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Inhibition of insulin secretion by interleukin-1β and tumour necrosis factor-α via an L-arginine-dependent nitric oxide generating mechanism

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Inhibition of glucose-induced insulin secretion by interleukin-1β (IL-1β), or IL-1β plus tumour necrosis factor-α (TNF-α), was less marked when rat islets of Langerhans were cultured for 12 h with these cytokines in L-arginine-free medium as opposed to medium containing L-arginine (1 mM). Inhibition of secretion by IL-1β was further alleviated when islets were maintained in L-arginine-free medium supplemented with *N*-ω-nitro-L-arginine methyl ester (NAME), while synergism between IL-1β plus TNF-α was completely abolished. Tissue culture medium nitrite levels were raised in islets treated with IL-1β or TNF-α (48 h). Cytokine-stimulated nitrite production was not observed in islets cultured with NAME (1 mM). In conclusion, an L-arginine-dependent nitric oxide generating mechanism is involved in the inhibition of insulin secretion by IL-1β, and accounts for the phenomenon of synergism between IL-1β and TNF-α.

Interleukin-1β; Tumour necrosis factor-α; L-Arginine; Nitric oxide; Insulin secretion; Rat

1. INTRODUCTION

Cytotoxic activated macrophages (CAM) induce metabolic dysfunction in target cells including the inhibition of mitochondrial respiration, aconitase activity and DNA replication, by a process dependent on the metabolism of L-arginine to nitrite, nitrate and L-citrulline [1,2]. The immediate precursor of nitrite and nitrate is the free radical nitric oxide (NO) derived from one of the N-terminal guanidino nitrogen atoms of L-arginine [2–6]. CAM-induced target cell inhibition, and NO generation are prevented by closely-related structural analogues of L-arginine [1–7]. The inhibitory activity of macrophage-derived NO may, in part, be due to the inactivation of iron-sulphur clusters at the active sites of mitochondrial enzymes [8,9].

Pretreatment of isolated rat islets of Langerhans with interleukin-1β (IL-1β) inhibits glucose-induced insulin secretion in a dose- and time-dependent manner [10,11]. This effect is potentiated synergistically when islets are exposed to IL-1β in combination with tumour necrosis factor-α (TNF-α) [12]. Furthermore, the combination of IL-1β and TNF-α has a cytolytic effect in long-term cultures of rat and human islet cell monolayers [13–15]. The mechanism(s) of action IL-1β and TNF-α on the islet β-cell remain largely unknown. Here, we investigate the involvement of an L-arginine-dependent-nitric oxide generating mechanism in the inhibition of insulin secretion by IL-1β and TNF-α in isolated rat islets of Langerhans.

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2. EXPERIMENTAL

2.1. *Materials*

Tissue culture reagents were from Gibco (UK). Dextran T70 was from Pharmacia (UK). Collagenase type XI, calf thymus DNA type I, L-arginine-HCl, and *N*-ω-nitro-L-arginine methyl ester (NAME) were from Sigma Chemicals (UK). Human recombinant interleukin-1β (code 86/552) and human recombinant tumour necrosis factor-α (code 87/650) were from NIBSC.

2.2. *Islet isolation and tissue culture*

Islets of Langerhans were isolated from groups of 4–6 Sprague-Dawley rats (200 g) by a collagenase digestion technique [16]. Islets were separated from pancreatic acinar tissue by centrifugation (800 × *g*, 5 min) in 23% dextran T70 (w/v) and collected using a finely drawn-out Pasteur pipette in a bicarbonate-buffered medium [17], pH 7.4, containing 2 mM glucose. Islets were then cultured for 48 h in RPMI-1640 tissue culture medium supplemented with 5% foetal calf serum, penicillin (60 000 U/l) and streptomycin (100 mg/l) prior to all experimental studies.

2.3. *Insulin secretion studies*

Islets were divided into three groups and transferred into fresh RPMI-1640 medium prepared without L-arginine, and (i) supplemented with L-arginine (1 mM), (ii) maintained without L-arginine, or (iii) supplemented with NAME (1 mM). Islets were then cultured for 12 h ± the addition of IL-1β, TNF-α, or IL-1β plus TNF-α (0.1 nM) as shown in Results. The culture medium was removed, and the islets rinsed twice in bicarbonate buffered medium [17], pH 7.4, containing 2 mM glucose, and preincubated in this medium for 1 h at 37°C. Groups of 5 islets were then transferred into 500 μl of medium containing 20 mM glucose and incubated for 30 min at 37°C. Aliquots of medium were radioimmunoassayed for insulin as described previously [18].

2.4. *Determination of nitrite and total islet DNA content*

Groups of 70–100 islets (in triplicate) were cultured in 120 μl of RPMI-1640 medium (i) containing L-arginine (1 mM), or (ii) without L-arginine but supplemented with NAME (1 mM), for 48 h ± the addition of IL-1β, TNF-α or IL-1β plus TNF-α (0.5 nM). Tissue culture

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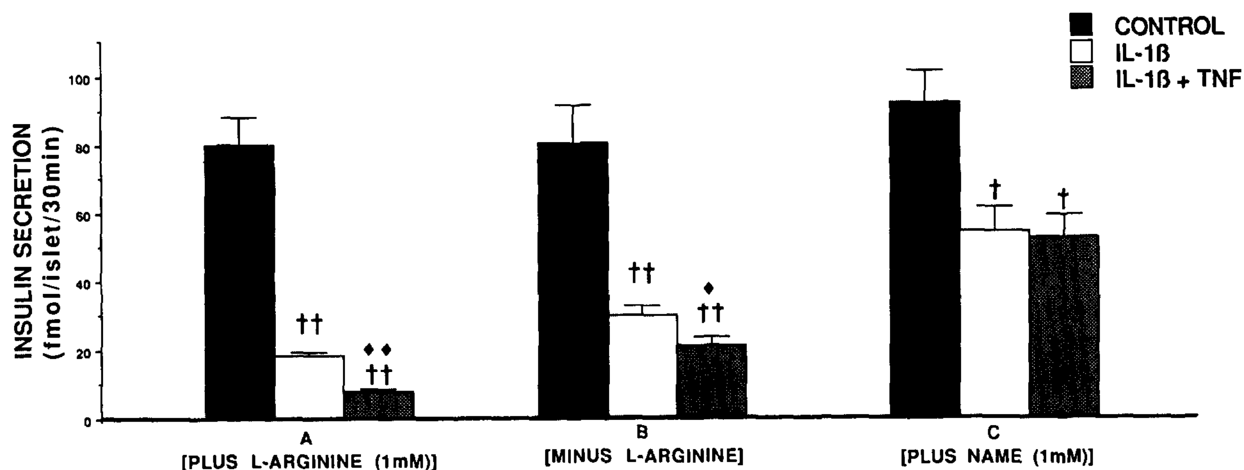

Fig. 1. L-Arginine requirement for the synergistic inhibition of insulin secretion by IL-1β and TNF-α. Groups of islets were cultured for 12 h ± the addition of cytokines (0.1 nM), in (A) RPMI-1640 medium containing L-arginine (1 mM). (B) L-arginine-free medium, or (C) L-arginine-free medium supplemented with NAME (1 mM). Islet insulin-secretory responsiveness was determined by a subsequent 20 mM glucose challenge (see section 2). Values are means ± SE from 3 separate experiments ($n=26$). Insulin secretion from control islets without cytokine treatment was not affected by the omission of L-arginine or the addition of NAME (1 mM). Insulin secretion significantly reduced vs control ($^{\dagger}P<0.01$ and $^{\dagger\dagger}P<0.0001$). IL-1β and TNF-α synergistically potentiated inhibition by IL-1β alone (♦ $P<0.05$, ♦♦ $P<0.0001$).

medium was then removed and stored at −20°C. Duplicate 50 μl aliquots of culture medium were mixed with 50 μl of the Griess reagent [19] and 200 μl distilled water and nitrite levels determined at an absorbance of 550 nM against a sodium nitrite standard curve. Islet DNA contents were determined as described previously [20].

2.5. *Statistical analysis*

Values are means ± SE from at least 3 separate experiments. Results were analysed using an unpaired Student's *t*-test.

3. RESULTS

Pretreatment of rat islets of Langerhans (12 h) with IL-1β (0.1 nM) inhibited insulin secretion induced by a 20 mM glucose challenge (19.2 ± 1.0 vs control 77.3 ± 5.5 fmol/islet/30 min, respectively, $n=46$ from 5 separate experiments, $P<0.0001$). In contrast, pretreatment with TNF-α (0.1 nM) did not affect insulin secretion (65.6±4.6 vs control 68.8±5.9 fmol/islet/30 min, $n=27$ from 3 separate experiments); however, pretreatment of islets with IL-1β plus TNF-α (0.1 nM) potentiated the inhibitory effect of IL-1β alone in a synergistic manner (8.1±0.8 vs 18.4±1.3 fmol/islet/30 min, respectively, $n=26$ from 3 separate experiments, $P<0.0001$). In Fig. 1, inhibition of secretion by IL-1β (0.1 nM), or by IL-1β plus TNF-α (0.1 nM) was less marked when islets were treated with these cytokines in L-arginine-free medium, although synergism between IL-1β and TNF-α clearly potentiated the inhibitory effect of IL-1β alone. The inhibitory effect of IL-1β was alleviated to a greater extent when islets were maintained in medium supplemented with NAME (1 mM), but most markedly, the synergistic inhibitory action of IL-1β plus TNF-α was completely abolished. To determine if the requirement for L-arginine was due to the generation of nitric oxide, we measured the accumulation of nitrite in the tissue culture medium from islets exposed to IL-1β and TNF-α (alone and in combination). Table I shows an 8-fold increase in nitrite release from islets treated with IL-1β (0.5 nM) for 48 h in the presence of L-arginine (1 mM), and a smaller (2-fold) but significant increase in nitrite release from TNF-α treated islets. Nitrite release was further increased in islets treated with IL-1β plus TNF-α (0.5 nM) compared to islets treated with either cytokine alone (in this instance in an additive rather than synergistic manner). Maintenance of islets for 48 h in medium containing NAME (1 mM) prevented cytokine-stimulated nitrite production. In a separate series of experiments we have found that maintenance of islets in L-arginine-free medium in the presence of NAME (1 mM) for up to 36 h does not affect the islet secretory response to a subsequent 20 mM glucose challenge (results not shown). Thus the lack of nitrite production following prolonged exposure to NAME does not appear to be due to

Table I

Nitrite production by cultured rat islets of Langerhans

Treatment	Nitrite production	(pmol/islet/48·h)
	(+) L-arginine	(+) NAME
Control	0.8 ± 0.18	0.9 ± 0.14
IL-1β	6.9 ± 0.96**	0.7 ± 0.13
TNF-α	1.8 ± 0.42*	0.6 ± 0.15
IL-1β + TNF-α	10.2 ± 1.11**,♦	1.1 ± 0.15

Groups of 70–100 islets were cultured for 48 h in RPMI-1640 medium containing L-arginine (1 mM), or without L-arginine supplemented with NAME (1 mM) ± the addition of cytokines (0.5 nM) as shown. Values are means ± SE of 3 separate experiments ($n=9$). In the presence of L-arginine, nitrite production was increased by all cytokine treatments (**$P<0.001$, *$P<0.05$) vs control, and was greatest with the combination of IL-1β + TNF-α, (♦$P<0.05$ vs either cytokine alone). Cytokine treatments did not affect nitrite production in the presence of NAME.

deleterious effects of this compound. Total islet DNA contents were not affected by the treatments shown in Table I (results not shown).

4. DISCUSSION

We have demonstrated the involvement of an L-arginine-dependent mechanism in the inhibition of insulin secretion by IL-1β, and have shown that such a mechanism accounts for the synergistic inhibition of insulin secretion observed between IL-1β and TNF-α. Raised nitrite production in IL-1β- and TNF-α-treated islets, which is prevented by the L-arginine analogue NAME, indicates that L-arginine is required as a substrate for the generation of NO. Previous studies have shown that IL-1β-mediated inhibition of insulin secretion is accompanied by a reduced rate of glucose oxidation [21] reflecting an impairment in the proximal steps of the Krebs cycle [22]. Furthermore, IL-1β-induced inhibition of glucose oxidation is potentiated by TNF-α [12]. As in CAM-induced target cell inhibition [1,8,9], L-arginine-derived NO may impair islet mitochondrial oxidative metabolism through the formation of nitrosyl-Fe complexes at the iron-sulphur clusters of key mitochondrial enzymes. Recently, IL-1β has been shown to inhibit glucokinase activity in an insulin secreting β-cell line [23]. Interaction of NO with functionally-essential thiol groups situated at the sugar binding site of this enzyme to yield the corresponding *S*-nitrosothiol, may account for this inhibition of enzyme activity [24]. Prolonged inactivation of such key sites may account for the inhibition of islet β-cell function, and islet β-cell death observed in IL-1β- and TNF-α-treated islets [13-15].

It is speculated that NO production may be an important contributory factor to IL-1β and TNF-α mediated inhibition of islet β-cell function and cell death, including that observed during the development of insulin-dependent diabetes mellitus.

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