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LETTERS TO THE EDITOR

The Effect of γ-Guanidinobutyramide on the Secretion and Synthesis of Insulin in vitro

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It has been found by Butterfield *et al.* [1] that γ-guanidinobutyramide (HL-523), a compound with structural similarities to arginine and phenethylbiguanide, plays the role of a regulator in the metabolism of carbohydrates and proteins. This substance is an intermediary in the metabolism of arginine to GABA in bacteria; and the free acid appears in the urine of humans after meals.

From experiments carried out on humans and animals, it is known that γ-guanidinobutyramide produces a significant decrease in the values of blood urea. This decrease is not related to any alteration in renal haemodynamics, but seems to derive from the urea cycle and the metabolism of the aminoacids.

Equally, γ-guanidinobutyramide produces very significant decreases of glycaemia in experimental animals, which in some cases cause hypoglycaemic shock. The hypoglycaemic effect is accompanied by a large glucose uptake by muscle, which leads us to the conclusion that under these experimental circumstances a secretion or release of insulin could have been produced.

With the object of clarifying this possible effect, we have studied the action of γ-guanidinobutyramide on the synthesis and release of insulin *in vitro* (secretion). For this we took advantage of an experimental model which we use in our laboratory; this allows us to reproduce biosynthetic and secretory sequences which occur *in vivo* in the animal. Other studies at the biochemical level and using optical and electron microscopy have validated this model.

We used rabbits of 1.5–2.0 kg body weight previously fasted for 24 hours with water *ad libitum*. After the animals were killed the pancreas was dissected *in situ* and removed. The gland was divided by a transverse cut into two equal portions, one corresponding to the region of the head and the other to that of the tail. Each of these portions was divided in turn into two others by a longitudinal cut, and a piece of each one of these sections was incubated in each vessel. Each vessel therefore contained a piece corresponding to the head of the pancreas and another to the tail.

Since a spontaneous secretion of insulin occurs after the excision of the pancreas, the two vessels with the pieces of pancreas were incubated for an hour as a control in order to determine the extent of this spontaneous secretion. After this initial incubation the pieces were placed into other vessels under the conditions to be described later on.

The incubation media was Krebs-Ringer II supplemented in all vessels with glucose (0.6 mg/ml), gelatine (0.6 mg/ml), sodium pyruvate (5mM), sodium fumarate (5mM) and sodium glutamate (5mM). The incubation vessels used to study the effect of γ-guanidinobutyramide (HL-523) contained this compound at a concentration of 1×10^{-3}M. Insulin was measured by the method of Hales and Randle [3]. The insulin in the pancreas was extracted and partially purified by the procedure of Coore and Randle [2].

Table 1 shows the values obtained for the secretion and synthesis of insulin using rabbit pancreas in the absence or presence of γ-guanidinobutyramide (1×10^{-3}M).

Table 1. *The Effect of γ-Guanidinobutyramide on the Secretion and Content of Insulin in rabbit Pancreas Incubated in vitro*

	Preincubation (μU/100 mg tissue)	Incubation (μU/100 mg tissue)	Amount of insulin in the pancreas (μU/100 mg tissue)
Control	87.8 (11)	50.8 (11)	2.92 (8)
With γ-guanidino butyramide	90.8 (11)	71.0 (11)	4.33 (8)
P	> 0.05	< 0.05	< 0.05

The numbers in parenthesis represent the number of experiments carried out

As can be seen, during the first incubation the amount of insulin measured in both media was the same ($P>0.05$), proving the correctness of the experimental model. However, after the addition of γ-guanidinobutyramide (1×10^{-3}M) a significant increase ($P<0.05$) in the secretion of insulin was observed. Similarly, the amount of insulin extracted from the pancreas incubated with the drug showed a significant increase ($P<0.005$).

These results support our hypothesis that one of the effects of γ-guanidinobutyramide is to favour the capacity of the biosynthesis of insulin by the pancreas,

together with an increase in the secretion of this hormone. These data could explain the decrease in the glycaemia observed in man and the various animals used in experiments, which at times produces hypoglycaemic shock.

The interesting point of these results is the fact that the concentrations used (1×10^3M) are much lower in relation to those doses used with other metabolites that stimulate insulin secretion. Thus, glucose, the most powerful physiological stimulant for the secretion of insulin, which possesses a molecular weight equal to that of γ-guanidinobutyramide, is used in concentrations of 3 mg/ml in incubation media, whereas the drug used in this study was added in the proportion of 0.18 mg/ml.

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References

1. Butterfield, W.J.H., Cox, B.D., Whichelow, M.J.: The metabolic effect on diabetes of γ-guanidinobutyramide. 4th Ann. Meeting Europian Association for the study of Diabetes Louvain. 1968.
2. Coore, H.G., Randle, P.J.: Regulation of insulin secretion studied with pieces of rabbit pancreas incubated *in vitro*. Biochem. J. **93**, 66—78 (1964).
3. Hales, C.N., Randle, P.J.: Immunoassay of insulin with insulin antibody precipitate. Biochem. J. **88**, 137—146 (1963).

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