A Species Difference in Nucleoside Phosphorylase Activity and Inosine-Stimulated Insulin Secretion in Isolated Islets of Langerhans

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Inosine is a potent stimulant of insulin release from rat but not from rabbit islets of Langerhans. Further investigation showed that nucleoside phosphorylase activity is exceptionally low in rabbit islets. The ability of inosine to promote insulin release seems to be related to islet nucleoside phosphorylase activity, which can display marked species differences.

Inosine has been shown to stimulate insulin secretion from isolated mouse islets of Langerhans (Capito & Hedekov, 1976) and insulin biosynthesis in isolated rat islets of Langerhans (Jain & Logothetopoulos, 1977). Capito & Hedekov (1976) demonstrated the presence, in homogenates of mouse islets of Langerhans, of a nucleoside phosphorylase capable of splitting inosine to hypoxanthine and ribose 1-phosphate, and concluded that an intermediate of inosine metabolism may trigger insulin release. The activity of a nucleoside phosphorylase may play a key role in stimulating insulin release by nucleosides.

In the present communication, nucleoside phosphorylase activity is reported in islets from two species, and correlated with the effects of inosine on insulin secretion.

Materials and Methods

Inosine, xanthine oxidase and bovine serum albumin fraction V were obtained from the Sigma Chemical Co., St. Louis, MO, U.S.A. Collagenase was from Boehringer, Mannheim, Germany. Insulin radioimmunoassay kits were obtained from CEA-IRE-SORIN Association (CIS), Saluggia, Italy.

All other reagents were of A.R. grade or the purest grade commercially obtainable.

Male white rabbits of a Himalayan strain weighing 2–3 kg were fed *ad libitum* with rabbit pellets and water. Fresh green food was also supplied daily. Male white Sprague–Dawley rats weighing 300–400 g were fed with a constant supply of rat and mouse cubes and water. Animals were killed by cervical dislocation and their pancreases removed. Islets were isolated by a collagenase digestion procedure described in detail by Howell & Taylor (1968).

To determine the insulin secreted, groups of five islets were placed in tubes containing 2 ml of a bicarbonate-buffered salt solution (Gey & Gey, 1936) previously gassed with O₂/CO₂ (19:1) and contain-

ing bovine serum albumin (1 mg/ml) with other additions as stated. The tubes were incubated with shaking at 37°C for 1 h, after which time the medium was removed, and the islets were stored at –5°C pending assay of insulin with the radioimmunoassay kits, with human insulin as standard.

The method used for the nucleoside phosphorylase assay was modified from that described by Bergmeyer *et al.* (1971). Some 200–300 islets were transferred to 50 µl of 0.05 M-sodium phosphate buffer, pH 7.4, and disrupted by ultrasonication for 15 s at 4°C. Nucleoside phosphorylase activity was measured in 1 ml of the above buffer containing inosine (0.26 mM) and xanthine oxidase [0.013 unit (µmol/min)/ml]. The reaction was started with the addition of 20 µl of the homogenate and the A₂₉₃ of the uric acid formed in this reaction was measured. Under these conditions, 1 unit of nucleoside phosphorylase is defined as the amount of enzyme that causes an increase in absorbance of 0.001/min at 293 nm.

The protein content of 10 µl samples of the homogenate was determined by the method of Lowry *et al.* (1951), with bovine serum albumin as standard.

Results and Discussion

Earlier work has shown that inosine is a potent secretagogue for insulin and greatly potentiates the effect of 5 mM-glucose in the mouse (Capito & Hedekov, 1976). In the present paper this has also been shown to be the case for rat islets (Table 1). Inosine is effective alone, and it also produces a 10-fold potentiation of the effects of 5 mM-glucose when tested at two different concentrations. By contrast, rabbit islets show an insignificant response to inosine, either when this is added to the preparation alone or in the presence of 5 mM-glucose (Table 1).

Thus there is a marked species difference in insulin release from isolated islets of Langerhans in response to the nucleoside inosine. Other species differences in the response of islets to a variety of agents are known

Table 1. *Effect of inosine on the rate of insulin release from isolated islets of Langerhans*

Groups of five islets from rabbit or rat were incubated for 1 h at 37°C in 2 ml of a gassed bicarbonate-buffered medium containing 1 mg of bovine serum albumin/ml and other additions as stated. The insulin released was assayed by radio-immunoassay (as described in the Materials and Methods section). Results are expressed as means $\pm$ S.E.M., with the numbers of observations in parentheses. A unit is 40 μ g of insulin.

Animal	Addition to medium	Insulin release (μ units/min per islet)
Rat		
Expt. 1	5 mM-Glucose	0.080 $\pm$ 0.007 (8)*
	5 mM-Inosine	0.171 $\pm$ 0.006 (8)*
	10 mM-Inosine	0.200 $\pm$ 0.006 (8)*
	20 mM-Glucose	1.135 $\pm$ 0.030 (4)*
Expt. 2	5 mM-Glucose	0.086 $\pm$ 0.012 (8)
	5 mM-Inosine + 5 mM-glucose	0.770 $\pm$ 0.033 (8)*
	10 mM-Inosine + 5 mM-glucose	0.830 $\pm$ 0.036 (4)*
	20 mM-Glucose	1.000 $\pm$ 0.062 (8)*
Rabbit		
Expt. 1	5 mM-Glucose	0.073 $\pm$ 0.008 (6)
	5 mM-Inosine	0.100 $\pm$ 0.032 (6)
	10 mM-Inosine	0.087 $\pm$ 0.006 (6)
	20 mM-Glucose	0.370 $\pm$ 0.026 (6)*
Expt. 2	5 mM-Glucose	0.032 $\pm$ 0.005 (8)
	5 mM-Inosine + 5 mM-glucose	0.056 $\pm$ 0.013 (8)
	10 mM-Inosine + 5 mM-glucose	0.045 $\pm$ 0.005 (8)
	20 mM-Glucose	0.210 $\pm$ 0.015 (8)*

* $P < 0.001$ for effects of inosine or 20 mM-glucose compared with 5 mM-glucose controls.

Table 2. *Activity of nucleoside phosphorylase in homogenates of the islets of Langerhans from rabbit and rat*
Islets of Langerhans (200–300) in 50 μ l of 0.05 M-sodium phosphate buffer, pH 7.4, were disrupted by ultrasonic treatment at 4°C. A portion (20 μ l) of the resulting homogenate was then taken and assayed for nucleoside phosphorylase activity. A further 10 μ l of homogenate was taken for protein determination by the method of Lowry *et al.* (1951). Results are expressed as means $\pm$ S.E.M., with the numbers of observations in parentheses. For the difference in enzyme activity between rat and rabbit islets $P < 0.001$.

Animal	Nucleoside phosphorylase activity (units/mg of protein)
Rat	27.50 $\pm$ 2.92 (4)
Rabbit	2.60 $\pm$ 0.52 (4)

to exist. For example, xylitol markedly stimulates insulin release in the dog (Kuzuya *et al.*, 1966), but it has no effect on insulin release in the mouse (Ashcroft *et al.*, 1972). Large species differences in the response to acetoacetate (Hawkins *et al.*, 1971) or to short-chain fatty acid have been recorded (Mann *et al.*, 1967).

Because of the apparent importance of nucleoside phosphorylase activity for the effect of inosine (Capito & Hedekov, 1976), the activity of this

enzyme was examined in islets from the two species. Table 2 shows that the activity of the nucleoside phosphorylase in rat islet homogenates is approx. 11 times that in rabbit islet homogenates when expressed per mg of protein. Differences in nucleoside phosphorylase activity may therefore mirror species differences in the ability of the nucleoside to promote insulin release.

It is unclear whether the effects of nucleosides on insulin biosynthesis and release (Jain & Logothetopoulos, 1977; Ismail *et al.*, 1977) are in any way related to the stimulatory effect of glucose on islets. The observed difference, however, in nucleoside phosphorylase activity between rabbit and rat islets suggests that, for inosine, the primary stimulus for insulin release is a metabolic product of the nucleoside.

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