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INTERLEUKIN-1ß INDUCES NITRIC OXIDE PRODUCTION AND INHIBITS THE ACTIVITY OF ACONITASE WITHOUT DECREASING GLUCOSE OXIDATION RATES IN ISOLATED MOUSE PANCREATIC ISLETS

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Summary: The aim of this investigation was to further characterize the process of interleukin-1ß (IL-1ß) induced nitric oxide production in isolated pancreatic islets. It was found that both IL-1ß and nitroprusside increased islet nitrite production. This effect was paralleled by inhibition of islet aconitase activity and glucose oxidation rates. Neither trifluoroperazinen or aminopterin could prevent the IL-1ß induced increase in nitrite production, aconitase inhibition and decrease in glucose oxidation rates. In a second series of experiments, isolated mouse pancreatic islets were exposed to IL-1ß for 24 h and subequently used for nitrite production, aconitase activity and glucose oxidation determinations. The islets responded to IL-1ß with an increased nitrite production and a decreased activity of aconitase, whereas the islet glucose oxidation rates were not decreased. It is concluded that IL-1ß in both rat and mouse islets induces nitric oxide formation and that this induction leads to the inhibition of the Krebs cycle enzyme aconitase. In rat islets this probably leads to an inhibited insulin secretion, whereas IL-1ß in mouse islets suppresses insulin secretion by a non-mitochondrial mechanism.

Recently, it was shown that the cytokine interleukin-1 (IL-1ß) during a 48 h exposure of isolated rat pancreatic islets increased the production of nitrite, an end-product of the highly reactive and short-lived nitric oxide radical [1]. In addition, the inhibitor of nitric oxide formation, NAME, could alleviate the IL-1 induced suppression of insulin release [1]. Moreover, we have found that IL-1 induced increase in islet nitrite production is an event which is probably preceded by an altered gene transcription, and that it may lead to the inhibition of the mitochondrial Krebs cycle enzyme aconitase [2]. This finding may explain the previously demonstrated inhibitory actions of the cytokine on the islet mitochondrial metabolism of glucose and thereby also the IL-1ß induced inhibition of the synthesis and release of insulin [3-4]. The enzyme nitric oxide synthase is known to

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catalyze the formation of nitric oxide from L-arginine and is known to exist in at least two different forms, one which can be isolated from macrophages and the other from brain cells [5]. The brain cell derived form was recently cloned and sequence analysis and expression of the nitric oxide gene in a kidney cell line revealed its requirements for FMN, FAD, NADPH, Ca^{2+} and calmodulin [6]. Furthermore, both the brain cell and the macrophage nitric synthase are known to be stimulated by the cofactor tetrahydrobiopterin [7].

To further characterize nitric oxide inhibition of the activity of the Krebs cycle enzyme aconitase, we have presently exposed islets to the nitric oxide generating drug nitroprusside and studied its effects on islet mitochondrial function. The effects of inhibitors of calmodulin and tetrahydrofolatreductase, i. e. trifluoroperazine and aminopterin, respectively on IL-1ß induced nitrite production were also studied. Finally, we have also compared the effects of IL-1ß on mouse islet nitrite production with that on rat islets, since mouse islets has previously been shown to not similarly be affected by IL-1ß with respect to glucose oxidation rates [8].

Materials and Methods

Chemicals The chemicals were obtained from the following sources. Boehringer Mannheim (Mannheim, Germany): collagenase from Clostridium histolyticum, type CLS (EC 3.4.24.3). Flow Laboratories (Irvine, UK): culture medium RPMI 1640, fetal calf serum (FCS) and L-glutamine. Sigma Chemical Co. (St. Louis, MO, USA): naphthylethylenediamine dihydrochloride, sulfanilamide, cis-aconitic acid, HEPES, nitroprusside, aminopterin and trifluoroperazine. Amersham International (Amersham, UK): D-[U-^{14}C]glucose. Human recombinant IL-1ß was kindly provided by Dr. K. Bendtzen (Copenhagen, Denmark). The biological activity of the IL-1ß preparation was 5 U/ng [9].

Islet isolation and culture Non-inbred male Sprague-Dawley rats were obtained from a local colony bred at the Biomedical Center, Uppsala, Sweden and non-inbred male NMRI mice were obtained from Anticimex, Sollentuna, Sweden. Pancreatic islets were isolated by collagenase digestion and Ficoll-gradient centrifugation [10]. Groups of 150-200 islets were pre-cultured free-floating for 3-5 days in RPMI 1640 supplemented with 10% FCS at 37°C [11]. The gas phase was humidified air and 5 % CO_2.

Cytokine and test agent treatment For the first series of experiments using rat islets, islets in groups of 100 were transferred to 5 ml of culture medium containing 10 % FCS with or without 5 ng/ml IL-1ß and 5 µM trifluoroperazine, 100 µM aminopterin or 100 µM nitroprusside and further incubated for 24 h. At times 6-7 h and 23-24 h, the islets were transferred to microwell plates containing 100 µl of the same incubation medium. After the first incubation period, the islets were returned to the 5 ml culture dish and the 100 µl incubation volumes were collected for nitrite measurements. After the second incubation period at 23-24 h, the islets were collected in Eppendorf tubes, washed in cold phosphate buffered saline (PBS) and sonicated in 100 µl redistilled water. In a second series of experiments, islets were taken already after the 6-7 h

incubation period for aconitase and glucose oxidation measurements (see below). Mouse islets were cultured and treated similarly for 24 h as given above. Medium insulin accumulation was determined using a radioimmunoassay technique [12].

Nitrite determination Culture media samples (90 µl) were deproteinized by the addition of 20 µl of 35 % sulfosalicylic acid. Samples were incubated for 30 min at 0°C and subsequently centrifuged for 20 min at 12000g. To the supernatants was added 10 µl of 0.5 % naphthylethylenediamine dihydrochloride, 5 % sulfanilamide and 25 % concentrated H_3PO_4 [13]. The reaction was carried out at 60°C for 15 min and the absorbance at 546 nm was measured against a standard curve.

Aconitase activity measurement To the islet homogenates were added 0.15 M NaCl, 30 mM Tris, pH 7.5, 0.2 % Triton X-100, 0.02 % BSA and the samples were centrifuged for 5 min at 12000g at 4°C. The supernatants were supplemented with 2 mM of cis-aconitic acid and the reaction rate was determined by the rate of decrease in absorbance at 240 nm during the first 2-3 min. The extinction coefficient used was 3.41 mM^{-1} cm^{-1} [14].

Islet glucose oxidation measurements For determination of glucose oxidation rates triplicate groups of 10 islets each were incubated for 90 min in glass vials containing 0.1 ml Krebs-Ringer bicarbonate buffer containing 10 mM HEPES (KRBH) supplemented with D-[U-^{14}C]glucose and nonradioactive glucose to a final concentration of 16.7 mM. The islets glucose oxidation was measured as described previously [15].

Statistical analysis Values were computed as means ± SEM and groups of data were compared by Student's paired t test.

Results

Effects of nitroprusside. Cultured rat islets responded to nitroprusside with an increased nitrite production both after 7 h and 24 h (Table 1). The nitroprusside effect on nitrite production was paralleled by an inhibition of aconitase activity, islet glucose oxidation rates and medium insulin accumulation (Table 1). However, the effects after 24 h were less evident and did not all reach the level of significance. This may be due to a gradual consumption and degradation of nitroprusside added to the culture media.

Effects of IL-1ß, aminopterin and trifluoroperazine. Addition of IL-1ß evoked a marked stimulation of the rat islet nitrite production both after 7 h and 24 h (Table 2). The cytokine also inhibited islet aconitase acitivity at both time points, by 38% at 7 h and by 78% at 24 h (Table 2). The concomitant addition of either aminopterin or trifluoroperazine did not prevent the IL-1ß induced effects on islet nitrite production and aconitase activity (Table 2).

IL-1ß also inhibited islet glucose oxidation rates and the medium insulin accumulation when determined after 24 h (Table 3). Again, aminopterin and triflouroperazine could not counteract the IL-1ß induced effects (Table 3). Trifluoroperazine by itself markedly inhibited the accumulation of insulin in the medium, an effect probably due to inhibition of calmodulin, a protein involved in the release of insulin.

TABLE 1

Effects of nitroprusside on rat islet nitrite production, aconitase activity, glucose oxidation and medium insulin accumulation

	Control		Nitroprusside	
	7 h	24 h	7 h	24 h
Nitrite production (pmol/100 islets x h)	0.15±0.10	0.23±0.09	0.90±0.05**	0.55±0.09*
Aconitase activity (pmol/islet x min)	21±3.3	16±6.3	12±1.8*	11±2.7
Glucose oxidation (pmol/10 islets x 90 min)	534±52	846±55	265±27**	663±62
Medium insulin accumulation (ng/40 islets x 24 h)	ND	2870±540	ND	1000±390*

Isolated rat islets were cultured for 7 or 24 h without or with nitroprusside (100 μM) and islet nitrite production, aconitase acitivity, glucose oxidation and medium insulin accumulation was determined. Values are means ± SEM for 6 experiments. ND denotes not determined. * and ** denote $P<0.05$ and $P<0.01$ when comparing vs. corresponding control islets, using Student's paired t test.

In mouse pancreatic islets IL-1ß exposure for 24 h increased nitrite production and decreased the activity of aconitase similar to the findings in rat islets (Table 4). The inhibition of aconitase was not as pronounced in the mouse islets as in the rats islets

TABLE 2

Effects of IL-1ß, aminopterin and trifluoroperazine on rat islet nitrite production and aconitase activity

	Nitrite production (pmol/100 islets x h)		Aconitase activity (pmol/islet x min)	
Addition	7 h	24 h	7 h	24 h
Control	0.15±0.10	0.33±0.10	21±3.3	16±6.3
+ IL-1ß	0.85±0.10**	1.0±0.25*	13±2.4*	3.6±2.7*
Aminopterin	0.25±0.10	0.22±0.10	22±4.5	16±4.5
+ IL-1ß	1.20±0.20**	1.40±0.30**	16±2.6*	1.8±0.8*
Trifluoroperazine	0.40±0.10	ND	22±3.6	ND
+ IL-1ß	0.95±0.15**	ND	15±1.5*	ND

Isolated rat islets were cultured for 24 h without or with IL-1ß (5 ng/ml) and in the presence or absence of aminopterin (100 μM) and trifluoroperazine (5 μM) and the islets were subsequently taken for measurements of nitrite production and aconitase activity. Values are means ± SEM for 5 experiments. ND denotes not determined. * and ** denote $P<0.05$ and $P<0.01$ when comparing vs. corresponding islets not exposed to IL-1ß, using Students paired t test.

TABLE 3

Effects of IL-1ß, aminopterin and trifluoroperazine on rat islet oxidation rates and insulin accumulation

Addition	Glucose oxidation (pmol/10 islets x 90 min)	Insulin accumulation (ng/islets x 24 h)
Control	846 ± 55 (6)	2870 ± 540 (6)
+ IL-1ß	337 ± 67 (6)**	1750 ± 410 (6)
Aminopterin	830 ± 75 (6)	2880 ± 650 (6)
+ IL-1ß	429 ± 51 (6)***	870 ± 200 (6)*
Trifluoroperazine	755 ± 87 (5)	490 ± 110 (5)
+ IL-1ß	238 ± 52 (5)***	640 ± 120 (5)

Isolated rat islets were cultured for 24 h without or with IL-1ß (5 ng/ml) and in the presence or absence of aminopterin (100 µM) and trifluoroperazine (5 µM) and the islet glucose oxidation rates and insulin accumulation to the media were subsequently determined. Values are means ± SEM with the number of experiments in parentheses. *, ** and *** denote P<0.05, P<0.01 and P<0.001, when comparing vs. corresponding islets not exposed to IL-1ß, using Student's paired t test.

(45% in mouse islets vs 78% in the rat). The glucose oxidation rates were not decreased by IL-1ß in mouse islets (Table 4).

Discussion

Injections of nitroprusside in mice have been reported to induce both alterations in the ß-cell structure as well as an inhibition of the release of insulin leading to a transient

TABLE 4

Effects of IL-1ß on mouse islet nitrite production, aconitase activity and glucose oxidation

Addition	Nitrite production (pmol/100 islets x h)	Aconitase activity (pmol/islet x min)	Glucose oxidation (pmol/10 islets x 90 min)
Control	0.09 ± 0.05 (5)	18 ± 1.4 (4)	941 ± 57 (5)
IL-1ß	0.80 ± 0.15** (5)	10 ± 1.6* (4)	948 ± 54 (5)

Mouse pancreatic islets in groups of 100 were cultured for 24 h without or with IL-1ß (5 ng/ml) and subsequently taken for measurements of nitrite production, aconitase activity and glucose oxidation. Values are means ± SEM with the number of experiments in parentheses. * and ** denote P<0.05 and P<0.01 when comparing vs. corresponding control, using Student's paired t test.

hyperglycemia [16]. Since nitroprusside is known to enhance cyclic GMP formation in islets [17], Boquist proposed that the nitroprusside induced effects may be caused by alterations in the islet guanosine nucleotide pools leading to altered activities of GTP sensitive enzymes such as glutamate dehydrogenase [16]. On the other hand, it is well known that nitroprusside upon reaction with biological molecules generates nitric oxide [18], and that nitric oxide has been shown to inhibit the activity of aconitase by binding to the iron atoms of the Fe-S clusters [19]. These two findings and our present results rather support the notion that an increased nitric oxide production in isolated islets, induced by either IL-1ß or nitroprusside, leads to inhibition of the Krebs cycle enzyme aconitase.

To investigate whether also nutrients, which control ß-cell mitochondrial events, may act via nitric oxide production and a subsequently altered aconitase activity, we presently studied the effects of perifusion of isolated islets with a high glucose concentration on nitrite production and aconitase activity. However, we were not able to detect any effects of glucose on islet nitrite production and aconitase activity indicating that the nutrient stimulated insulin release seems not be mediated by alterations in this pathway (results not shown). This is in agreement with a recent preliminary report by Jones et al. [20].

In view of previous studies indicating that the brain form of nitric oxide synthase, but not the macrophage form, requires Ca^{2+} and calmodulin for its activity [19], the finding that the calmodulin inhibitor trifluoroperazine did not prevent the IL-1ß induced production of nitrite, may suggest that IL-1ß induced nitric oxide synthase in islet cells resembles the macrophage form of the enzyme. Because higher concentrations of trifluoroperazine than 5 μM severly impaired islet cell viability during culture, we presently used a modest concentration of the compound. Therefore, it cannot be excluded that this concentration and the concentration of aminopterin (100 μM) used, which also failed to prevent IL-1ß induced nitite production, both were insufficient to effectively inhibit the IL-1ß induced nitric oxide synthase activity.

It has previously reported that mouse islets respond to IL-1ß with an impaired insulin production, i.e. a reduction in insulin mRNA, proinsulin biosynthesis, insulin content and release, which was not caused by a decreased glucose metabolism [8]. We thus suggested that the cytokine, in the mouse islet, acts primarily by affecting the expression of the insulin gene and other putative genes involved in the regulation of insulin release. The finding in the present investigation, that IL-1ß induced nitrite production and inhibition of aconitase in mouse islets occured without impaired

glucose oxidation appears somewhat contradictory. In this context, it should be pointed out that the IL-1ß induced decrease in aconitase acitivity was less pronounced in mouse islets than in rat islets, and therefore may is still be sufficient to sustain an unaltered mitochondrial function. Furthermore, mouse aconitase may be more resistant to inactivation than the rat aconitase, which is in line with previous findings showing that streptozotocin and alloxan, two ß-cell toxins of which one is known to inhibit aconitase [22], act more potently in rat islets than in mouse islets [23,24]. Three possible explanations for this putative species difference in sensitivity to aconitase inhibitors can be envisaged. Firstly, the mouse mitochondria may possess a higher reducing potential than rat mitochondria (reducing agents such as cysteine effectively restore the activity of aconitase toghether with ferreous ions in vitro [25]). Secondly, the mouse mitochondria may have better defence mechanisms against nitric oxide, e.g. the superoxide radical has been shown to inactivate nitric oxide [26] Thirdly, the mouse aconitase enzyme may be structurally different from the rat aconitase. Naturally, these questions need further attention to be resolved.

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