Volume 261, number 1, 97-100 FEBS 08112 February 1990

Insulin-secreting β-cells possess specific receptors for interleukin-1β

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Received 13 December 1989

The effect of the cytokine interleukin-1β on the insulin secretory responsiveness of single β-cells (HIT-T15) was investigated. In the short-term, IL-1β induced a dosage-dependent stimulation of insulin release. In contrast, in the long-term, IL-1β, inhibited both basal and secretagogue-stimulated insulin secretion. We also demonstrate the simultaneous presence of specific high and low affinity binding sites for IL-1β on β-cells. IL-1β, which has been implicated in the pathogenesis of insulin-dependent diabetes, may therefore mediate its opposing effects on β-cells through a specific plasma membrane receptor.

Insulin secretion; HIT-T15; Insulin-dependent diabetes mellitus; Interleukin-1β; Interleukin-1β receptor

1. INTRODUCTION

Insulin-dependent diabetes is characterised by inflammation of the pancreas, insulitis, the severity of which correlates with cumulative destruction of the insulin-secreting β cells. Macrophages are the first immune effector cells observed in insulitis, probably resulting in high local concentrations of the cytokine IL-1β, which has been implicated as the main effector of the autoimmune destruction of β cells [1,2].

IL-1β has contrasting effects on insulin secretion and the onset of diabetes, dependent on both time of exposure and dosage. In vivo, the administration of high concentrations of IL-1β accelerates, whereas lower doses reduce the rate of disease onset in the BB rat [3]. In addition, insulin release from the perfused pancreata of normal animals is inhibited after repeated injection of IL-1β for 5 days [4]. In the short-term, IL-1 is hypoglycaemic when injected into animals [5,6]. Numerous studies have demonstrated the in vitro effects of IL-1β on isolated islets of Langerhans [1,2,7-10]. In general, high concentrations of IL-1β severely inhibit insulin biosynthesis, secretion and glucose metabolism and are cytotoxic, as revealed by electron microscopy [9] or ^{51}Cr leakage from labelled cells [10]. However, in contrast, lower doses of IL-1β, over shorter periods of exposure, stimulate insulin secretion from isolated islets.

During inflammation of the pancreas or insulitis, the characteristic lesion of type I diabetes, immune destruction is specific for β cells. However, to date there has been only one report dealing with the effects of IL-1β on single β cells, in this case the RIN m5F cell line [11]. Further, the mechanism of action of IL-1β remains obscure, although disruptions in mitochondrial function have been implicated [12-15], possibly mediated by the generation of toxic oxygen-free radicals [1,2].

In the present study, we have investigated the effects of IL-1β on insulin secretion from HIT-T15 cells, a clonal cell line of pure β cells. We demonstrate, for the first time, the presence of specific receptors for IL-1β on single, insulin-secreting β cells. This observation defines a specific pathway for β-cell destruction mediated by IL-1β and may therefore have implications in the search for novel therapeutics for insulin-dependent diabetes.

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2. EXPERIMENTAL

2.1. *Materials*

Tissue culture media, sera, antibiotics and plastics were purchased from Gibco Laboratories (New York, NY, USA). Recombinant human IL-1β was from Collaborative Research (Bedford, MA, USA) or from Glaxo Institute of Molecular Biology, Geneva, Switzerland. ^{125}I-IL-1β was from Amersham Corporation (Arlington Hts, IL, USA). Rat insulin standards were from Novo Laboratories (Danbury, CT, USA). All other reagents were purchased from Sigma Chemical Co. (St Louis, MI, USA).

2.2. *Methods*

HIT-T15 cells (generously provided by Dr A.E. Boyd III, Baylor College of Medicine, Houston, TX, USA) were routinely cultured in RPMI 1640 containing 11 mM glucose and supplemented with foetal calf serum and antibiotics as we have described [16]. For studies of insulin release, cells were passaged 2 days prior to each experiment and seeded with 4×10^5 cells per well in 24-well multiwell plates. On the day of the experiment, culture medium was aspirated and replaced with medium supplemented with additions as shown. At timed intervals, aliquots were removed, centrifuged briefly to sediment any detached cells and stored at −20°C prior to assay. Insulin release was measured by enzyme-linked immunosorbent assay (ELISA) [17], using a Bio-Rad 2550 plate reader and Microplate Manager Macintosh software. DNA was measured fluorometrically [18]. In order to com-

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00145793/90/$3.50

bine and compare data from several experiments, insulin release rates are reported as a percentage of the mean rate of control wells in the same well and are given as mean ± SE [16]. Mean absolute values of insulin release are reported where appropriate. Each test condition was assayed at least in triplicate, with 3 replicate wells per experiment. Data were analysed by analysis of variance (ANOVA).

For specific IL-1β binding studies, HIT-T15 cells were cultured as described above. Cells were washed with 2 × 0.5 ml PBS, pH 7.4, then incubated in 0.5 ml PBS/0.5% (w/v) glucose/0.5% (v/v) BSA, pH 7.6 (incubation buffer), containing 100000 cpm ^{125}I-IL-1β (sp. act. 774291 dpm/pmol) and increasing amounts of IL-1β, in triplicate wells. The experiment was repeated 4 times. Incorporation of IL-1β was maximal at 3 h at 22°C (data not shown). Following incubation, the buffer was aspirated and the cells washed with 4 × 0.5 ml of ice-cold incubation buffer. The cells were then solubilised with 0.5 ml 0.1 M NaOH, and the cell-associated radioactivity determined by liquid scintillation counting. Non-specific binding was defined as the residual radioactivity bound in the presence of 0.25 μM IL-1β. Binding isotherms were analysed by non-linear least-squares curve fitting using the programme LIGAND [19] to determine the maximal number of sites and the equilibrium dissociation constant (K_d).

3. RESULTS AND DISCUSSION

IL-1β induced opposing effects on HIT-T15 insulin secretion, dependent on both dosage and time, which are largely comparable to previously reported observations with isolated islets [1,2,7,8]. Overall, HIT cells proved to be more sensitive to the effects of IL-1β than islets, as shown by the lower doses required to trigger either the stimulatory or inhibitory responses. This may simply be because a monolayer of single β cells (HIT cells) presents less of a diffusion barrier to cytokines than does an aggregate mixture comprised of both β and non-β cells (islets).

In long-term incubations (48 h; fig.1a), IL-1β in the range 0.005–50.0 U/ml, induced a dosage-dependent inhibition of insulin release. Maximal inhibition (30% of control) was evident at 0.5 U/ml. The inhibitory threshold was between 0.01 and 0.05 U/ml IL-1β. That this effect represents a specific inhibition of insulin secretion is supported by two additional observations. First, HIT-T15 DNA content (768.8 ± 23.8 ng/10^6 cells (n = 8)) was not affected by IL-1β. Therefore, the reduced levels of insulin accumulated in the medium during a 48 h exposure to cytokine are not merely due to a reduced number of cells. In addition, the subsequent insulinotropic response to secretagogues was also shown to be impaired after a 48 h preincubation with IL-1β. Here, cells were preincubated in the presence or absence of IL-1β (25 U/ml or ~1 pM) for 48 h, washed, and then challenged with the adenylate cyclase activator, forskolin (10μmol/l), or the sulphonylurea, glibenclamide (20 μg/ml), for a total of 90 min (fig.1b). In control incubations, both forskolin and glibenclamide significantly ($P<0.001$) potentiated insulin release. However, following preincubation with IL-1β, control, forskolin- and glibenclamide-stimulated secretory responses were all significantly ($P<0.01$) inhibited. IL-1β therefore also inhibits insulinotropic activity that is potentiated

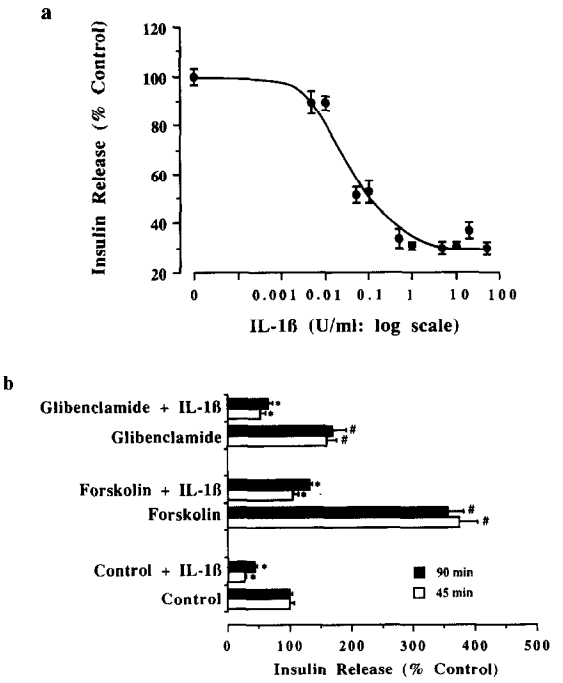

Fig.1. Long-term (48 h) inhibitory effect of IL-1β on HIT-T15 insulin secretion (a) dosage-dependent inhibition ($P<0.001$, ANOVA) of insulin release following a 48 h incubation with IL-1β (0.005–50.0 U/ml). Insulin release was measured by ELISA. Mean absolute control insulin release: 8621.7 μU/ml (n = 6). (b) Effect of preincubation with IL-1β on secretagogue responsiveness of HIT-T15 cells. Cells were preincubated in the presence or absence of 25 U/ml IL-1β for 48 h, washed, then challenged with forskolin (10 μM) or glibenclamide (20 μg/ml) for a total of 90 min. Insulin release was measured by ELISA. In controls, forskolin and glibenclamide potentiated insulin release ($^{\#}P<0.01$, ANOVA). However, preincubation with IL-1β induced significant inhibition ($^{*}P<0.01$, ANOVA) of control, forskolin- and glibenclamide-stimulated insulin release. Mean absolute values for control insulin release: 45 min, 450.0 μU/ml (n = 3); 90 min, 1572.9 μU/ml (n = 3).

by either increased β-cell cAMP or through regulation of the ATP-dependent potassium channel.

In contrast to the inhibition of insulinotropic responses seen in long-term experiments, IL-1β (0.1–50.0 U/ml) induced an overall 2-fold potentiation of insulin release in short-term incubations (45, 90 min; fig.2), similar to the potentiated secretory response reported previously in islets [7,8]. The question as to how IL-1 may have opposing effects on β cell function, and whether these events are mediated by corresponding or disparate mechanisms, still remains largely unanswered.

Zawalich and colleagues [20–22] have shown that IL-1 actuates glucose-dependent, stimulatory or inhibitory effects on β cell function, either of which may be mediated by phosphoinositide (PI)-derived second messengers. Thus, prior short-term exposure of islets to IL-1 potentiated the first-phase insulin secretory response to a subsequent glucose challenge [20]. In contrast, prolonged exposure ultimately resulted in defects

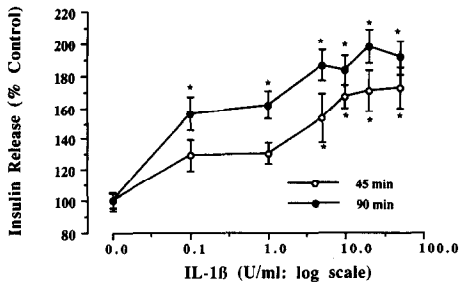

Fig.2. Short-term insulinotropic effect of IL-1β in HIT-T15 cells. Dosage-dependent stimulation ($P<0.01$, ANOVA) of HIT cell insulin release over 90 min incubation with IL-1β (0.1–50.0 U/ml). Insulin release was measured by ELISA. Mean absolute values for control insulin release: 45 min, 422.6 μU/ml ($n = 3$); 90 min, 2792.8 μU/ml ($n = 3$).

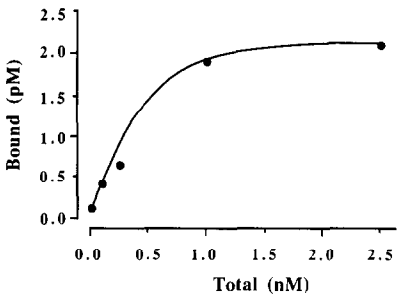

Fig.3. IL-1β binding isotherm for HIT-T15 cells. Binding of IL-1β to HIT cells was specific and saturable. Analysis by non-linear least-squares curve fitting revealed binding sites of high and low affinity. Data are mean ± SE ($n = 3$) for a single assay, representative of a total of 4 experiments. Where SE bars are not present, they are smaller than mean experimental values.

in both first- and second-phase secretory responses to glucose. This potentiatory effect on glucose-stimulated insulin secretion was subsequently extended to stimulation by cholecystokinin, tolbutamide, leucine or glyceraldehyde, and in each case was accompanied by PI hydrolysis [21,22]. However, prolonged exposure to IL-1 appears to desensitize the cell to glucose, an event which is accompanied by an impairment in PI hydrolysis [22]. One consequence of this impaired PI hydrolysis would be a reduced β-cell diacylglycerol (DAG) content and, consequently reduced activity of protein kinase C. The observation that 1-monooleoyglycerol (a diacylglycerol kinase inhibitor which increases DAG content, thereby activating protein kinase C), significantly restored insulin release inhibited by IL-1 is consistent with this proposition [22]. A second messenger role for Ca^{2+} in insulin secretion is now firmly established. IL-1 is known to increase intracellular Ca^{2+} in human neutrophils, and may also mobilise membrane-associated Ca^{2+} [8]. β Cell calcium homeostasis is clearly an area that may be associated with the short-term potentiation of insulin release by IL-1, and deserves further study. The impairment of intracellular Ca^{2+} homeostasis in the long-term inhibition of insulin secretion by IL-1β is suggested by the observation that an 18 h exposure to IL-1β has been shown to inhibit the voltage-dependent uptake of Ca^{2+} in islets [23].

In their compelling model of type I diabetes, which views IL-1β as the main effector cell associated with β-cell destruction, Nerup and colleagues have alluded to the considerable circumstantial evidence implicating free radical generation as a major component of cytokine damage [1,2,7]. Recently, Sumoski et al. furnished support in favour of this hypothesis by showing that a combination of the free radical scavengers, dimethylthiourea (DMTU; 1 mM) and citiolone (3 mM), offered protection against cytokine lysis of rat islet monolayers [24]. However, at best, protection was only 25–30% above controls, suggesting that although induction of oxygen-free radicals by IL-1β is possible, it cannot account for all of the damage to β cells. Further, other workers have found no effect with DMTU [14]. Likewise, in preliminary studies we have not found any protective effects of free radical scavengers, including DMTU, on cytokine-induced damage in HIT-T15 cells (unpublished observations). This point will only be resolved if oxygen-free radicals can be unequivocally demonstrated in single β cells. In an attempt to achieve this, we are currently applying electron spin resonance to HIT-T15 cells in the presence of cytokines.

In addition to its effects on insulin-secreting β cells, IL-1 invokes a copious number of endocrine and immunological effects in a myriad of cell types [25]. Further, although the biochemical mechanisms remain ambiguous, it is now known that many of these cells bind IL-1 in a specific and saturable manner [25]. In other words, the capacity to respond to IL-1 normally correlates with the presence of specific plasma membrane receptors. Accordingly, we have looked for IL-1 receptors on HIT-T15 cells. Fig.3 shows that HIT-T15 cells bind IL-1β in a specific and saturable manner. This is consistent with the presence of specific plasma membrane receptors for IL-1 in pancreatic β cells. Moreover, Scatchard analysis (data not shown) of the binding data revealed a curvilinear profile. This suggested two categories of binding site: a high affinity site (K_d = 0.203 ± 0.032 ($n = 4$) nM) with an average of 450 ± 122 sites per cell ($n = 4$), and a low affinity site (K_d = 1.410 ± 0.758 ($n = 4$) nM) with an average of 1332 ± 355 ($n = 4$) sites per cell. β cells may therefore simultaneously possess both high and low affinity receptors for IL-β, as do many T-cell lines and other normal cells (which also show similar ranges in K_d values and numbers of binding sites) [25].

The demonstration of specific receptors for IL-1β on insulin-secreting β cells provides a further step towards gaining an understanding of how cytokines may explicitly mediate autoimmune destruction during the progression of insulitis. Studies aimed at defining (i) the regulation of receptor activity and expression, and (ii) the second messengers linking receptor activity to the biological responses of β cells, may reveal novel thera-

peutic approaches for the treatment of insulin-dependent diabetes.

Acknowledgements: We are grateful to Dr A.E. Boyd III (Baylor College of Medicine) for providing HIT-T15 cells, and Dr Alan Shaw (Glaxo Institute for Molecular Biology) for providing human recombinant IL-1β.

REFERENCES

[1] Nerup, J., Mandrup-Poulsen, T., Molvig, J., Helquist, S., Wogensen, L. and Egeberg, J. (1988) Diabetes Care 11 (Suppl. 1), 16–23.
[2] Nerup, J., Mandrup-Poulsen, T., Molvig, J. and Spinas, G. (1988) in: The Pathology of the Endocrine Pancreas in Diabetes (Lefebvre, P. and Pipeleers, D., eds) pp. 71–84, Springer-Verlag, Berlin.
[3] Jacobs, C., Vertrees, S., Wilson, D., Stenger, D., Dower, S., Baker, P. and Lermark, A. (1989) Endocrine Society 71st Annual Meeting, Seattle, WA, USA, 982A.
[4] Nerup, J., Wogensen, L. and Kolb-Bachofen, V. (1989) Diabetologia 32, 355A.
[5] del Rey, A. and Besedovsky, H. (1987) Am. J. Physiol. 253, R794–R798.
[6] del Rey, A. and Besedovsky, H. (1989) Horm. Res. 31, 94–99.
[7] Mandrup-Poulsen, T. (1988) Danish Med. Bull. 35, 438–460.
[8] McDaniel, M.L., Hughes, J.H., Wolf, B.A., Easom, R.A. and Turk, J.W. (1988) Diabetes 37, 1311–1315.
[9] Mandrup-Poulsen, T.J., Egeberg, J., Nerup, J., Bendtzen, K., Nielsen, J.H. and Dinarello, C.A. (1987) Acta Pathol. Microbiol. Immunol. Scand. Sect. C 95, 55–63.
[10] Pukel, C., Baquerizo, H. and Rabinovitch, A. (1988) Diabetes 37, 133–136.
[11] Sandler, S., Bendtzen, K., Eizirik, D.L., Sjoholm, A. and Welsh, N. (1989) Immunol. Lett. 22, 267–272.
[12] Sandler, S., Andersson, A. and Hellerstrom, C. (1987) Endocrinology 121, 1424–1431.
[13] Eizirik, D.L., Sandler, S., Hallberg, A., Bendtzen, K., Sener, A. and Malaisse, W.J. (1989) Endocrinology 125, 752–759.
[14] Sandler, S., Bendtzen, K., Hakan Borg, L.A., Eizirik, D.L., Strandell, E. and Welsh, N. (1989) Endocrinology 124, 1492–1501.
[15] Eizirik, D.L. and Sandler, S. (1989) Diabetologia 32, 769–733.
[16] Ashcroft, S.J.H., Hammonds, P. and Harrison, D.E. (1986) Diabetologia 29, 727–733.
[17] Kekow, J., Ulrichs, K., Muller-Ruchholtz, W. and Gross, W.L. (1988) Diabetes 37, 321–326.
[18] Hinegardner, R.T. (1971) Anal. Biochem. 39, 197–201.
[19] Munsen, P.J. and Rodbard, D. (1980) Anal. Biochem. 107, 220–229.
[20] Zawalich, W.S. and Diaz, V.A. (1986) Diabetes 35, 1119–1123.
[21] Zawalich, W.S., Dierolf, B. and Zawalich, K.C. (1989) Endocrinology 124, 720–726.
[22] Zawalich, W.S., Zawalich, K.C. and Rasmussen, H. (1989) Endocrinology 124, 2350–2357.
[23] Wolf, B.A., Hughes, J.H., Florholmen, J., Turk, J.W. and McDaniel, M.L. (1989) FEBS Lett. 248, 35–38.
[24] Sumoski, W., Baquerizo, H. and Rabinovitch, A. (1989) Diabetologia 32, 792–796.
[25] Dinarello, C.A. and Savage, N. (1989) Crit. Rev. Immunol. 9, 1–20.