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EFFECT OF SLAFRAMINE ON EXOCRINE GLAND FUNCTION*†

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Abstract—The administration of slaframine to animals results in a sustained increase in secretory activity by the exocrine glands. A quantitative study of pancreatic secretions was undertaken to better define the consequences of the stimulation of exocrine glands by slaframine. After a brief delay, presumably due to the bioactivation of slaframine, there is a substantial and prolonged increase in the rate of secretion. The increased activity of the digestive enzymes in the pancreatic fluid is not accompanied by an equal increase in the total protein content, suggesting a specific increase in the synthesis of the digestive enzymes. The increased protein synthesis was confirmed by measurement of the incorporation of labeled amino acids into pancreatic proteins. The increased rate of secretion is accompanied by a decreased concentration of mucoproteins, but only a very slight decrease in viscosity was observed. These results indicate that slaframine results in a prolonged stimulation of pancreatic secretory activity and is selective in stimulating the synthesis of digestive enzymes.

EXCESSIVE salivation in cattle fed certain legume forages is due to infestation of such forages with a fungus.[1] The fungus, *Rhizoctonia leguminicola*,[2] when grown on extracts of red clover hay, results in the production of an alkaloid, isolated as the picrate[3] or Mayer's salt,[4] which will cause salivation. This alkaloid has been characterized as 1-acetoxy-6-amino-octahydroindolizine[5, 6] and given the name slaframine.[5] The isolated compound itself has no biological activity before activation by the liver.[7] This bioactivation has been suggested as the basis for the prolonged activity of slaframine, the slow metabolism by the liver resulting in the prolonged production of the active metabolite.[7]

Various properties of slaframine, such as its specificity toward the exocrine glands and its prolonged activity, suggest that the compound may be useful as a research tool in the study of exocrine gland function or as a chemotherapeutic for diseases involving exocrine gland insufficiency. For these reasons, a rather extensive study of the effect of slaframine on pancreatic function was undertaken and will be reported in this communication.

METHODS AND MATERIALS

Chemical. Slaframine was isolated from pure cultures of *Rhizoctonia leguminicola*[4] and purified by repeated crystallization as the dipicrate before conversion to the

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dicitrate for use. ^{14}C-leucine was purchased from the Calbiochem Corp., Los Angeles, Calif. Viodenum was a gift of the Viobin Corp., Monticello, Ill. Benzoyl-arginine-methyl-ester (BAME), benzoyl-tyrosine-ethyl-ester (BTEE), and polyoxyethylene-sorbitan-monolaurate (Tween 20) were purchased from the Sigma Chemical Co., St. Louis, Mo.

Enzyme assays. Lipase activity was assayed by potentiometric titration using a Radiometer Titrigraph at pH 8·2 with Tween 20 as a substrate. The assays were run at 25° in 0·05 M sodium acetate containing 0·075 M calcium chloride and 20% (v/v) Tween 20. The substrate was made up in 0·05 M sodium acetate buffer, pH 4·5, and titrated to pH 8·2 immediately before use. Spontaneous hydrolysis rates were then determined for subtraction from enzymatic rates.

Amylase activity was estimated by the method of Bernfeld,[8] wherein reducing groups liberated from soluble starch are measured spectrophotometrically by the reduction of 3,5-dinitrosalicylic acid.

Trypsin and chymotrypsin activities were assayed spectrophotometrically according to the method of Hummel[9] using BAME and BTEE, respectively, as substrates. The enterokinase preparation used for the activation of trypsinogen and chymotrypsinogen was prepared from Viodenum. Viodenum (2%, w/v) was incubated for 30 min at 37° in 0·1 M Tris-HCl buffer containing 0·1 M calcium chloride of pH 7·2. The incubation mixture was centrifuged at 12,000 *g* for 20 min and the supernatant used as the source of enterokinase. Activation was then accomplished by mixing the solutions to be activated with equal volumes of the enterokinase preparation and incubating at 37° for 30 min. Pure pancreatic fluid was diluted 1 :10 before activation.

Protein assays were performed by the method of Lowry *et al.*[10] and mucoprotein was estimated by assaying for *N*-acetylneuraminic acid by the method of Svennerholm.[11] Viscosity measurements were made with a No. 1 Ubbelohde Viscosimeter at 20°.

Effect of slaframine on the rat pancreas. Experiments to determine the effect of slaframine on the pancreas were performed in a similar manner to those of Farber and Sidransky.[12] In these experiments 200 g males (Holtzman strain) were injected with slaframine (0·6 mg/kg) and sacrificed at various time intervals for removal of the pancreas to determine the enzyme levels in the tissue. Immediately upon removal, the pancreas was placed in ice-cold isotonic saline. Extraneous tissue was trimmed away and the pancreas was blotted to remove excess liquid and weighed. After homogenization with a glass homogenizer in 25 vol. of 0·15 M potassium chloride containing 0·1% (v/v) Triton X-100, the homogenates were centrifuged at 12,000 *g* for 30 min and the supernatant was used immediately for the various assays described above. All manipulations were performed at 0–5°.

Effect of slaframine on the pancreatic activity of goats, calves and sheep. Quantitative pancreatic activity was studied by placing permanent cannulae in the pancreatic ducts of goats, calves and sheep. (All surgery was performed by Dr. R. M. Cook, Department of Dairy Science, Michigan State University.) Cannula loops were inserted into the intestine for the purpose of returning the pancreatic fluid during intervals when collections were not being taken. During collection periods, pancreatic fluid was collected for at least 30-min periods. The total volume collected was then divided by the time of the collection period to obtain an average flow rate, expressed as milliliters per minute. All collections were made directly into a flask kept on ice and all samples were kept at 0° for immediate assays (within 1 or 2 days).

The protein-synthesizing activity of the pancreas was determined by injecting ^{14}C-leucine into the jugular vein and measuring the incorporation of radioactivity into the proteins of the pancreatic fluid according to the method of Casjens and Morris.[13]

RESULTS AND DISCUSSION

The administration of slaframine to animals results in a specific stimulation of exocrine glands. The salivary glands and the pancreas seem to be the most susceptible; however, with high doses, all exocrine glands are stimulated. The reason for the specificity for the exocrine glands is not clear, but it suggests a uniqueness in the receptors of the glands. The increased activity is quite prolonged; salivary activity in the cat remains high for 5–8 hr,[7] while the pancreatic flow in the calf remains well above normal for about 10 hr. The prolonged activity may be due to the fact that slaframine requires activation by the liver, thus resulting in the prolonged production of the active metabolite.[7] It is also possible that the active metabolite has a high

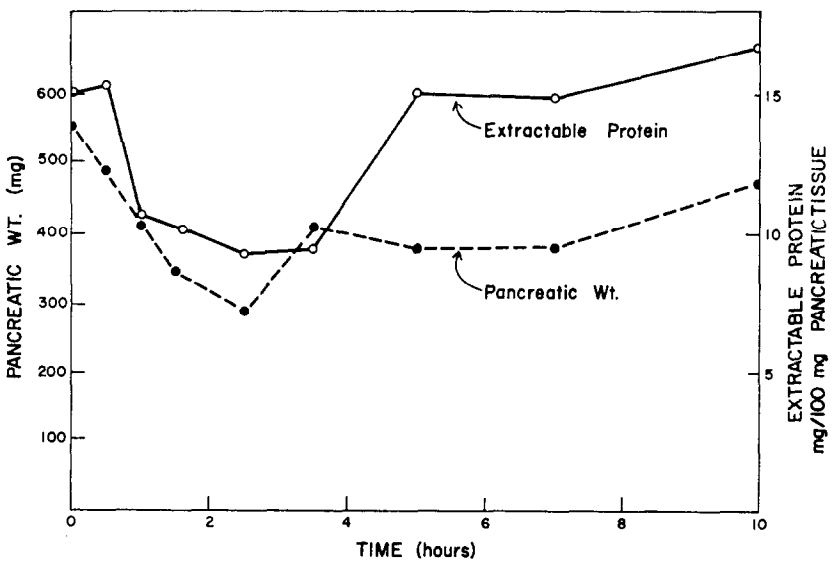

FIG. 1. Changes, with time, in pancreatic weight and extractable pancreatic protein of rats stimulated with slaframine (0·6 mg/kg). The points are means of four animals sacrificed at the time interval indicated.

affinity for the receptor and upon binding continues to stimulate. This possibility is suggested by the fact that atropine will not reverse the action of slaframine after the exocrine glands are in a hyperactive state, but will completely prevent the action of slaframine if given prior to slaframine.* Slaframine does contain an acetate ester and it is possible that inactivation of the active metabolite could involve hydrolysis by an esterase. However, a variety of esterases were inactive toward slaframine. Deacetyl-slaframine is completely devoid of activity.

* Unpublished experiments.

In rats, the effect of slaframine on the pancreas is similar to that found by Farber and Sidransky[12] for other cholinomimetics. The effect of slaframine, however, was more pronounced and prolonged. The amount of protein extractable from the pancreas fell markedly and returned to normal at about 5 hr after slaframine injection (Fig. 1). Pancreatic weight decreased immediately to about 50 per cent of controls (Fig. 1). At about the time extractable protein returned to normal (5 hr), pancreatic weight returned partially to normal (Fig. 1). At this time, it was also possible to observe a change in the physical appearance of the gland. Chymotrypsin activity in the pancreatic extracts decreased to almost insignificant levels before returning to normal (Fig. 2). The same pattern was seen for all of the digestive enzymes assayed. The activity of the digestive enzymes returned to normal shortly after the amount of extractable protein had increased, but before pancreatic weight returned to normal. These results suggest that the gland secretes all of the stored enzymes. Then the gland responds to a hyperactive state which results in the synthesis of new enzymes.

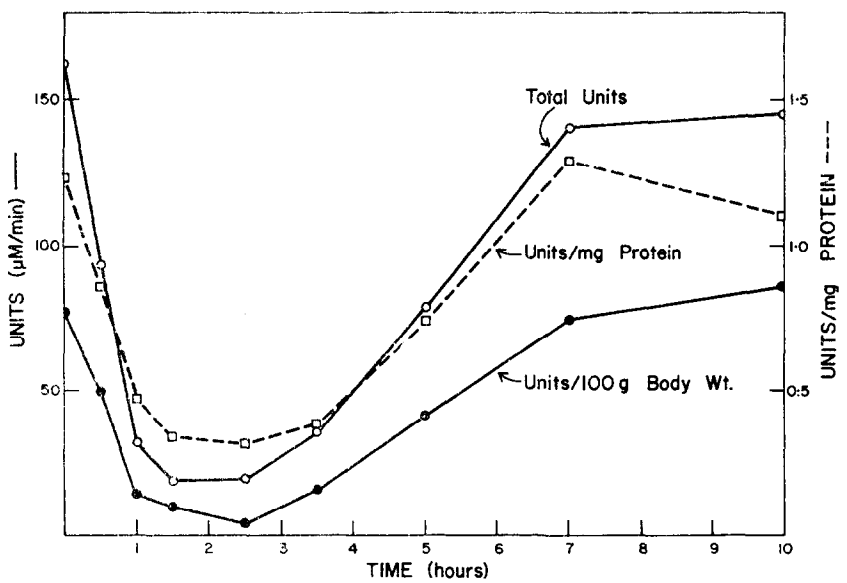

FIG. 2. Changes, with time, in the chymotrypsin activity of pancreatic extracts of rats stimulated with slaframine (0·6 mg/kg). The values are the mean activities of the extracts obtained from the pancreas of four animals sacrificed at the time interval indicated and are expressed as total units, units/mg protein, and units/100 g body weight. One unit is equivalent to 1 μmole substrate hydrolyzed/min at pH 7·8 and 25°.

Quantitative studies of pancreatic activity in goats, calves and sheep showed that after a delay, presumably required for the activation of slaframine, flow rates increased and remained high for several hours (Fig. 3). Species and animal differences were sometimes significant and seemed to reflect the animals' ability to activate slaframine,[7] which correlates well with their ability to metabolize drugs in general.[14] The activity of all digestive enzymes increased dramatically. Trypsin activity of the pancreatic fluid collected from a goat is illustrated in Fig. 4. The results are presented as total

units secreted per unit time and as units per milligram of protein (i.e. specific activity). Enzyme activity increased dramatically at first, presumably due to a small amount of stored enzyme. This initial increase was followed by a rather constant activity 3–4 times normal. During this time the specific activity of the enzymes continued to increase, suggesting a stimulated synthesis of digestive enzymes.

The stimulation of protein synthesis was confirmed by the administration of

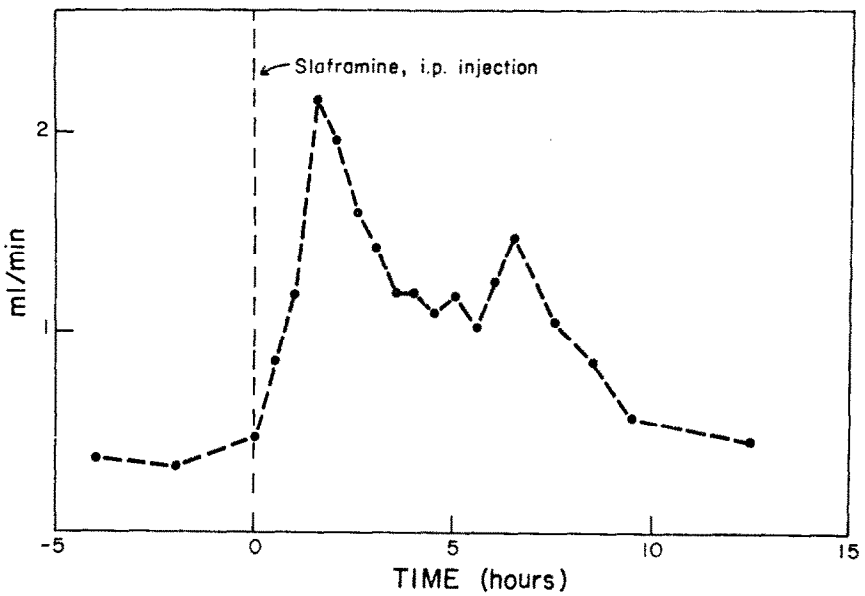

FIG. 3. Effect of slaframine on the volume of pancreatic fluid secreted by a goat. The values were obtained by dividing the total volume collected in the time period by the length of the time period and expressed as ml/min. Slaframine (0·1 mg/kg) was administered i.p. as the dicitrate.

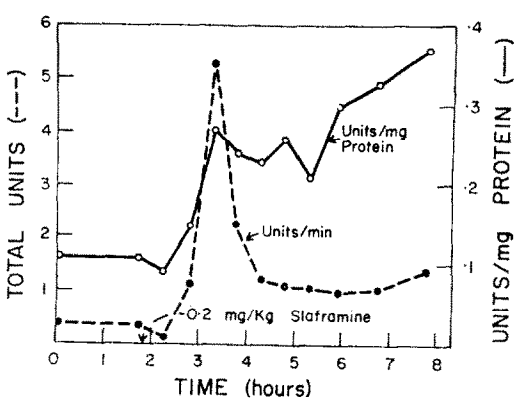

FIG. 4. Trypsin activity of pancreatic fluid collected from a goat stimulated with slaframine. Activity is expressed as total units/min (obtained by dividing the total units of enzyme activity secreted during the collection period by the length of the collection period in minutes) and as units/mg protein. Slaframine (0·2 mg/kg) was administered i.p. as the dicitrate. One unit is equivalent to 1 μmole substrate hydrolyzed/min at pH 7·8 and 25°.

^{14}C-leucine and following the incorporation of radioactivity into pancreatic proteins. In control experiments, the incorporation of radioactivity reached a peak soon after the injection of the radioactive precursor and decreased exponentially. However, after the administration of slaframine, the exponential decrease in incorporation of radioactive amino acid was interrupted by a second peak of incorporation (Fig. 5). This second peak of incorporation correlated with the increased specific activity of digestive enzymes, suggesting that the pancreas was specifically synthesizing digestive enzymes.

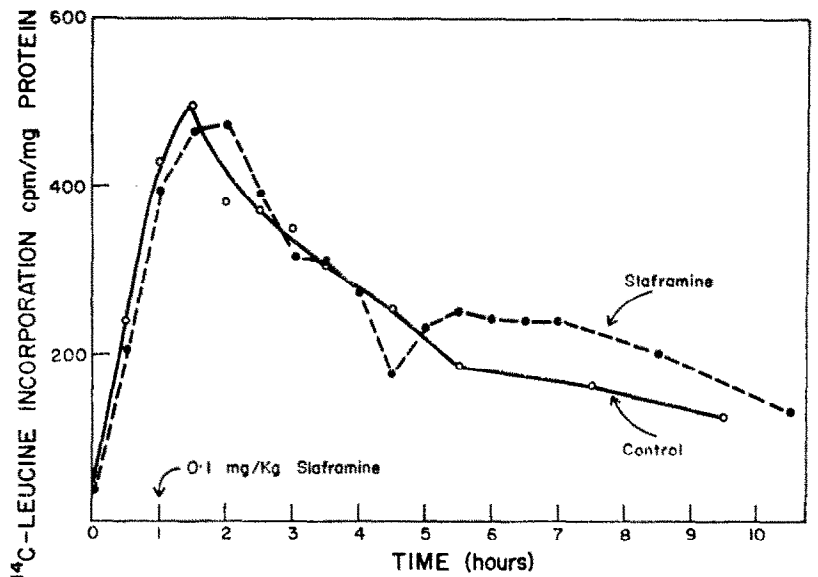

FIG. 5. Effect of slaframine (0·1 mg/kg) on the incorporation of ^{14}C-leucine into pancreatic protein by the calf.

The physical nature of the pancreatic secretion was studied in the calf, since the calf possesses a pancreatic duct independent of the bile duct and pure pancreatic fluid could be collected. Only minor changes in the nature of the secretions could be observed. Neither protein content, pH, nor viscosity changed significantly. However, mucoprotein content, determined as *N*-acetylneuraminic acid, decreased to about 50 per cent of normal (Fig. 6). This corresponded to approximately a doubling in flow rate, suggesting a simple dilution effect.

In one experiment, bile flow was also measured in the calf by independent cannulation of the bile duct. No changes in flow rate due to slaframine could be observed. The same results were evident in animals with common bile and pancreatic ducts, for a dilution of the bile could be observed when pancreatic flow was stimulated.

The results reported in this communication indicate that slaframine is a potent stimulator of exocrine gland activity as judged by the response of the pancreas. In the rat, there is a decrease in pancreatic weight and a dramatic decrease in the activity of the digestive enzymes extractable from the pancreas. In goats, calves and sheep, there is an increase in flow rates accompanied by an increase in the activity of all the

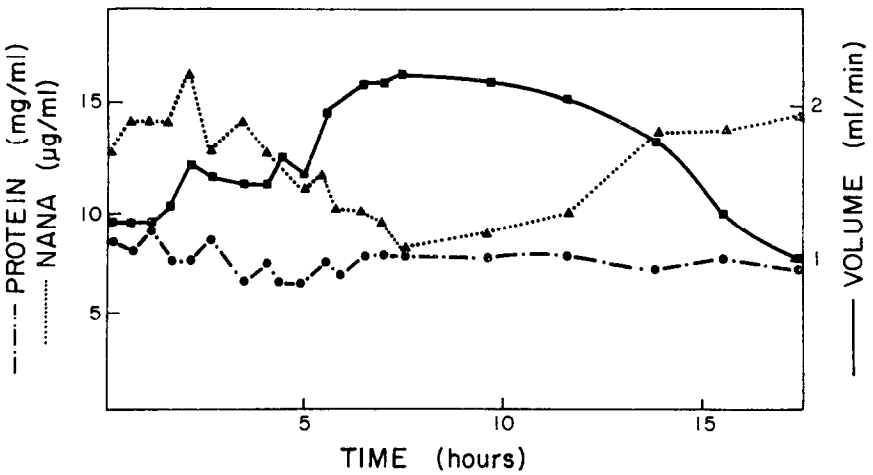

FIG. 6. Changes, with time, in the volume, protein and *N*-acetylneuraminic acid (NANA) content of pancreatic fluid secreted by a calf stimulated with slaframine. Slaframine (0·2 mg/kg) was was administered i.p. as the dicitrate.

digestive enzymes studied. In addition to an increase in total activity, there is also an increase in the specific activity of the digestive enzymes.

While such parameters as pH, viscosity and protein content do not change markedly, there is a decrease in the concentration of mucoproteins. This decrease corresponds to the increase in flow rates, suggesting a simple dilution phenomenon.

The fact that the stimulation is not merely secretory activity is emphasized by the fact that there is also an increased incorporation of ^{14}C-amino acids into pancreatic proteins after the administration of slaframine. Presumably, this incorporation is into the digestive enzymes.

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