EFFECT OF ALLOXAN ON ARGININE- AND LEUCINE-INDUCED INSULIN SECRETION IN ISOLATED ISLETS

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1. Introduction

The insulin secretion by arginine and leucine appears to be mediated through different mechanisms. By using isolated rat islets perifused in vitro, arginine (15 mM) was shown to provoke no active insulin release except for a tiny first phase [1]. Leucine (19 mM), however, stimulated sustained insulin secretion with apparent biphasic pattern [2]. Leucine-induced insulin secretion is dependent on extracellular calcium and its insulinotropic action is associated with an increased uptake of calcium [2]. By adding glucose (8.3 mM) to arginine (15 mM), the insulin secretion was three times above the baseline secretion [1]. Thus, arginine is the most potent glucose dependent amino acid for stimulating insulin secretion [3]. The object of this communication was to study arginine- and leucine-induced insulin secretion with and without prior exposure to alloxan and further, to clarify the possible difference of alloxan effect on amino acid-induced insulin secretion.

2. Materials and methods

Two hundred islets were isolated from one fed Sprague-Dawley rat (200–300 g) by the standard collagenase technique [4]. The perifusion system was identical to that described in the studies on alloxan inhibition of insulin secretion [5]. In brief, two chambers were perifused simultaneously: one without alloxan exposure served as the control and the other with alloxan exposure as the experimental chamber. The chambers were maintained at 37°C and perifused at a flow rate of 0.7–0.8 ml/min. The perifusate was collected at one- or five-minute intervals and the insulin content of aliquots was determined by the radioimmunoassay and was expressed as μU/islet/min [5]. The total insulin secretion by the amino acids was calculated as mU/100 islets/60 min [5].

Exposure to alloxan: Alloxan monohydrate (Sigma Chemical, St. Louis) was dissolved at a final concentration of 20 mg% or 40 mg% in previously warmed (37°C) and gassed (95% O_2; 5% CO_2) Krebs-Ringer bicarbonate media immediately prior to perifusion. L-arginine hydrochloride and L-leucine (both Sigma Chemical) were dissolved in bicarbonate media and were gassed to maintain at pH 7.4 prior to use.

3. Results

Leucine (4 mg/ml = 30.49 mM) induced biphasic insulin secretion with first and second phases occurring at 6 min and 30 min, respectively (fig.1). Prior exposure to alloxan (20 mg%) for 5 min diminished leucine-induced insulin secretion to below 1 μU/islet/min; however, small bursts of insulin secretion were noted during the exposure to alloxan, immediately after changed to leucine medium from glucose medium (1 mg/ml) as well as 20–40 min perifusion with leucine (fig.1). The exposure to alloxan (40 mg%) for 5 min produced similar, but more, inhibition of subsequent insulin secretion (fig.1). The relative insulin secretion after exposure to 20 mg% and 40 mg% alloxan, was 52% and 39% of the controls, respectively (table 1).

Arginine hydrochloride (4 mg/ml = 18.98 mM) alone induced small but an apparent biphasic secretion profile occurring at 5 min and 25 min, respectively

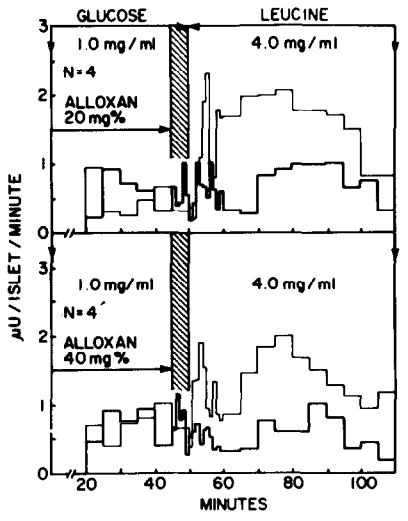

Fig.1. Two perifusion chambers were perifused simultaneously with a 1 mg/ml glucose medium for either 50 min (control chambers) or 45 min (experimental chambers). The control chambers (thin line) were subsequently followed by perifusion with a 4 mg/ml leucine for 60 min while the experimental chambers (thick line) were introduced with alloxan solution for 5 min and were followed by a 4 mg/ml leucine for 60 min. Upper: 20 mg% alloxan exposure in the experimental chambers. Lower: 40 mg% alloxan exposure in the experimental chambers.

(fig.2). The addition of a non-stimulatory level of glucose (0.6 mg/ml) to the arginine solution augmented insulin secretion to 8.560 mU/100 islets/60 min, an 85% increase compared to arginine alone (table 1). Prior exposure to alloxan (20 mg%) had little effect on arginine and arginine–glucose induced insulin secretion, maintaining 92% and 96% of the control secretion, respectively (table 1). Furthermore, prior exposure to alloxan (40 mg%) did not significantly diminish subsequent arginine–glucose induced insulin secretion, maintaining 87% of the control secretion (table 1).

4. Discussion

The dynamics of insulin secretion by arginine and leucine have been widely studied with the use of perfusion of isolated pancreas [6–10], pancreatic pieces [3] as well as perifusion of isolated islets [2,6, 11,12]. There was discrepancy on insulin secretion by the experimental methods between the isolated pancreas and isolated islets. Namely, Norfleet et al. reported that arginine (15 mM) alone had no effect on the insulin secretion by perifused isolated islets, but that it clearly stimulated insulin secretion in a perfused isolated pancreas [6]. Gerich et al. however, had shown only monophasic insulin release in response to argine by perfused isolated pancreas [8]. By perifusing isolated islets, Charles et al. also demonstrated that argine (15 mM) alone caused a tiny first phase of insulin secretion [12]. The addition of non-stimulatory

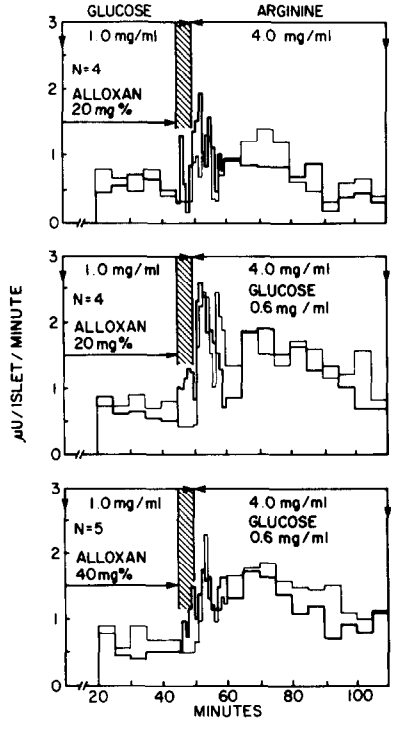

Fig.2. Two chambers were perifused simultaneously with a 1 mg/ml glucose medium for either 50 min (control chambers) or 45 min (experimental chambers). The control chambers (thin line) were subsequently followed by perifusion with a 4 mg/ml arginine (upper) or a 4 mg/ml arginine plus 0.6 mg/ml glucose (middle and lower) for 60 min. The experimental chambers (thick line) were introduced with alloxan solution for 5 min and were followed by a 4 mg/ml arginine (upper) or a 4 mg/ml arginine plus 0.6 mg/ml glucose (middle and lower). Upper: 20 mg% alloxan exposure in the experimental chambers. Middle: 20 mg% alloxan exposure in the experimental chambers. Lower: 40 mg% alloxan exposure in the experimental chambers.

Table 1
Effect of alloxan on arginine- and leucine-induced insulin secretion

Group of Experiment		5 Min exposure to alloxan (mg%)	Insulin secretion (mU/100 islets/60 min) Mean ± S.E.	Effect *P* Values	Secretion (%)
I	Leucine	0	8.61 ± 0.50 (4)[b]		100
		20	4.50 ± 0.87 (4)	$P<0.01$[c]	52 ± 10
II	Leucine	0	8.08 ± 0.31 (4)		100
		40	3.13 ± 0.06 (4)	$P<0.001$	39 ± 1
III	Arginine	0	4.64 ± 0.41 (4)		100
		20	4.25 ± 0.27 (4)	$P<0.40$	92 ± 6
IV	Arginine–glucose	0	8.56 ± 0.67 (4)		100
		20	8.22 ± 0.77 (4)	$P<0.70$	96 ± 9
V	Arginine–glucose	0	8.52 ± 0.52 (5)		100
		40	7.42 ± 0.34 (5)	$P<0.10$	87 ± 4

[a]Control chambers were initially perifused with a 1 mg/ml glucose medium for 50 min, then with a 4 mg/ml medium of leucine, arginine or arginine plus glucose (0.6 mg/ml) for 60 min. Experimental chambers were initially perifused with a 1 mg/ml glucose medium for 45 min, then with alloxan solution (20 mg% or 40 mg%) for 5 min, followed by a 4 mg/ml medium of leucine, arginine of arginine plus glucose (0.6 mg/ml) for 60 min.

[b]Numbers in parentheses are the numbers of the experiments

[c]*P* values by paired *t*-test

levels of glucose to arginine solution markedly augmented insulin secretion both in perfused isolated pancreas [8,10] and perifused isolated islets [12].

The present results of insulin secretion, by arginine alone, showed a small but apparent biphasic pattern, probably due to the higher concentration of arginine 18.98 mM (4 mg/ml) used than 15 mM (2.6 mg/ml) [1] as well as to a subtle difference in the procedure used in isolating rat islets. Arginine-induced insulin secretion is not accompanied by an elevation of islet tissue cAMP levels, while glucose- and tolbutamide-induced insulin secretion is modulated at least partially through increased cAMP levels of islets, particularly the initial phase of insulin secretion [1]. The initial phase of glucose-induced insulin secretion was accompanied by the elevated cyclic adenosine 3,5-monophosphate (cAMP) levels in isolated islets [1,13]. Tolbutamide increased islet tissue cAMP levels by decreasing phosphodiesterase activity [15]. The increased phosphodiesterase activity by arginine may correspond to the non-elevated levels of cAMP during arginine-induced insulin secretion [14]. If arginine stimulates insulin secretion through a mechanism other than adenylate cyclase–cAMP system, this will be the system which is not affected by prior exposure to alloxan as documented in this study.

Amino acids appear to stimulate insulin release by binding to a specific transport molecule on the beta cell membrane [15]. Each individual amino acid should be further studied for its insulinotropic mechanism, since there is an essential difference between arginine and leucine in terms of insulin secretion after prior exposure to alloxan.

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