

# Studies on Synergism between Glucose and Amino Acids with Respect to Insulin Release *in vitro* \*

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The mutual enhancement of the effect of insulin release by glucose and amino acids is not clearly understood. Present *in vitro* studies with isolated rat islets were undertaken to elaborate the role of amino acids on insulin release, particularly their interaction with glucose as well as among each other, which has been reported to lead to synergism in the human subjects.

In the presence of 8.3 mM glucose, both arginine, as well as, leucine potentiated the effect of glucose which increased progressively with the increasing concentrations of the amino acid. This effect of arginine was not synergistic in nature because arginine did not stimulate insulin release in the absence of glucose.

The effect of glucose and leucine was found to be additive and not synergistic.

No synergism was exhibited by any of the amino acid pairs tested in the present study. Thus both phenylalanine and lysine did not potentiate the effect of either arginine or leucine. Arginine showed a mild, but significant potentiating effect on leucine-stimulated insulin release.

It is suggested that synergism between glucose and amino acids and between certain amino acid pairs reported in man may not be due to the direct effects of these stimuli on the beta cells, but some other factors *in vivo* may be involved.

## Introduction

It has been shown that in adult human subjects, glucose and amino acids as well as certain amino acid pairs act synergistically on insulin release [1, 2]. Levin *et al.* [3] noted such a response when glucose was administered to normal subjects prior to the administration of arginine. Similarly, in premature infants, Grasso *et al.* [4] found synergistic effect between glucose and an amino acid mixture. This mutual enhancement of the effect on insulin release by glucose and amino acids was suggested to be due to a direct effect of these stimuli on the beta cells [1, 2].

Use of *in vitro* techniques offers considerable advantage over the complex system of intact animal and helps to evaluate the direct influence of various substances on the pancreas, whereas, in the *in vivo* system a modification of such responses is possible due to an obligatory presence of glucose and other humoral factors. Isolated islets seem to offer a suitable system for such studies and, therefore, it

was decided to assess the direct effect of various agents on the beta cells by using isolated rat islets. A model simulating the *in vivo* conditions with respect to glucose was prepared by adding 8.3 mM glucose to the incubation medium which is approximately equivalent to the normal plasma glucose level of the rat [5]. It was thought that such a model could provide a suitable basis for the comparison of insulin releasing effects of amino acids *in vitro* in rat with those obtained *in vivo* in man because a significant correlation ( $P < 0.02$ ) has been shown between the relative capacity of amino acids to stimulate insulin release in man *in vivo* and from rat pancreatic pieces *in vitro* [6].

## Materials and Methods

Male rats of Sprague-Dawley strain weighing between 300–350 gm were used in these studies. The animals were reared at the Animal House of the Jinnah Postgraduate Medical Centre, Karachi. They were fed *ad libitum* on the standard rat cube diet supplied by the Lever Brothers (Pakistan) Ltd., and were not fasted prior to sacrifice.

Islets were isolated by the collagenase digestion method of Lacy and Kostianovsky [7] and pre-incubated for 30 minutes at 37 °C in lots of 80 to 90 islets in 5 ml of the modified Krebs-bicarbonate

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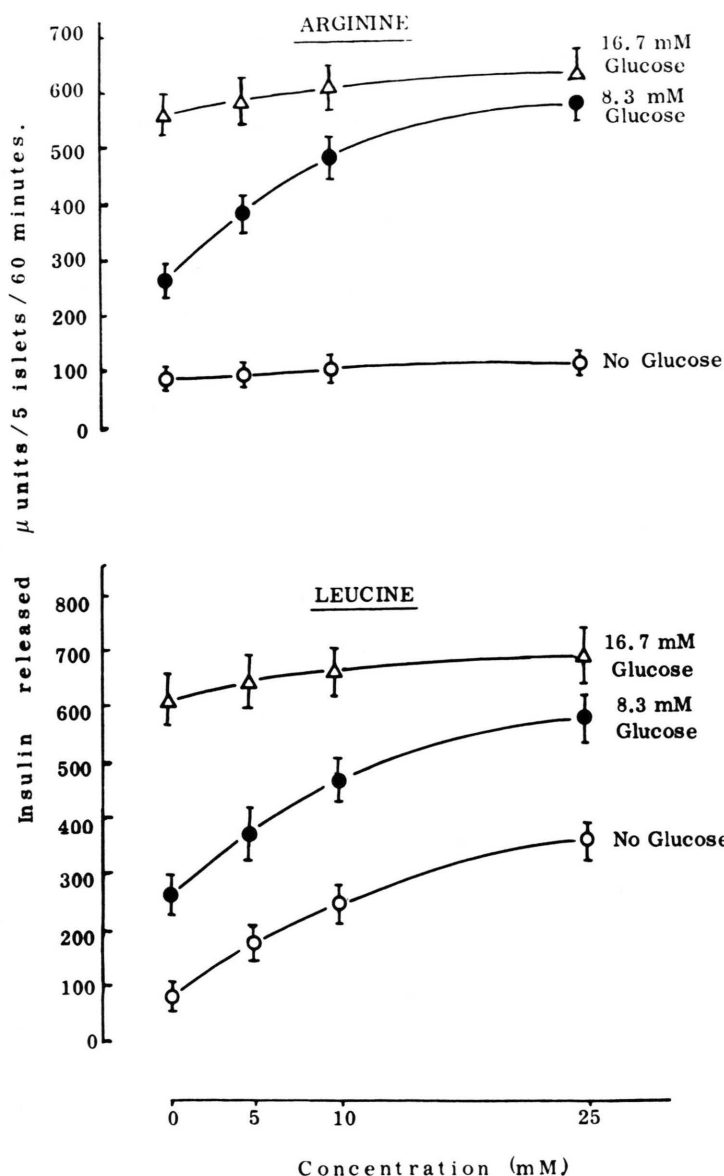


Fig. 1. The effect of arginine or leucine on insulin release by isolated rat islets in the presence of glucose. The insulin released by the islets in the presence of amino acid alone (○—○), and in the presence of glucose 8.3 mM (●—●) or 16.7 mM (△—△) is expressed as mean  $\pm$  s. e. m.

buffer [8] containing 2 mg/ml bovine serum albumin and 1.67 mM glucose. The media were gassed with 95% oxygen and 5% carbon dioxide to a final pH of 7.4. Five islets were transferred to each flask containing 1 ml of fresh medium supplemented with various concentrations of glucose and/or amino acids and incubated for 60 minutes at 37 °C in a metabolic shaker.

Insulin released into the medium was assayed by the radio-immunoassay method of Hales and Randle [9] using the radio-immunoassay kit supplied by the

Radiochemical Centre, Amersham, Buckinghamshire, U. K. Ox insulin was used as a standard and the values reported are bovine insulin equivalents. On account of reports of some discrepancy between the binding characteristics of bovine and rat insulin in the range of 50 to 250  $\mu$ units/ml [10], the insulin samples were diluted appropriately to yield values to less than 50  $\mu$ units/ml.

In all experiments, the rate of insulin release has been expressed as  $\mu$ units/5 islets/60 minutes. The differences in the mean rates of secretion in response

Table I. Effect of arginine in presence of leucine on insulin release from isolated rat islets of Langerhans. The values are expressed as  $\mu$  units of insulin released by 5 islets in 60 minutes  $\pm$  s.e.m. The number of observations is given in parenthesis.

Addition to the medium	Leucine		
	Absent	5 mM	10 mM
Control	95 $\pm$ 4 (24)	182 $\pm$ 17 (18)	272 $\pm$ 24 (15)
Arginine (5 mM)	98 $\pm$ 5 (24)	238 $\pm$ 20 * (18)	348 $\pm$ 23 * (15)
Arginine (10 mM)	108 $\pm$ 7 (24)	—	367 $\pm$ 33 * (15)
Arginine (25 mM)	117 $\pm$ 8 (24)	—	355 $\pm$ 27 * (15)

\*  $P < 0.05$  as compared with the control.

Table II. Effect of amino acids in the presence of arginine or leucine on insulin release from isolated rat islets of Langerhans. Insulin released into the medium was expressed as  $\mu$  units/5 islets/60 minutes  $\pm$  s.e.m. The number of observations is shown in parenthesis.

Addition to the medium	Arginine (5 mM)		Leucine (5 mM)	
	Absent	Present	Absent	Present
Control	95 $\pm$ 4 (24)	98 $\pm$ 5 (18)	—	195 $\pm$ 18 (15)
Phenylalanine	95 $\pm$ 5 (11)	104 $\pm$ 7 (15)	87 $\pm$ 5 (18)	244 $\pm$ 19 (18)
Lysine	94 $\pm$ 7 (12)	102 $\pm$ 6 (15)	87 $\pm$ 5 (14)	230 $\pm$ 20 (17)

to various stimuli of insulin secretion were compared by the students "t" test. To assess whether the effect of glucose and amino acids was additive or synergistic, the individual values of insulin secreted in response to the two stimuli in each set were randomly tabulated and then the values for the two stimuli in each set were added to calculate the mean and the standard error. This mean was compared with the combined response of the two stimuli obtained experimentally by applying the students "t" test.

## Results

In the presence of low concentration of glucose (1.67 mM), both arginine (118  $\pm$  8  $\mu$ units) and leucine (197  $\pm$  10  $\mu$ units) did not show any significant increase in insulin release as compared with their

effect in the absence of glucose (100  $\pm$  8 and 168  $\pm$  14  $\mu$ units respectively). Fig. 1, shows the effect of 8.3 mM and 16.7 mM glucose in the presence of varying amounts of arginine or leucine. Arginine, as well as, leucine potentiated the effect of 8.3 mM glucose, which increased progressively with the increasing concentrations of the amino acid while, with 16.7 mM glucose there was no further enhancement of insulin release. Arginine at 5, 10 or 25 mM concentration significantly enhanced the insulinotropic effect of leucine at 10 mM concentration (Table I). Effect of 5 mM leucine was also significantly enhanced by 5 mM arginine. Phenylalanine and lysine failed to enhance the insulinotropic effect of either arginine or leucine (Table II).

## Discussion

Arginine 5, 10 and 25 mM produced no significant release of insulin in the absence of glucose. In the presence of 8.3 mM glucose, arginine potentiated the effect of glucose which increased progressively with increasing concentration of the amino acid (Fig. 1). In the presence of high glucose (16.7 mM), arginine failed to potentiate the insulinotropic effect of glucose. It therefore appears that arginine facilitates the effect of glucose at the moderate glucose concentration or the concentration of glucose normally prevalent in the blood, but has no further effect when the pancreatic beta cells are already stimulated maximally by the high glucose concentration. These findings are in contrast with the *in vivo* observations of Floyd *et al.* [2] who found arginine to be a potent insulinotropic amino acid which showed synergism when infused along with glucose. However, in the living system pancreas is being constantly perfused by glucose. Hence the insulinotropic effect of arginine *in vivo* may be considered compatible with glucose dependence of this amino acid *in vitro*. This argument is further substantiated by observation that in man, insulin response to arginine was obliterated when the amino acid was administered during a state of induced hypoglycemia [11], suggesting that a normal blood glucose level is a prerequisite for the induction of insulin release by arginine *in vivo*.

Leucine stimulated insulin release in the absence of glucose as well as in its presence (Fig. 1). The gradual rise of insulin released in response to the increasing concentrations of leucine (5, 10 and 25 mM)

was further increased in the presence of moderate glucose (8.3 mM) concentration. The failure of leucine to enhance the effect of high glucose concentration appears to be due to some saturation phenomenon. The combined effect of glucose and leucine seemed to be additive and not synergistic because this response did not significantly differ from the individual responses of the two stimuli added together. These *in vitro* findings are not in agreement with results of Floyd *et al.* [2], who obtained a synergistic effect by intravenous infusion of glucose and leucine in man.

Floyd *et al.* [1], have shown a synergistic insulinotropic effect in man by arginine and leucine but obtained no such effect with leucine and histidine. Present results show a significant enhancement of leucine-stimulated insulin release by arginine (Table I) but phenylalanine and lysine did not potentiate the effects of either arginine or leucine (Table II).

The present *in vitro* findings, therefore, show a considerable potentiating but not synergistic effect of arginine on glucose-stimulated insulin release because this amino acid showed no insulinotropic

effect in the absence of glucose. Moreover, the combined effect of glucose and leucine was only additive and no synergism was shown by any of the amino acid pairs tested in the present study, though arginine exhibited a mild but significant potentiating effect ( $P < 0.05$ ) on leucine-stimulated insulin release.

In the light of the above discussion, it may be concluded that the synergism observed by Floyd *et al.* [1, 2], *in vivo* may not be due to the direct effects of these stimuli on the beta cells but as has been suggested by the authors themselves some unidentified humoral factor(s) might be responsible for this synergism.

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