GLUCAGON-RELEASING ACTIVITY OF GUANIDINE COMPOUNDS IN MOUSE PANCREATIC ISLETS

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1. Introduction

Structure–function studies performed to investigate the betacytotropic effect of arginine, have suggested that the guanidino group may be involved in triggering insulin release, since several compounds that contain this radical within their molecule are capable of stimulating the secretion of insulin, both in vivo [1] and in vitro [2].

Given that arginine also behaves as a potent glucagon secretagogue, whose mechanism of action remains unknown, we have examined the effect of guanidine and two guanidine derivatives (guanidinoacetic acid and creatine) on the secretory activity of the alpha-cell in a preparation of isolated mouse pancreatic islets.

2. Methods and materials

Four-week old Swiss male mice, fasted for 14 h, were used as pancreas donors. In each experiment a pool of 8 pancreases was employed. The islets were isolated by the collagenase technique [3] as modified by Coll-García and Gill [4]. Batches of 10 islets were incubated for 60 min in 2 ml of a bicarbonate-buffered salt solution [5] (gas phase, 95:5, O_2 + CO_2) containing 0.2% bovine serum albumin (Cohn fraction IV), 2000 U of kallikrein-trypsin inhibitor (Trasylol, Bayer) and 3.3 mM glucose ('basal medium'). In order to verify the releasing activity of the islets, on each day arginine hydrochloride was used as a control secretagogue. All incubations were performed in a water bath at 37°C and at 60 strokes per min. At the end of the incubation, an aliquot of each sample was diluted 1:4 in 0.2 M glycine containing 0.25% human albumin and 1% normal sheep serum (pH 8.8) and immediately frozen at −20°C until the time of analysis. Full details of this method will be published elsewhere [6].

Radioimmunoassay was used to estimate glucagon [7] and insulin [8]. The values were read against beef–pork glucagon (Eli Lilly) and mouse insulin (Novo) standards. Statistical differences were calculated by unpaired t-test analysis.

Collagenase was purchased from Boehringer Mannheim GmbH; arginine hydrochloride, guanidine HCl, guanidinoacetic acid and bovine serum albumin were obtained from Sigma Chem. Co. Glucagon antiserum (30K) was a generous gift from Dr R. H. Unger, Dallas.

3. Results

As expressed in table 1, glucagon release by mouse pancreatic islets incubated in the basal medium, was fairly constant in the three groups of experiments. The addition of 10 mM arginine induced, in all cases, a marked increase of glucagon output, the increments ranging from 140% to 410% of the basal values.

When 10 mM guanidine was incorporated into the medium, glucagon release amounted to 110% above the basal value. Guanidinoacetic acid and creatin, both at 10 mM, were also effective stimuli of glucagon secretion, the increments being 50% and 140%, respectively.

At the glucose concentration employed (3.3 mM), 10 mM arginine elicited significant increments of insulin release, varying from 180% to 590%. While

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Table 1
Effect of arginine, guanidine, guanidinoacetic acid and creatine on glucagon and insulin release by incubated mouse pancreatic islets. These substances were used at 10 mM (means ± SEM)

	Glucagon (pg/10 islets/60 min)	Insulin (ng/10 islets/60 min)
Basal medium (N = 40)	309 ± 22	1.4 ± 0.15
Guanidine (N = 40)	[a]654 ± 77[b]	[a]1.5 ± 0.2
Arginine (N = 40)	1571 ± 169[b]	3.9 ± 0.3[b]
Basal medium (N = 60)	321 ± 14	1.3 ± 0.1
Guanidinoacetic acid (N = 84)	[a]480 ± 21[b]	[a]4.2 ± 0.2[b]
Arginine (N = 36)	766 ± 39[b]	9.0 ± 0.7[b]
Basal medium (N = 70)	200 ± 10	1.3 ± 0.1
Creatine (N = 97)	[a]479 ± 70[b]	[a]3.6 ± 0.2[b]
Arginine (N = 42)	897 ± 70[b]	7.6 ± 0.9[b]

N = number of incubation vials.
[a] *P* vs. basal value <0.0001.
[b] *P* vs. arginine value <0.00001.

guanidinoacetic acid and creatine augmented the insulin-releasing activity of the islets (220% and 180%, respectively), no effect of guanidine on the secretion of this hormone was observed.

4. Discussion

Guanidine and the two guanidine derivatives tested, display a clear-cut glucagonotropic effect in the preparation of mouse pancreatic islets. Therefore, it seems reasonable to consider that the guanidino group may be in part responsible for the glucagon-releasing activity of arginine.

Parenteral administration of arginine analogs, metabolizable and nonmetabolizable, has been previously described to increase the levels of circulating glucagon in rats and dogs [9,10]. The fact that these compounds possess a guanidino residue, would support the concept that recognition of this radical by the alpha-cell may promote glucagon release.

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