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Interleukin-1 induces rapid and transient expression of the c-fos proto-oncogene in isolated pancreatic islets and in purified β-cells

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The effect of interleukin-1β (IL-1) on expression of c-fos mRNA in isolated rat pancreatic islets was examined. Accumulation of c-fos mRNA was demonstrable after 30 min of exposure to IL-1, peaked by 60 min, and declined thereafter. Fluorescence-activated cell sorting (FACS) of dispersed islet cells was employed to localize the accumulation of c-fos mRNA to the β-cell. Cycloheximide did not influence the induction of c-fos mRNA by IL-1. Accumulation of c-fos mRNA therefore appears to be an early signal transduction event in the β-cell and a component of the cellular mechanism(s) by which IL-1 influences β-cell function.

Interleukin 1; Pancreatic islet; β-cell; c-fos; Insulin secretion

1. INTRODUCTION

An impressive body of evidence suggests that the disease insulin-dependent diabetes mellitus (IDDM) has an underlying autoimmune component [1]. Although the molecular mechanisms which contribute to the pathogenesis of IDDM are not yet understood, a recent hypothesis is that the soluble immune mediator, interleukin 1 (IL-1), plays a role in the destruction of pancreatic β-cells that is the hallmark pathologic lesion of the disease [2]. IL-1, alone or in concert with other cytokines, has been reported to cause lysis of β-cells in vitro under some conditions [3-6]. In addition, IL-1 exerts potent stimulatory and inhibitory effects on insulin secretion from isolated islets under conditions in which cell viability is maintained [7-12]. These latter effects of IL-1 on islets have been taken to imply that IL-1 may play a physiologic role in the modulation of insulin secretion [8,11].

Although disruptions in mitochondrial function [9,13-15] and the generation of toxic-free radicals [16,17] have been proposed to contribute to the functional effects of IL-1 on islets, the molecular mechanisms whereby IL-1 exerts its complex effects on insulin secretion and β-cell viability are largely unknown. The recent demonstration that β-cells possess specific receptors for IL-1 suggests that some or all of the effects of IL-1 on the β-cell may be the result of specific receptor-mediated pathways [18]. Little is known about the mechanism of action of IL-1 following binding of the cytokine to its receptor, but the observation that IL-1 and its receptor are translocated to the nucleus in the murine T cell line EL-4 indicates that IL-1 may directly modulate the transcription of specific genes in target cells [19]. Helqvist has demonstrated that IL-1 induces protein synthesis in isolated islets [20], and we have recently demonstrated that inhibition of insulin secretion by IL-1 is prevented by an inhibitor of gene transcription (actinomycin D) [21]. These findings underscore the potential importance of new gene products in the functional effects of IL-1 on the β-cell.

The identities of specific genes whose expression is modulated by IL-1 in the β-cell have not yet been determined, but a great deal of recent attention has been focused on the early response genes that are activated by IL-1 in other cells and on the roles that the protein products of these genes play in the biological effects of the cytokine. In fibroblasts and in endothelial cells, IL-1 induces rapid, transient expression of the c-fos proto-oncogene [22,23]. The protein product of the c-fos gene (c-Fos) has been proposed to function as a transcriptional regulator which couples early signal transduction events to long-term nuclear events that result in growth, differentiation, or the acquisition of new functional properties by the target cell [24,25].

In this study, the effect of IL-1 on c-fos mRNA expression has been examined in pancreatic islets isolated from rats. Our results indicate that c-fos mRNA is rapidly induced in isolated islets and in purified β-cells by IL-1, and they suggest that IL-1 has the capacity of

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initiate nuclear events which may be involved in the functional effects of IL-1 on insulin secretion.

2. MATERIALS AND METHODS

2.1. *Isolation of rat islets and purification of islet β-cells by autofluorescence-activated cell sorting*

Islets were isolated from 12–15 rats fed ad libitum by collagenase digestion, as previously described [26]. Isolated islets were dispersed into individual cells by treatment with dispase, and the freshly dissociated cells were subjected to autofluorescence-activated cell sorting (FACS) using a FACS-IV instrument [27,28]. An argon laser illuminated the cells at 488 nm, and emission was monitored at 510–550 nm. This technique yields two populations of cells. One population consists of highly purified β-cells (>90%). The second population is predominantly α-cells (70%) but also contains some δ-cells and a few β-cells [27,28].

2.2. *Incubation of isolated islets and FACS-purified islet cells with IL-1*

Isolated islets and FACS-purified islets cells were placed in 2.5 ml Petri dishes containing CMRL-1066 tissue culture medium with recombinant human interleukin-1β (Cistron, Pine Brook, NJ) (5U/ml) or the control vehicle. Either 600 islets/dish or 5×10^5 dispersed, purified cells/dish were used. In some experiments, cycloheximide (10 μM) was added. The dishes were incubated at 37°C under an atmosphere of 95% air/5% CO_2 for a predetermined time interval. At the end of this interval, the islets or FACS-purified cells were harvested, placed in 1.5 ml microcentrifuge tubes, and centrifuged in a table-top microcentrifuge for 15 s. The supernatant was discarded, and 500 ml guanidine isothiocyanate was added. Isolation of RNA and Northern blot analysis were then performed as described [29]. Total cellular RNA isolated from PC-12 rat pheochromocytoma cells stimulated with nerve growth factor served as a positive control for c-fos expression [29].

3. RESULTS

IL-1 was found to induce rapid, transient accumulation of c-fos mRNA in isolated rat islets (Fig. 1). The c-fos mRNA was not detected in untreated islets (lane 1), but detectable levels were achieved after 30 min of exposure to IL-1 (lane 2). Accumulation of c-fos mRNA was maximal after 60 min of exposure to IL-1 (lane 3) and declined rapidly thereafter (lanes 4–7).

The islet is a heterogeneous cell population which contains a variety of endocrine cells, each of which represents a potential target for IL-1. In order to localize the accumulation of c-fos mRNA to the β-cell and to demonstrate that the induction of c-fos mRNA does not require the presence of non β-cells, we examined the effect of IL-1 on the accumulation of c-fos mRNA in islet cells which had been purified by fluorescence-activated cell sorting (FACS). Fig. 2 demonstrates that IL-1-induced c-fos mRNA accumulation in intact isolated islets (lanes 1 and 2) and in FACS-purified β-cells (lanes 3 and 4) but not in FACS-purified α-cells (lanes 5 and 6). This finding suggests that IL-1 induces c-fos expression by a direct effect on the β-cell, rather than by an action on an intermediary cell.

A characteristic feature of c-fos and other 'immediate-early' response genes is that their induction by

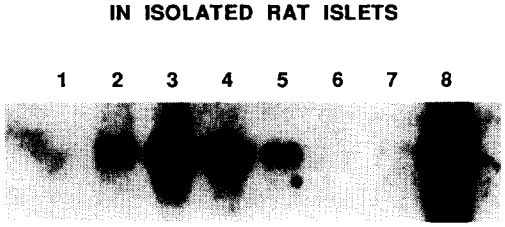

Fig. 1. Effect of IL-1 on expression of c-fos in isolated pancreatic islets. Isolated rat islets were incubated in CMRL-1066 tissue culture medium containing IL-1 (5 U/ml) for 0 min (lane 1), 30 min (lane 2), 60 min (lane 3), 90 min (lane 4), 2 h (lane 5), 4 h (lane 6), or 8 h (lane 7). Northern analysis for c-fos was then performed as described in section 2. Total RNA isolated from PC-12 rat pheochromocytoma cells stimulated with nerve growth factor served as a positive control for c-fos expression (lane 8).

growth factors or differentiation-inducing agents occurs under conditions where protein synthesis is inhibited [32]. These 'immediate-early' response genes therefore appear to be directly coupled to signal transduction events, and their expression does not require the synthesis of transcription-activating proteins. In order to examine what role protein synthesis plays in the induction of c-fos by IL-1, isolated rat islets were cultured with either IL-1 (5 U/ml) or with vehicle in the presence or absence of cycloheximide (10 μM) for 1 h. At this concentration, cycloheximide completely inhibited islet protein synthesis, as assessed by the incorporation of [^{35}S]methionine into total islet TCA-precipitable protein (not shown). Total islet RNA was isolated, and Northern analysis was performed as described above in section 2. Fig. 3 demonstrates that cycloheximide had no effect on the accumulation of c-fos mRNA induced by IL-1 (lanes 2 and 4). This finding suggests that IL-1-induced accumulation of c-fos mRNA does not require intermediary protein synthesis, and it is consistent with a role for c-fos as an 'immediate-early' gene in the response of the β-cell to IL-1. Cycloheximide alone induced c-fos to a small degree (lane 3). This may reflect that c-fos transcription is controlled by a suppressor protein whose synthesis is block-

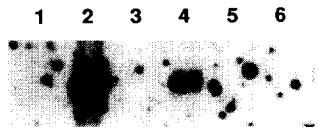

Fig. 2. Effect of IL-1 on expression of c-fos mRNA in FACS-purified islet cells. Isolated rat islets (lanes 1 and 2), a FACS-purified islet cell population enriched for β-cells (lanes 3 and 4), and a FACS-purified islet cell population enriched for α-cells (lanes 5 and 6) were treated with vehicle (odd-numbered lanes) or with IL-1 (5 U/ml) (even-numbered lanes) for 60 min. Northern analysis for c-fos was then performed as described in section 2.

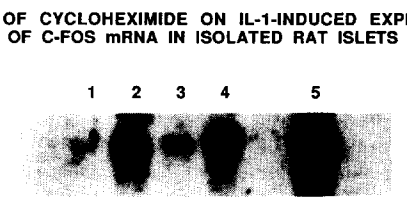

Fig. 3. Effect of cycloheximide on IL-1-induced expression of c-fos mRNA in isolated rat islets. Isolated islets were treated with vehicle (lane 1), IL-1 (5 U/ml) (lane 2), cycloheximide (10 μM) (lane 3), or IL-1 + cycloheximide (lane 4) for 60 min. Northern analysis for c-fos mRNA was then performed as described in section 2.. Total RNA isolated from PC-12 rat pheochromocytoma cells stimulated with nerve growth factor served as a positive control for c-fos expression (lane 5).

ed by cycloheximide or that cycloheximide stabilizes the c-fos transcripts by blocking the synthesis of enzymes involved in mRNA degradation.

4. DISCUSSION

The c-fos proto-oncogene is a member of the class of inducible genes termed 'immediate-early' genes [30–32]. The protein product of the c-fos gene is thought to function as a transcriptional regulator which couples extracellular signals to alterations in cellular phenotype by regulating the expression of target genes [30,31]. Induction of c-fos occurs in response to many growth factors and differentiation-inducing agents [24,25]. For example, c-fos expression has been associated with the transition from G_0 to G_1 that occurs when quiescent fibroblasts are stimulated with epidermal growth factor [24,25]. In addition, c-fos is thought to play a role in the functional response of macrophages to colony stimulating factors [24,25] and in the prolonged functional changes induced in neurons by nerve growth factor and neurotransmitters [32]. In this study we have provided the first demonstration that IL-1 induces accumulation of c-fos mRNA both in isolated pancreatic islets and in FACS-purified pancreatic β-cells. Our results indicate that induction of c-fos mRNA accumulation occurs through a direct interaction of IL-1 with the β-cells and does not require the synthesis of protein(s). The latter finding suggests that the c-fos gene product may directly couple early transmembrane signals to long-term nuclear responses which influence islet function.

The precise role that induction of c-fos plays in the functional effects of IL-1 on the β-cell has not yet been established. Our recent demonstration that a pharmacologic inhibitor of mRNA synthesis (actinomycin D) prevents the inhibition of insulin secretion otherwise induced by IL-1 suggests that Il-1-induced gene transcription is required for the development of the effects of IL-1 on insulin secretion from pancreatic islet β-cells [21]. It is therefore possible that the protein product of the c-fos gene (c-Fos) regulates the expression of genes whose products mediate the inhibitory influence of IL-1 on insulin secretion. It is also possible that c-Fos induces the transcription of specific genes whose products are involved in the reported cytotoxic effects of IL-1 on the β-cell [3–6]. Recently, Helqvist demonstrated that IL-1 induces the synthesis of several proteins in islets [20]. The identities of these proteins have not yet been determined, but the observation that IL-1 induces new protein synthesis in islets underscores the potential importance of specific gene products as mediators of IL-1 action. Identification of the β-cell gene(s) whose transcription is regulated by the c-fos gene product will lead to a better understanding of the mechanism of action of IL-1 on the β-cell and may yield insight into the pathogenesis of IDDM.

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REFERENCES

[1] Maclaren, N.K. (1981) in: The Islets of Langerhans (Cooperstein, S.J. and Watkins, D. eds.) pp. 453–466, Academic, New York.
[2] Nerup, J., Mandrup-Poulsen, T. and Molvig, J. (1987) Diabetes Metab. Rev. 3, 779–802.
[3] Mandrup-Poulsen, T., Bendtzen, K., Nielsen, J.H., Bendixen, G. and Nerup, J. (1985) Allergy 40, 424–429.
[4] Mandrup-Poulsen, T., Bendtzen, K., Nerup, J., Dinarello, C.A., Svenson, M. and Nielsen, J.H. (1986) Diabetologia 29, 63–67.
[5] Bendtzen, K., Mandrup-Poulsen, T., Nerup, J., Nielsen, J.H., Dinarello, C.A. and Svenson, M. (1986) Science 232, 1545–1547.
[6] Pukel, C., Baquerizo, H. and Rabinovitch, A. (1989) Diabetes 37, 133–136.
[7] Zawalich, W.S. and Diaz, V.A. (1986) Diabetes 35, 1119–1123.
[8] Comens, P.G., Unanue, E.R., Lacy, P.E. and McDaniel, M.L. (1987) Diabetes 36, 963–970.
[9] Sandler, S., Andersson, A. and Hellerstrom, C. (1987) Endocrinology 121, 1424–1431.
[10] Spinas, G.A., Mandrup-Poulsen, T., Molvig, J., Baek, L., Bendtzen, K., Dinarello, C.A. and Nerup, J. (1986) Acta Endocrinol. 113, 551–586.
[11] McDaniel, M.L., Hughes, J.H., Wolf, B.A., Easom, R.A. and Turk, J.W. (1988) Diabetes 37, 1311–1315.
[12] Hughes, J.H., Easom, R.A., Wolf, B.A., Turk, J. and McDaniel, M.L. (1989) Diabetes 38, 1251–1257.
[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, 769–773.
[16] Nerup, J., Mandrup-Poulsen, T., Molvig, J., Helquist, S., Wogenson, L. and Egeberg, J. (1988) Diabetes Care 11 (Suppl. 1), 16–23.
[17] 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.

[18] Hammonds, P., Beggs, M., Beresford, G., Espinal, J., Clarke, J. and Mertz, R.J. (1990) FEBS Lett. 261, 97–100.
[19] Curtis, B.M., Widmer, M.B., DeRoos, P. and Qwarnstrom, E.E. (1990) J. Immunol. 144, 1295–1303.
[20] Helqvist, S., Hansen, B.S., Johannesen, J., Andersen, H.V., Nielsen, J.H. and Nerup, J. (1989) Acta Endocrinol. 121, 136–140.
[21] Hughes, J.H., Watson, M.A., Easom, R.A. and McDaniel, M.L. (1990) Diabetes (Abstract) in press.
[22] Lin, J.X. and Vilcek, J. (1987) J. Biol. Chem. 262, 11908–11911.
[23] Colotta, F., Lampugnani, M.G., Polentarutti, N., Dejana, E. and Mantovani, A. (1988) Biochem. Biophys. Res. Commun. 152, 1104–1110.
[24] Muller, R. (1986) Biochim. Biophys. Acta 823, 207–225.
[25] Verma, I.M. and Graham, W.R. (1987) Adv. Cancer Res. 49, 29–52.
[26] McDaniel, M.L., Colca, J.R., Kotagal, N. and Lacy, P.E. (1983) Methods Enzymol. 98, 182–200.
[27] Pipeleers, D.G. (1984) in: Methods in Diabetes Research (Larner, J. ed.), pp. 185–211, John Wiley and Sons, New York.
[28] Wang, J.L. and McDaniel, M.L. (1990) Biochem. Biophys. Res. Commun. 166, 813–818.
[29] Milbrandt, J. (1986) Proc. Nat. Acad. Sci. USA 83, 4789–4793.
[30] Curran, T. and Morgan, J.I. (1987) Bioessays 7, 255–261.
[31] Curran, T. and Franza Jr., B.R. (1988) Cell 55, 395–397.
[32] Morgan, J.I. and Curran, T. (1989) Trends Neurosci. 12, 459–462.