

Insulins of Hystricomorph Rodents

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SUMMARY

The ability of a guinea pig antibeef/pork insulin serum to neutralize the endogenously secreted insulins of five species of hystricomorph rodents has been studied. Endogenous insulins of the tuco-tuco, casiragua and degu were apparently not neutralized, but endogenous insulins of the cuis and chinchilla did appear to be neutralized. *DIABETES* 22: 851-53, November, 1973.

Antibovine insulin antibodies inactivate endogenously secreted insulin in those species whose insulins do not differ substantially from bovine insulin. A state of transient acute diabetes, characterized by raised blood glucose levels, can be provoked in the mouse,¹ cat, rat, rabbit,² dog, sheep and pig³ by intraperitoneal injection of serum from guinea pigs immunized with crystalline bovine insulin. Hyperglycemia does not occur in guinea pigs (*Cavia porcellus*) injected with guinea pig antibovine insulin serum,² indicating that neutralization of circulating guinea pig insulin has not taken place. When the insulin of the coypu (*Myocastor coypus*) was tested in the mouse in the presence of a considerable excess of bovine insulin antibody, glycogen content in the hemidiaphragm in vitro was increased¹ and blood glucose levels in vivo were lowered.⁴ It has been reported also that capybara (*Hydrochoerus hydrochaeris*) insulin is not neutralizable by bovine insulin antibodies.

The guinea pig and the coypu both possess insulins whose primary structures are markedly different from those of all other mammalian species studied.^{6,7} The guinea pig has nineteen and the coypu twenty-two amino acid residues, which differ from the fifty-one of beef insulin; guinea pig and coypu insulins differ

from each other in seventeen places.⁷ The structure of capybara insulin is not known, but it is reasonable to suppose, since it is not neutralizable, that its primary structure also differs from that of bovine insulin. If it is assumed that the extensive amino acid substitutions have not altered the tertiary structure of the molecule, it seems likely that the antigenic sites of these insulins have been altered.

The guinea pig, coypu and capybara are all rodents of the suborder Hystricomorpha. It was of interest, therefore, to investigate the cross-reactivity of the insulin of some other hystricomorphs to bovine insulin antibodies.

MATERIALS AND METHODS

The hystricomorph species used were the chinchilla, degu, tuco-tuco, casiragua and cuis (table 1). Breeding colonies of these species were available at the Wellcome Institute of Comparative Physiology, Zoological Society of London, and details of their management have been described.⁸⁻¹⁰ The domestic guinea pig and the Wistar laboratory rat were used as controls since they were known to possess insulins that, respectively, do not and do cross-react.

Active healthy animals were not fasted beforehand, but food was withheld during each experiment. Blood samples (0.05 ml.) were obtained by cardiac puncture from the guinea pig, from the tail vein of the rat and by retrobulbar technic^{11,12} from the other species. Halothane (Fluothane, I.C.I.) was used to produce light anesthesia during blood sampling of the tuco-tucos, degu and casiragua. Immediately after an initial bleeding, test animals were injected intraperitoneally with 1 ml. of a commercially available guinea pig antibeef/pork insulin serum (Wellcome Reagents Ltd.) (table 1). This antiserum is capable of neutralizing at least one international unit of ox insulin, measured by the effects on glucose uptake and glycogen formation in the isolated rat hemidiaphragm.¹³ It was not possible to do a dose response test for all the species involved, but preliminary tests showed that 1.0 ml. of the antiserum used was effective in 2 kg. rabbits. An equal number of con-

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TABLE 1

Species of the rodent suborder Hystricomorpha¹⁶ whose insulins have been compared with that of the ox

Superfamily	Family	Species	Common name	No. used	Av. wt.	Trend of response to antiserum	Source
Cavioidea	Caviidae	<i>Cavia porcellus</i>	Guinea pig	1	?	Normoglycemia	Armin et al. ² (1960)
		<i>Galea musteloides</i>	Cuis	8	400 gm.	Normoglycemia	Present study
	Hydrochoeridae	<i>Hydrochoerus hydrochaeris</i>	Capybara	7	350 gm.	Hyperglycemia	Present study
Octodontoidea	Capromyidae	<i>Myocastor coypus</i>	Coypu	1	11.5 kg.	(Not neutralized in vitro)	Davidson et al. ⁵ (1969)
	Ctenomyidae	<i>Ctenomys talarum</i>	Tuco-tuco	7	6.2 kg.	(Not neutralized in vitro)	Davidson et al. ⁴ (1968)
	Octodontidae	<i>Octodon degus</i>	Degu	7	150 gm.	Normoglycemia	Present study
	Echimyidae	<i>Proechimys guairae</i>	Casiragua	11	250 gm.	Normoglycemia	Present study
Chinchilloidea	Chinchillidae	<i>Chinchilla laniger</i>	Chinchilla	?	?	(Neutralizable ? in vitro)	Davidson et al. ⁵ (1969)
				10	400 gm.	Hyperglycemia	Present study

tol animals received 1 ml. of fresh, normal guinea pig serum. The animals were used in six separate experiments, but none was used more than once.

A preliminary study, using at least three animals of each species and sampling at three times within the first hour, showed that the highest blood glucose levels¹⁴ in any one animal in the test occurred at one, two or four hours after injection of antiserum. Blood glucose levels were therefore measured at these times in subsequent tests and the results were expressed as percentages of the initial values. The mean of the cumulated results was calculated to depict the over-all recorded hyperglycemia (figure 1).

RESULTS AND DISCUSSION

The experimental approach and presentation show clearly the expected difference in response between the guinea pig and the rat and the similarity of the response of the tuco-tucos, degu and casiragua to that of the guinea pig and of the chinchilla and cuis to that of the rat.

Armin et al.² demonstrated¹ that the highest rate of increase in blood glucose concentration and the highest blood glucose values were obtained¹ in fed conscious rats. The same authors also state that anesthesia has no appreciable effect upon the response of fed rats. Previous work^{10,15} has also illustrated that light anesthesia and/or the sampling technic did not produce hyperglycemia in animals of the species used in the present experiments. Therefore, nutritional status and anesthesia were unlikely to have biased the results. Nonspecific

foreign protein reactions associated occasionally with hyperglycemia have been reported² in animals receiving repeated injections of serum, but since none of the present animals was used twice, any hyperglycemia recorded¹ could not be attributed to anaphylaxis.

Since optimal doses of antiserum for the species used were not known, we elected to use a standard dose which was known to be effective in the rat and 2 kg. rabbit. The average body weights of all the hystricomorphs tested was much less than 2 kg., so it was probable that there was an excess of antibody present. No appreciable change in blood glucose levels was apparent in the lightest animals (tuco-tuco and degu), but hyperglycemia was common in the chinchilla and cuis. Because of the scatter of the results and the small number of animals available for study, statistical tests were not applied. However, the direction of the responses observed suggests that the casiragua, degu and tuco-tuco, like the guinea pig, secrete an insulin which does not appear to cross-react with bovine insulin antibodies. The endogenous insulins of the chinchilla and cuis did appear to cross-react and were neutralized. This finding in the chinchilla supports a report that the pancreatic insulin of this species is neutralized by serum of guinea pigs immune to bovine insulin.⁵

Pancreases from these species are being kept for comparison of the primary structure of insulins that cross-react with antibody and those that do not, in order to provide information relating to the antigenic determinants of the insulin molecule. It is possible that not all hystricomorphs possess insulins whose antigenic de-

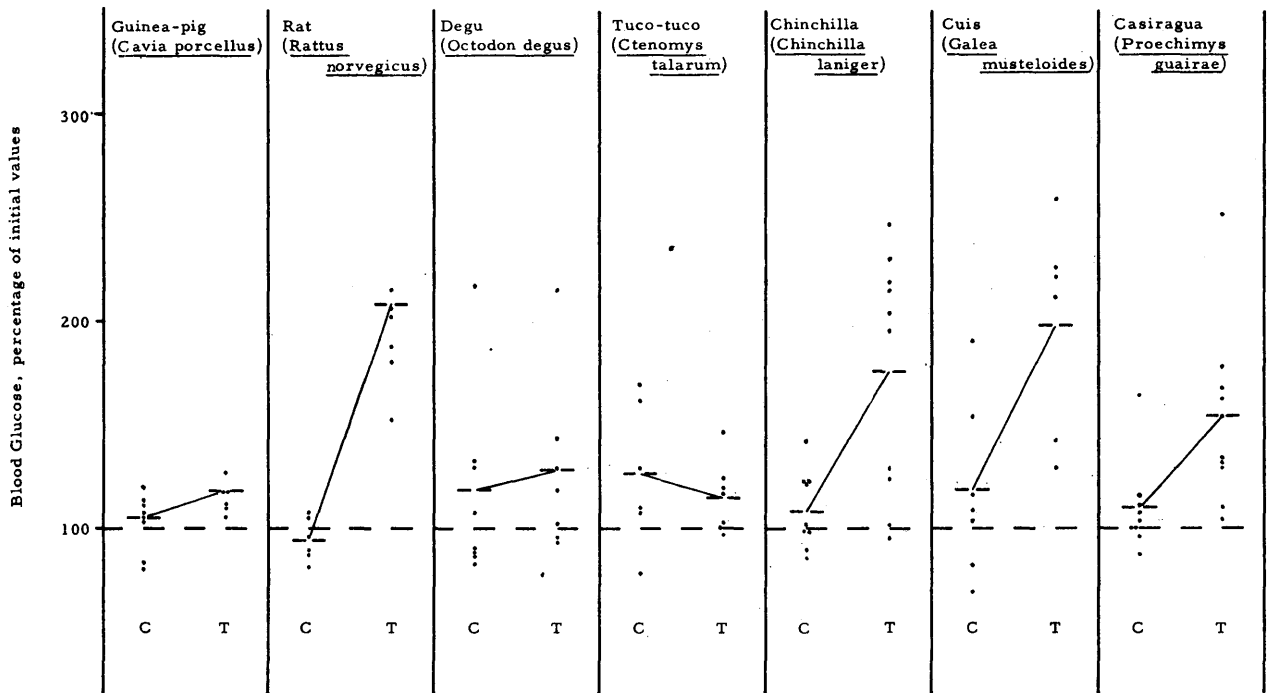


FIG. 1. The effect of guinea pig antibovine insulin serum on six hystricomorph species and the rat. The mean of one, two and four hour cumulated blood glucose values are expressed as percentages of the initial values. T = test (guinea pig anti-insulin serum); C = control (normal guinea pig serum).

terminants differ from those of other mammals, and at present, there is little apparent taxonomic correlation with the trend of response to serum of guinea pigs immunized to beef insulin.

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