Failure of Guinea Pig Antibodies to Beef Insulin, Chicken Insulin, and Cod Insulin to Neutralize Capybara Insulin

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SUMMARY

Guinea pig and coypu insulins are the only mammalian insulins known to be non-neutralizable by antibody to beef insulin. A third non-neutralizable mammalian insulin, that of the capybara (carpincho, or hydrochoerus hydrochoerus) has been identified. Capybara pancreas yielded 0.5 U. extractable insulin per gram. An amount of guinea pig antibody to beef insulin sufficient to neutralize thirty times as much beef insulin did not significantly alter the increase in glycogen content produced by pancreatic capybara insulin in the mouse hemidiaphragm in vitro. Both 50 mU. capybara insulin and 50 mU. capybara insulin plus 150 mU. antibody to beef insulin when injected into mice produced about the same number of convulsions and lowered the blood glucose to about the same mean level as did 50 mU. beef insulin alone. Mice injected with 50 mU. beef insulin plus 150 mU. antibody to beef insulin did not convulse and had a mean blood glucose of 156 mg. per 100 ml. Antibody to chicken insulin and antibody to cod insulin did not significantly alter the increase in glycogen content produced by pancreatic capybara insulin in the mouse hemidiaphragm in vitro.

Capybara pancreatic extract cross-reacted with guinea pig antiserum to beef insulin in the passive cutaneous anaphylaxis test. DIABETES *18*:212-15, April, 1969.

Two mammalian insulins (guinea pig and coypu) are known to be non-neutralizable by antibody to beef insulin.[1] The following observations indicate that there is a third mammalian insulin that is not neutralized by guinea pig antibody to beef insulin, namely that of the capybara (carpincho, or hydrochoerus hydrochoerus). In this study, it was also noted that capybara insulin was not neutralized by guinea pig antibodies to chicken insulin and to cod insulin.

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MATERIALS AND METHODS

Preparation and assay of capybara pancreatic insulin activity

An overnight fasted, 11.5 kg. female capybara was anesthetized with Nembutal and exsanguinated through a carotid cannula. The pancreas, which weighed 14.8 gm., was removed surgically and frozen between slabs of solid carbon dioxide. Later the pancreas was extracted with acid alcohol and prepared for testing by the dialysis method.[2]

The pancreatic extract was assayed for its insulin activity content by the mouse hemidiaphragm insulin assay.[3] It contained 0.50 U. insulin per gram.

Preparation of guinea pig sera containing antibodies to beef insulin, to chicken insulin, and to cod insulin

Guinea pig antibodies to beef insulin (Ab_1), to chicken insulin (Ab_2), and to cod insulin (Ab_3) were prepared by the method of Moloney and Coval[4] and their neutralizing potency determined as previously[3] described. Each milliliter of the immune serum to beef insulin was capable of neutralizing 600 milliunits (mU.) beef insulin, each milliliter of the immune serum to chicken insulin was capable of neutralizing 600 mU. of chicken insulin (one milliliter was also capable of neutralizing 600 mU. beef insulin), and each milliliter of the immune serum to cod insulin was capable of neutralizing 600 mU. of cod insulin.

Determination of insulin activity in solutions containing capybara insulin with and without guinea pig antibody to beef insulin (Ab_1), with and without guinea pig antibody to chicken insulin (Ab_2), and with and without guinea pig antibody to cod insulin (Ab_3)

The hormonal activities of beef and capybara insulins in the absence and presence of guinea pig antibody to beef insulin (Ab_1) were compared by measuring three insulin-responsive metabolic variables: (1) increase in glycogen of mouse hemidiaphragm in vitro, (2) convulsions in mice, and (3) blood glucose lowering in mice. The hormonal activities of beef and capybara insulins (as measured by glycogen increase in mouse hemidiaphragm) in the absence and presence of guinea pig antibody to chicken insulin (Ab_2) and in the absence and presence of guinea pig antibody to cod insulin (Ab_3) were also compared.

1. *Mouse hemidiaphragm method*: Aliquots of extracts of capybara pancreas containing 0.1 mU., 0.2 mU., and 0.4 mU. were mixed with aliquots of guinea pig serum containing enough antibody to neutralize 3 mU. beef insulin and tested.

Aliquots of extracts of capybara pancreas containing 0.3 mU. were mixed with aliquots of antibody to beef insulin (Ab_1), of antibody to chicken insulin (Ab_2) and of antibody to cod insulin (Ab_3), in amounts sufficient to neutralize 3 mU. of either beef (Ab_1), chicken (Ab_2), or cod (Ab_3) insulin.

The mean relative glycogen contents of hemidiaphragms incubated in buffer, in buffer with antibody, in a solution of beef insulin with and without antibody, and in a solution of capybara insulin with and without antibody were determined.

2. *Mouse convulsion method*: Twenty-gram Canadian Breeding Laboratory albino male mice were deprived of food but not water for four hours and then randomly assigned to one of four test groups of eleven mice each. One group was injected subcutaneously with 50 mU. beef insulin alone; one group was injected with 50 mU. beef insulin plus 150 mU. antibody to beef insulin; one group was injected with 50 mU. capybara insulin alone; one group was injected with 50 mU. capybara insulin plus 150 mU. antibody to beef insulin. Immediately after injection, the mice were grouped and placed in compartments of a constant temperature bath at 38° C. The mice were observed for convulsions for a period of 120 minutes.

3. *Mouse blood glucose lowering method.* Mice that convulsed were immediately removed from the compartment of the bath and decapitated with scissors. Blood was collected for glucose determination by the glucose oxidase method.[5] Mice that did not convulse were decapitated 120 minutes after injection and blood was collected for glucose determinations.

Test of cross-reactivity between capybara pancreatic extracts and beef insulin antiserum in the passive cutaneous anaphylaxis reaction in guinea pigs

Normal depilated guinea pigs were injected intradermally with 0.1 ml. aliquots of various sera from beef insulin-immune guinea pigs. The guinea pigs were challenged intravenously twenty to twenty-two hours later with 5 mU. capybara insulin in 1 ml. saline containing 5 mg. pontamine sky blue 6 BX. The appearance of a blue spot at the site of intradermal injection of antiserum within ten to twenty minutes after intravenous injection of an insulin preparation indicates that an antigen-antibody reaction has taken place.[6] Local tissue anaphylaxis increases vascular permeability, and this in turn causes leakage of pontamine sky blue into the skin. Although a positive reaction between an antibody to a highly purified insulin and a highly purified insulin indicates that a reaction has taken place between insulin and the antibody to insulin, the presence of noninsulin antibodies or of impurities in the injected insulin preparation can also give a positive PCA reaction. The extracted capybara insulin used in this study was not highly purified.

EXPERIMENTAL RESULTS

Guinea pig antibody to beef insulin completely neutralized the glycogen content-increasing effect of beef insulin; antibody to beef insulin did not significantly alter the glycogen content-increasing effect of pancreatic capybara insulin (figure 1). Table 1 compares the

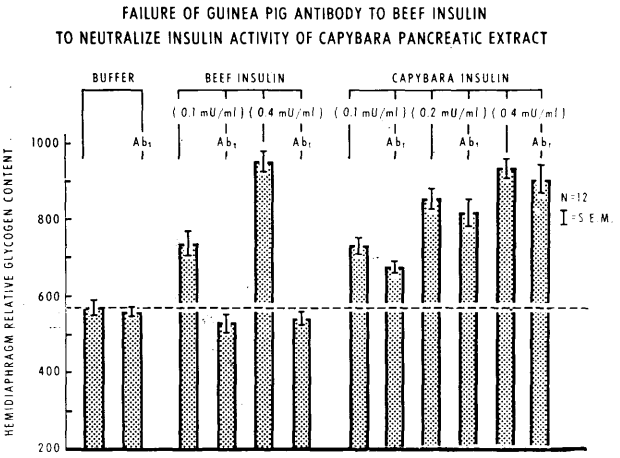

FIG. 1. Each incubation tube contained sufficient guinea pig antibody to beef insulin (Ab_1) per milliliter to neutralize 3 mU. beef insulin. S.E.M. = standard error of the mean. The glycogen content of mouse hemidiaphragms incubated in the presence of capybara insulin alone and capybara insulin plus beef insulin antibody were not significantly different at P = .05.

TABLE 1

Convulsions and terminal blood glucose levels in mice after injections of beef and capybara insulin with and without antibody to beef insulin.

	50 mU. beef insulin	50 mU. beef insulin + 150 mU. beef insulin antibody	50 mU. capybara insulin	50 mU. capybara insulin + 150 mU. beef insulin antibody
Number of mice that convulsed	10 of 11	0 of 11	9 of 11	11 of 11
	Mean ± S.E.M.	*Mean ± S.E.M.*	*Mean ± S.E.M.*	*Mean ± S.E.M.*
Terminal blood glucose (mg./100 ml.)	20 ± 2	156 ± 15	21 ± 4	18 ± 1

effects of beef insulin and pancreatic capybara insulin injected with and without antibody to the effects of an equivalent amount of beef insulin alone in producing convulsions and in lowering the blood glucose of mice. Both capybara insulin alone and capybara insulin plus antibody to beef insulin when injected into mice produced about the same number of convulsions and lowered the blood glucose to about the same mean level as did beef insulin alone. Mice injected with beef insulin plus antibody to beef insulin did not convulse and had blood glucose levels that were slightly above the normal fasting level. Beef insulin was neutralized not only in the presence of antibody to beef insulin, but was also neutralized in the presence of antibody to chicken insulin (figure 2). It was not neutralized in the presence of antibody to cod insulin (figure 2). Capybara insulin was not neutralized by antibody to beef insulin, by antibody to chicken insulin, or by antibody to cod insulin (figure 2).

In the PCA test, 5 mU. of capybara pancreatic extract gave a moderate to strong reaction with beef insulin antiserum in each of four guinea pigs. Average diameter of blue spots was 25 mm. with a range of diameters between 15 and 30 mm.

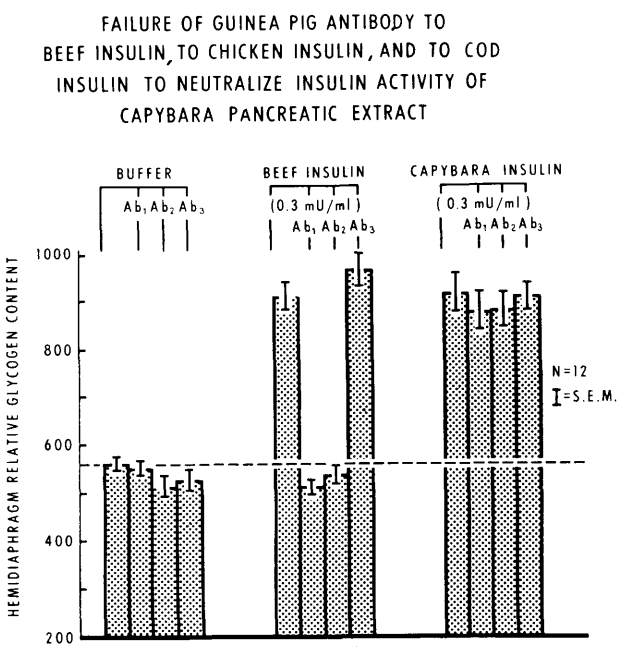

FIG. 2. Each incubation tube contained sufficient guinea pig antibody to beef insulin (Ab_1), to chicken insulin (Ab_2), or to cod insulin (Ab_3) per milliliter to neutralize 3 mU. beef insulin (Ab_1), chicken insulin (Ab_2), or cod insulin (Ab_3). S.E.M. = standard error of the mean.

DISCUSSION

The results of the current study indicate that the hormonal activity of pancreatic capybara insulin as measured by the mouse convulsion test and the mouse blood glucose-lowering test is not significantly altered by a three-fold excess of antibody to beef insulin, nor is it significantly altered by a thirty-fold excess of beef insulin antibody when glycogen synthesis by the mouse hemidiaphragm is the metabolic parameter of insulin action used. Hormonal activity of pancreatic capybara insulin was not significantly altered by the presence of a ten-fold excess of antibody to chicken or cod insulin.

A previous report[1] indicated that cross-reactivity of exogenous and endogenous guinea pig and coypu insulins with beef insulin antibody is undetectable in the hormone neutralization tests that have been carried out. The results of this study indicate that there is no significant cross-reactivity of exogenous capybara insulin with beef insulin antibody in the hormone neutralization tests that were carried out. There was also no evidence of cross-reactivity between capybara pancreatic extract and the antibodies to chicken or cod insulin.

Most mammalian pancreatic insulins, including beef, sheep, pig, horse, whale, dog, cat, rabbit, rat, Chinese hamster, mouse, monkey, and man are neutralizable by serum of guinea pigs immune to beef insulin. Re-

cently the pancreatic insulin of the chinchilla and the muskrat[7] have been shown to be neutralizable by serum of guinea pigs immune to beef insulin. Many endogenous mammalian insulins are neutralizable by antibody to beef insulin, either when the native serum is tested in vitro or when the antibody is injected into an animal with neutralization of the animal's endogenous insulin and creation of an antibody-induced diabetic state.

The two previously reported non-neutralizable mammalian insulins differ markedly in structure from that of beef insulin, both guinea pig insulin and coypu insulin having about eighteen amino acid residues that differ from those in beef insulin. The structure of capybara insulin is not known, but its non-neutralizability in the presence of beef insulin antibody suggests that it too differs considerably in structure from beef insulin. Its non-neutralizability in the presence of chicken and cod insulin antibodies suggests that it differs significantly in structure from those insulins as well.

There are several possible explanations for the fact that immune guinea pig serum possessed PCA reactivity but did not possess measurable hormone neutralizing activity in the presence of capybara insulin: (1) the IgA class of antibodies is responsible for a positive PCA test, whereas the class (or classes) of antibodies responsible for hormone neutralization is (are) not known; thus different classes of antibodies may be involved in PCA reactivity and in neutralization of hormonal activity[1]; (2) cross-reaction between capybara insulin and neutralizing antibodies may occur, but binding affinity may be so low that quantitative binding of capybara insulin to living tissue is not significantly affected[1]; (3) there may have been a cross-reacting haptenic site in the impurities present in the capybara pancreatic extracts; (4) antibodies to impurities in the beef insulin used to immunize the guinea pigs may have reacted with impurities in the capybara pancreatic extract. These problems can be resolved only by preparing highly purified capybara insulin and different classes of guinea pig antibodies to highly purified beef insulin, and then carrying out parallel PCA tests and quantitative antibody-insulin binding studies in the presence and absence of living tissue.

ACKNOWLEDGMENT

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