INFLUENCE OF INTRAJUGULAR ADMINISTRATION OF INSULIN, GLUCAGON AND PROPIONATE ON VOLUNTARY FEED INTAKE OF SHEEP1,2

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Summary

The effect of intrajugular injections of insulin, glucagon and propionate, administered singly and in combination, as possible peripheral feedbacks in the control of feed intake in wethers was studied. A complete mixed diet (25% chopped hay:75% cracked corn) was fed ad libitum. The treatments were saline, 6 mU insulin/kg body weight (BW), 9 ng glucagon/kg BW and 1.3 mg propionate/kg BW. In Exp. 1, five wethers were given the treatments at the beginning of each spontaneous meal over a 24-hr period, and total daily feed intakes were measured. The average number of injections per sheep for a 24-hr period was eight. In Exp. 2, the effects of the treatments on plasma concentrations of insulin, glucagon, propionate and glucose at 15, 30, 60 and 120 min after injection were measured in six other wethers. In Exp. 1, insulin (P<.01), glucagon (P<.01), insulin plus propionate (P<.05) and glucagon plus propionate (P<.05) decreased 24-hr feed intake by 18.5, 15.8, 11.0 and 11.8%, respectively, compared to the saline control. In Exp. 2, plasma insulin concentrations were increased (P<.05) at 15 min after the injection of glucagon, to 2.0 times the pretreatment values. Insulin and glucagon concentrations in plasma were increased only slightly (P<.10) after administration of glucagon plus propionate. No treatments affected glucose or propionate concentrations in the plasma. Increases in plasma concentrations of insulin, glucagon and propionate may interact directly or initiate other mechanisms involved in the short-term control of feed intake by sheep on a concentrate diet. (Key Words: Feed Intake, Insulin, Glucagon, Propionate, Glucose, Sheep.)

I ntrod uction

Level of feed intake is a major factor limiting production in ruminants (Balch, 1976), but the nature of the physiological factors controlling feed intake is not well understood. Various blood metabolites and hormones have been suggested to be possible short-term signals in the control of meal feeding (Baile, 1975; Bassett, 1975; Bhattacharya and ALulu, 1975; Chase et al., 1977a,b). Recently, we reported that when insulin was administered to sheep in amounts producing blood concentrations within physiological ranges, feed intake was decreased (Deetz and Wangsness, 1980). We concluded, therefore, that insulin may function as part of a humoral feedback mechanism to bring about meal termination. However, as it may function in the control of feeding, insulin should be considered along with other hormones and metabolites, such as glucagon and the VFA. More specifically, insulin secretion and hepatic gluconeogenesis (Bassett, 1975; Chase et al., 1977b) are associated closely with meal feeding. Furthermore, it has been suggested that a balance of insulin and glucagon may be important in glucose homeostasis (Bassett, 1975); and propionate, a major gluconeogenic precursor and possible stimulator of insulin secretion, has been impli-

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cated in the control of feeding (Bhattacharya and Alulu, 1975). Thus, the present study was conducted to determine whether injections of insulin, glucagon and propionate, administered singly and in combination, to produce physiological increases of each hormone in plasma, would influence feed intake.

The specific objectives were: (1) to determine the effect of the treatments on 24-hr (total daily) feed intake, and (2) to determine the effect of the treatments on plasma concentrations of the corresponding constituents during a meal.

Materials and Methods

Animals and Feeding. Crossbred wether sheep averaging 39.3 kg body weight (BW) were used to study the effect on feed intake of intrajugular injection of physiological amounts of insulin, glucagon and propionate, administered singly and in combination. A complete mixed diet (25% chopped hay:75% cracked corn), similar to the concentrate diet used in previous work (Deetz and Wangsness, 1980), was fed ad libitum. Animals were adjusted to the diet 10 days before the initiation of an experiment, after which they were fitted with indwelling jugular catheters 3 days before the start of an experiment. The same catheter was used in each sheep for injection and blood sampling, and an external extension catheter enabled stress-free injection of the treatments and blood sampling. The catheter was flushed with saline immediately after injection of a treatment to minimize contamination of the blood samples. Sheep were housed in individual pens in a room with a controlled environment (24 C at 40% relative humidity) and were separated, with each having its own feed and water buckets. Removable feed buckets facilitated feed weighbacks.

Treatments and Sampling Schedule. Calculation of insulin\(^5\), glucagon\(^6\) and propionate\(^7\) doses were based on average literature values for basal blood concentrations of 38.9 \(\mu\)U/ml plasma (Trenkle, 1970; Lofgren and Warner, 1972; Berzins and Manns, 1974; Bhattacharya and Alulu, 1975; Bassett, 1975), 177 pg/ml plasma (Bassett, 1975; Brockman and Johnson, 1977) and 2.5 mg/100 ml plasma (Evans et al., 1975), respectively, and on a plasma volume of 51.1 ml/kg BW (MacFarlane et al., 1959; Hodgetts, 1961). Treatment amounts were 6 mU insulin/kg BW, 9 ng glucagon/kg BW and 1.3 mg propionate/kg BW; these were designed to produce plasma concentrations of each constituent within a physiological range normally observed in ruminants before eating. Physiological saline (9%, 10 ml) was used as the control and as the carrier for the treatments. A quantity of sodium propionate calculated to be equivalent to the acid form required to prepare the propionate treatment was dissolved in saline (pH 7.6). In previous work (Deetz and Wangsness, 1980), the injection of 6 mU insulin/kg BW produced three times the basal, prefeeding insulin concentration at 15 min after injection; this dose decreased feed intake in sheep 1 hr after injection, and this was the insulin dose used in the present study.

Exp. 1. This study was conducted to determine the effects of intrajugular administration of the treatments on 24-hr (total daily) feed intake. The sheep were given the treatments via Harvard infusion pumps at the beginning of each meal occurring over a 24-hr period. Automated injections, 3.15 ml in volume and 1 min in duration, were administered at the beginning of each meal. The other two sheep were injected on the alternate day, thus providing a day on which no treatments were administered and eliminating possible residual effects from the previous injections.

A meal was defined as 1 min of eating activity preceded by 20 min of noneating activity (Wangsness et al., 1976). Each wether had to break a photobeam located across its feed bucket for a minimum of 1 min in order to receive an injection. After receiving an injection, the animal was required to remove its head from the feed bucket for a minimum of 20 min, a procedure which reset a series of electronic timers; if, after the waiting period, the animal broke the photobeam for 1 min, it received another injection. Feed was removed 1 hr before the beginning of an experimental day; therefore, an infusion could be administered 1 min after initiation of the first

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\(^5\) Regular Iletin\(^\text{®}\) (bovine-porcine Neutral Regular Insulin), Eli Lilly and Co., Indianapolis, IN 46206.

\(^6\) Glucagon (bovine-porcine glucagon as the solubilized hydrochloride), Eli Lilly and Co., Indianapolis, IN 46206.

\(^7\) #U335, J. T. Baker Chemical Co., Phillipsburg, NJ 08865.
meal. Treatments were assigned randomly, and total daily feed intakes were recorded on the noninjection day so that it could determine whether intakes returned to pre-experimental levels.

Exp. 2. This experiment was conducted to determine the effects of the treatments on plasma concentrations of insulin, glucagon, propionate and glucose during a meal. Feed was removed 1 hr before intrajugular injection of the treatments. The sheep were refed immediately after injection. As in Exp. 1, between each treatment day, there was one day on which no treatment was administered.

Blood was sampled at 15, 30, 60 and 120 min after injection of the treatment and meal initiation. A blood sample was obtained 15 min before injection of the treatment. Blood samples (10 ml) were immediately placed on ice in heparinized tubes. Each catheter was flushed with 10 ml of .9% saline after treatment and blood sampling. Heparinized saline (150 U/ml) was maintained in the catheter during nontreatment periods and was removed before the beginning of an experimental day. Benzamidine (7.8 mg/ml whole blood) was added to each tube to retard glucagon degradation.

Plasma Analyses. Hematocrits were measured at each blood sampling time. Blood was centrifuged at 1,110 × g at 10 C for 20 min to remove plasma. Plasma was stored at -60 C in three individual vials to avoid the need to thaw and refreeze repeatedly. Plasma glucose was analyzed by the Sigma #510 glucose oxidase procedure. Insulin was assayed by a double antibody radioimmunoassay procedure described previously (Vasilatos and Wangsness, 1980). Intra- and interassay coefficients of variation for insulin were 3.7 and 5.5%, respectively. Plasma glucagon was analyzed by competitive binding radioimmunoassay with antibovine-porcine glucagon. Dextran-coated charcoal was used for the final separation. Bovine-porcine glucagon was the standard. The intra- and interassay coefficients for the glucagon radioimmunoassay were 8.7 and 8.3%, respectively (R. J. Martin, personal communication). The minimum detectable limit of the assay was 20 pg/tube. Cross-reactivity of the pancreatic bovine-porcine glucagon antibody has previously been reported (Unger et al., 1961; Eisentraunt et al., 1968). Plasma propionate was measured by a modified version of the methods of Erwin et al. (1961) and Simkins (1965) as summarized by Chase et al. (1977a).

Statistical Procedures. Feed intake, plasma insulin, glucagon, glucose and propionate data were analyzed for treatment effects with a split-plot Design (Steel and Torrie, 1960). Analyses were performed with Statistical Analysis System (SAS) programs maintained at The Pennsylvania State University Computation Center (Barr et al., 1976). Preinjection values of the plasma constituents for each variable were used as covariates in the analysis of treatment effects. One-way analysis of variance was used to test for time effects within treatment for each variable. Treatment means were tested by Least Significant Difference (Steel and Torrie, 1960).

Results

Feed Intake. The effects of intrajugular injections of physiological amounts of insulin, glucagon and propionate, administered singly and in combination, on 24-hr (total daily) feed intake are shown in figure 1 (Exp. 1). Insulin and glucagon decreased (P<.01) 24-hr feed intake by 18.5 and 15.8%, respectively, in comparison to that observed with the saline control. Insulin plus propionate and glucagon plus propionate also decreased (P<.05) 24-hr feed intake, by 11.0 and 11.8%, respectively. The average number of infusions per sheep for a 24-hr period was eight, and intakes returned to preexperimental amounts on non-injection days.

Plasma Insulin. Insulin concentrations in plasma (figure 2, Exp. 2) were increased (P<.05) only at 15 min after injections of insulin and insulin plus propionate and were slightly increased (P<.10) after injection of glucagon plus propionate. Concentrations of insulin in plasma after administration of
the respective treatments were increased to 2.0, 2.1 and 2.0 times the preinjection values.

**Plasma Glucagon.** Glucagon concentrations in plasma (figure 3, Exp. 2) were increased (P<.01) only at 15 min after injection of glucagon and were slightly increased (P<.10) after injection of glucagon plus propionate. The concentrations of glucagon in plasma after administration of the respective treatments were increased to 2.0 and 1.5 times the preinjection values.

There was a slight increase in insulin and glucagon in plasma at 15 min after meal initiation and after administration of the other treatments. Glucose and propionate in plasma were not influenced by the treatments, and hematocrits were stable during blood sampling.

**Discussion**

Table 1 is presented to facilitate an overview discussion of feed intake and plasma hormone responses to the various treatments infused intrajugularly.

The decrease in feed intake induced by insulin alone as a blood-borne feedback signal involved in the overall mechanism(s) controlling meal feeding and the observed increase in plasma insulin agree with findings from a previous report (Deetz and Wangsness, 1980). In that report, the insulin dosage, 6 mU/kg BW, appeared to decrease feed intake during a meal, and the decrease was maximal at 15 min after intrajugular injection; this was the insulin dose used in the present study. Blood was sampled at 15 min after injection of the treatments and meal initiation in both studies. If blood samples had been obtained sooner than 15 min after injections, higher plasma concentrations of the injectates probably would have been observed. Thus, the intake responses may have been related to earlier events occurring between 0 and 15 min after injection. This blood sampling schedule, however, does not negate the feed intake responses observed in both studies.

The rapid increase in plasma insulin observed within 5 min after initiation of a spontaneous meal in ruminants (Bassett, 1975; Chase et al., 1977a,b) provides indirect support for the concept that rapid blood changes are at least associated with meal feeding. The present study provides no direct evidence about the mechanism(s) by which insulin decreases feed intake. However, it is possible that the insulin effect, and effects of the other treatments in the present study, could be associated with tissue utilization of glucose and(or) gluconeogenesis.

Baile and Martin (1971) reported that a high dose of insulin (67 U/meal) administered intravenously did not influence feeding behavior, meal duration or meal frequency in sheep. The highest insulin dose (74 U/meal) used in a previous study (Deetz and Wangsness, 1980)
HUMORAL INFLUENCE ON FEEDING IN SHEEP

Figure 3. Plasma glucagon at 15 min in sheep fed a concentrate diet and given insulin (I, 6 mU/kg BW), glucagon (G, 9 ng/kg BW), propionate (P, 1.3 mg/kg BW) and a saline control (S) intrajugularly at the initiation of the meal. Means (± SEM) within treatment bearing different superscripts differ (P<.01, except GP, P<.10). Plasma concentrations at 0 min represent preinjection values.

The possibility exists that insulin decreased feeding in this study by indirectly affecting the glucose utilization rate, even though a change in plasma glucose was not detected.

The effects of insulin on gluconeogenesis, especially in ruminants, as related to the control of feeding, are not well understood. Even though insulin is known to decrease hepatic glucose output in sheep (Bassett, 1978), some workers have suggested that prandial increases in insulin mediate satiation by increasing hepatic uptake of existing small amounts of propionate in the portal circulation (Bhattacharya and Alulu, 1975). The latter hypothesis is suggested by our observation that insulin plus propionate decreased feed intake even though a change in plasma propionate was not detected. It is possible that plasma propionate would have been increased if blood samples had been obtained sooner than 15 min after injection.

A consideration of possible relationships between insulin and glucagon was of central importance. In ruminants, the relationships are not as well documented as they are in nonruminant animals. The extent of endogenous insulin release during alimentation is determined largely by the integrated action of vagal reflex activity, gastrointestinal hormones and pancreatic glucagon. This was evidenced in this study by the fact that insulin concentrations were slightly increased in plasma even after the administration of saline. Glucagon causes insulin release from the beta-cells and raises blood glucose, first by glycogenolysis and second by gluconeogenesis. Bassett (1975) has summarized the studies that point toward interactions of insulin and glucagon in peripheral tissues and their involvement in hepatic metabolism. Glucagon also has been shown to stimulate glucose

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aBy comparison with intake observed after administration of saline control.
output by the liver in nonruminants (Unger, 1971; deCastro et al., 1978). Thus, it is possible that the glucagon plus propionate treatment (table 1) was associated with enhanced hepatic gluconeogenesis, with a slight resultant increase in plasma insulin and, therefore, an increase in peripheral glucose utilization. However, it would appear that the concentration of propionate administered with glucagon was not sufficient to induce through the mechanisms described above a depression in feed intake beyond that induced by glucagon alone.

Reports on the possible involvement of propionate as a feedback to terminate a meal have been inconclusive (Baile, 1971; Baile and Forbes, 1974; Anil and Forbes, 1977; Papas and Hatfield, 1978) and suggest that propionate alone does not influence feed intake. In the present study, the injection of propionate in physiological amounts over a 24-hr period also did not affect feed intake.

The significant effects of insulin and glucagon on feed intake, discussed above, along with the absence of the effect of propionate alone, suggest that insulin, glucagon and propionate may interact in the complex mechanism(s) controlling meal feeding in sheep. Of importance in future studies will be the measurement of feeding responses under undisturbed feeding situations after the administration of treatment(s) that produce changes in blood hormone and metabolite levels (Chase et al., 1977b) and changes in tissue metabolism that animals normally undergo.

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