seven days for group 3.

[6]Values after seven days of Treatment period II.

Appendix Table A2. Fecal lipids, treatment periods I and II[1].

Group	Treatment	N	Fecal lipids (g)		
			Average daily		Total
			Initial[3]	Final[4]	
			Treatment period I[5]		
1	Saline	7	0.08 ± 0.02	0.09 ± 0.01	1.34 ± 0.11
2	PZ	7	0.10 ± 0.02	0.13 ± 0.01	1.74 ± 0.25
3	PZ-SRIF (100 µg/kg)	8	0.10 ± 0.01	0.11 ± 0.01	1.63 ± 0.14
			Treatment period II[5]		
1	PZ-SRIF (100 µg/kg)	7	0.09 ± 0.01	0.10 ± 0.02	1.58 ± 0.08
2	PZ	8	0.13 ± 0.02	0.13 ± 0.02	1.96 ± 0.23
2	PZ[6]	8	0.13 ± 0.01	0.17 ± 0.02	1.09 ± 0.13
3	PZ-SRIF (200 µg/kg)	8	0.11 ± 0.01	0.15 ± 0.02	0.98 ± 0.08

[1]Means ± SEM.

[2]Treatments of saline, protamine zinc (PZ) and protamine zinc somatostatin (PZ-SRIF) were administered as two daily subcutaneous injections.

Appendix Table A2 (cont'd.).

[3]A three day average prior to beginning the treatment period.

[4]A three day average at the end of the treatment period.

[5]Treatment period I lasted sixteen days; Treatment period II lasted fourteen days for groups 1 and 2 and seven days for group 3.

[6]Values after seven days of Treatment period II.

Appendix Table A3. Effect of chronic protamine zinc somatostatin (PZ-SRIF) treatment on immunoreactive insulin content of and secretion by isolated pancreatic islets[1]

	Insulin content µU/islet	Insulin secretion[2] µU/islet/2 hours			
		Glu 50	Glu 50 + Arg	Glu 300	Glu 300 + Arg
Normal[3]	1491 ± 149	138 ± 15	456 ± 29	615 ± 36	618 ± 28
PZ-SRIF[4]	674 ± 60[1]	24 ± 15[7]	36 ± 29[7]	309 ± 36[7]	436 ± 28[7]
24 hours post[5] PZ-SRIF	869 ± 60[6]	52 ± 15[7]	71 ± 29[7]	292 ± 36[7]	349 ± 28[7]

[1]Means ± SEM where the mean for islet insulin content represents values for twenty normal islets and twelve islets each for PZ-SRIF and 24 hours post PZ-SRIF; where each mean of insulin secretion represents values for eight islets.

[2]Islets incubated for 2 hours in 1) low glucose, 50 mg/100 ml (50 Glu); 2) low glucose plus 200 mg/100 ml arginine (Glu 50 + Arg); 3) high glucose, 300 mg/ml (300 Glu); and 4) high glucose plus 200 mg/100 ml arginine, (Glu 300 + Arg).

[3]Islets from untreated comparable weight rats.

[4]Islets from rats treated with 100 µg/kg PZ-SRIF for sixteen days followed by 200 µg/kg PZ-SRIF for seven days isolated and incubated immediately after treatment.

[5]Islets from rats treated with 100 µg/kg PZ-SRIF for sixteen days followed by 200 µg/kg PZ-SRIF for seven days isolated and incubated 24 hours post treatment.

[6]Significantly lower ($P<0.05$) than normal islets.

[7]Significantly lower ($P<0.01$) than normal islets.

Appendix Table A4. Effect of chronic protamine zinc somatostatin (PZ-SRIF) treatment on immunoreactive glucagon content of and secretion by isolated pancreatic islets[1]

	Glucagon content pg/islet	Glucagon secretion[2] pg/islet/2 hours			
		Glu 50	Glu 50 + Arg	Glu 300	Glu 300 + Arg
Normal[3]	3657 ± 515	747 ± 102	2538 ± 382	698 ± 140	1803 ± 290
PZ-SRIF[4]	1992 ± 421[6]	107 ± 102[7]	252 ± 382[7]	164 ± 140[7]	314 ± 290[7]
24 hours post PZ-SRIF[5]	2983 ± 421	232 ± 102[7]	435 ± 382[7]	261 ± 140[7]	355 ± 290[7]

[1]Means ± SEM where the mean for islet glucagon content represents values for eight normal islets and twelve islets each for PZ-SRIF and 24 hours post PZ-SRIF; where each mean of glucagon secretion represents values for eight islets.

[2]Islets incubated for 2 hours in 1) low glucose, 50 mg/100 ml (50 Glu); 2) low glucose plus 200 mg/100 ml arginine (Glu 50 + Arg); 3) high glucose, 300 mg/ml (300 Glu); and 4) high glucose plus 200 mg/100 ml arginine, (Glu 300 + Arg).

[3]Islets from untreated comparable weight rats.

[4]Islets from rats treated with 100 µg/kg PZ-SRIF for sixteen days followed by 200 µg/kg PZ-SRIF for seven days isolated and incubated immediately after treatment.

[5]Islets from rats treated with 100 µg/kg PZ-SRIF for sixteen days followed by 200 µg/kg PZ-SRIF for seven days isolated and incubated 24 hours post treatment.

[6]Significantly lower ($P<0.05$) than normal islets.

[7]Significantly lower ($P<0.01$) than normal islets.

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