transactions

The cytoprotective actions of long-chain mono-unsaturated fatty acids in pancreatic β-cells

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Abstract

Chronic exposure of pancreatic β-cells to long-chain fatty acids can cause loss of secretory function and enhanced apoptosis by a process of 'lipotoxicity', which may be a contributory factor to the rising incidence of Type 2 diabetes in humans. However, when incubated *in vitro*, β-cells respond differentially to long-chain saturated and mono-unsaturated fatty acids, suggesting that these molecules may regulate cell functionality by different mechanisms. In particular, it is clear that, whereas saturated fatty acids [e.g. palmitate ($C_{16:0}$)] exert detrimental effects on β-cells, the equivalent mono-unsaturated species [e.g. palmitoleate ($C_{16:1}$)] are well tolerated. Indeed, mono-unsaturated species are potently cytoprotective. The present review explores the differential effects of these various fatty acids on β-cell viability and considers the possible mechanisms involved in cytoprotection by mono-unsaturates.

Introduction

It has been argued that the dramatically increasing incidence of diabetes mellitus represents one of the principal threats to human health in the 21st century [1]. On this basis, it is critical to the well-being of individuals that a better understanding is gained of the processes that underlie this escalation. Strong evidence indicates that the rise in diabetes (especially Type 2 diabetes) is correlated with increasing levels of obesity and that a contributory factor to the development of diabetes is an elevation in circulating non-esterified fatty acids [2,3]. These molecules appear to promote the deterioration in peripheral insulin-sensitivity, which is a primary feature of the condition [4,5], and they may also be responsible for the gradual decline in β-cell function that inevitably occurs during the longer-term progression of Type 2 diabetes [6]. Thus the idea has emerged that a rise in non-esterified fatty acids is detrimental to the long-term maintenance of good health and there is considerable epidemiological and experimental evidence to support this concept. However, this generalization overlooks the possibility that different species of fatty acids may exert differential effects on target tissues within the body and it is important that the responses to individual fatty acids are understood since these may vary. In general, it appears to be true that a rise in saturated fatty acids leads to loss of tissue insulin-sensitivity and to declining β-cell function, but it is less clear whether a similar response follows from an increase in unsaturated species. Since changing dietary habits lead to altered ratios of the various fatty acid species *in vivo* [7,8], it seems probable that interactions between the saturates and unsaturates will be important determinants of the overall outcome at the level of relevant tissues.

In order to address the possibility that different species of fatty acids may contribute differentially to the decline in pancreatic β-cell function seen in Type 2 diabetes, we have examined the effects of individual species of saturated and unsaturated fatty acids *in vitro* [9–13]. The results are striking in that they reveal dramatic differences between the effects of long-chain saturated fatty acids [e.g. palmitate ($C_{16:0}$) or stearate ($C_{18:0}$)] versus the equivalent mono-unsaturated species [palmitoleate ($C_{16:1}$) or oleate ($C_{18:1}$)]. The nature of these differences forms the basis of this brief review.

Changes in β-cell viability associated with exposure to saturated fatty acids versus MUFAs (mono-unsaturated fatty acids)

The principal dogma that lies at the heart of the concept that a rise in non-esterified fatty acids may contribute to β-cell loss in Type 2 diabetes relates to a process that is termed 'gluco-lipotoxicity' [3]. This postulates that a combination of rising blood glucose and non-esterified fatty acid levels represents a particularly toxic mix that collaborates to induce the demise of the β-cell. This hypothesis is supported by a large body of evidence, but the precise mechanisms by which β-cell death is induced remain unclear. Therefore, to address this, we have studied the loss of viability of cultured rodent β-cells during exposure to fatty acids *in vitro* at a fixed (11 mM) glucose concentration. It is clear that use of such a model system cannot entirely reproduce the situation that develops *in vivo*, but it has the distinct advantage that the effects of individual fatty acids can be studied either alone or in defined combinations.

Exposure of cultured β-cells to long-chain saturated fatty acids produces a dramatic change in viability [9,10,14–16]. Thus, within only a few hours of exposure of cells

Key words: apoptosis, long-chain mono-unsaturated fatty acid, obesity, palmitate, palmitoleate, Type 2 diabetes.

Abbreviations used: ERK, extracellular-signal-regulated kinase; GPCR, G-protein-coupled receptor; MUFA, mono-unsaturated fatty acid; PI3K, phosphoinositide 3-kinase.

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to physiologically relevant concentrations of palmitate or stearate, evidence of loss of viability can be detected. This is manifest as a rapid activation of effector caspases [13] coupled with a series of other changes indicative of induction of apoptosis [including (i) redistribution of phosphatidylserine, (ii) condensation and margination of nuclear chromatin etc.]. Thus the cells respond to the burden imposed on them by an elevated level of saturated fatty acids by entering apoptosis. However, this response is not uniform in terms of its specificity, suggesting that it does not represent a non-specific mechanism to *any* saturated fatty acid. Thus β-cells tolerate the presence of elevated concentrations of myristate ($C_{14:0}$), laurate ($C_{12:0}$) or octanoate ($C_{8:0}$) much better than they tolerate palmitate [9]. Indeed, they can survive relatively prolonged incubation in the presence of these shorter chain species, whereas even short-term exposure to palmitate is detrimental. Therefore it is clear that, to the β-cell, not all saturated fatty acids are equivalent and that these cells respond differentially to incubation in the presence of species having even quite modest changes in chain length (i.e. from $C_{16:0}$ to $C_{14:0}$). The molecular basis of these differential responses still awaits an adequate explanation (and has been rarely explored).

An even more striking difference is observed when β-cells are exposed to long-chain MUFAs. In this case, rather than undergoing apoptosis, cells incubated in the presence of palmitoleate or oleate show minimal signs of distress [9,12,17,18]. Their ability to proliferate in culture is preserved (or even enhanced) and they display none of the signs of apoptosis that accompany the exposure to saturated molecules. More dramatically still, co-incubation with palmitate in the simultaneous presence of palmitoleate is not associated with cytotoxicity. Therefore the response of the cells to saturated fatty acid molecules is modified according to the presence or absence of mono-unsaturated species. It is noteworthy that similar effects are not seen if toxic saturates (e.g. palmitate) are provided in combination with 'non-toxic' saturates (e.g. myristate). Under these conditions, cell death still ensues. By contrast, viability is preserved when palmitate and palmitoleate are used in combination. This leads to the important conclusion that there is a unique aspect to the handling of mono-unsaturates by β-cells whereby they are able to override the pro-apoptotic stimulus that normally derives from exposure to saturates. Hence, it appears that palmitoleate is directly anti-apoptotic in these cells. In support of this, it has been shown that the ability of palmitoleate to promote β-cell viability is not limited simply to its capacity to overcome the cytotoxic actions of saturated fatty acids. Rather, it has a more general anti-apoptotic action which also extends to other stimuli. For example, the loss of viability induced by exposure to pro-inflammatory cytokines or by withdrawal of the survival factors present in serum is also attenuated by palmitoleate [9].

On the basis of these considerations, it is clear that there is a fundamental difference in the way β-cells respond to long-chain saturated fatty acids versus long-chain MUFAs. More importantly, the results lead to the conclusion that treatment of cells with mono-unsaturates can be cytoprotective (at least under the model conditions employed *in vitro*). As a consequence, it may be inappropriate to generalize the proposition that an elevation of fatty acids is inevitably associated with β-cell loss *in vivo* since, conceivably, the final outcome may be influenced by the ratio of saturated to unsaturated species and by the cumulative response to these species over time.

Cytoprotection by long-chain MUFAs

As indicated above, the ability of saturated fatty acids to promote β-cell death varies according to the chain length. Similarly, incubation of cells with mono-unsaturates also elicits differential responses when molecules of varying chain length are employed. For example, a reduction in chain length [from $C_{16:1}$ to $C_{14:1}$ (myristoleate)] is associated with a notable decrease in anti-apoptotic potency [13]. This might imply that β-cells are simply rather inefficient in their handling of C_{14} fatty acid species, although an alternative possibility should also be considered, namely, that, at least in the case of mono-unsaturates, this difference betrays an important feature of the underlying mechanism involved. This is illustrated most clearly when the responses to various other fatty acid derivatives are also considered (below).

Treatment of β-cells with alkyl esters of long-chain MUFAs does not lead to loss of viability. More importantly, these molecules retain the anti-apoptotic activity of their unmodified counterparts, suggesting that metabolism of the mono-unsaturates is not a prerequisite for their cytoprotective activity [13]. This concept is brought into particular focus when the potency of methylated palmitoleate is compared with that of native palmitoleate, since this reveals that the two are essentially identical. It must be accepted that this result is not, in itself, conclusive, since it is possible that methyl-palmitoleate might become de-methylated during the incubation period, and that released free palmitoleate then mediates a cytoprotective effect. However, additional studies indicate that this is not the case. For example, exposure of cells to methyl-palmitoleate fails to alter either β-cell phospholipid disposition or the triacylglycerol content of the cells, whereas both of these parameters are modified by palmitoleate [11]. Thus these results are consistent with the expectation that methyl-palmitoleate should be metabolically inert and they imply that any release of free palmitoleate during the incubation period is minimal. As a consequence, they lead to the inevitable conclusion that structural, rather than metabolic, determinants are most important for conferring the cytoprotective efficacy of mono-unsaturates.

The proposition that the structural features of mono-unsaturates are critical for their anti-apoptotic actions immediately prompts an additional consideration, relating to the recent description of a class of cell-surface receptors for fatty acid molecules [19]. Among these, various members of the GPCR (G-protein-coupled receptor) family have attracted particular attention as possible mediators of some of the effects of fatty acids in defined cell types. In this context, the fatty acid GPCR, GPR40, is abundantly

expressed in β-cells [20] and has been proposed as a potential target for the cytoprotective actions of mono-unsaturates [21]. However, the pharmacology of GPR40 suggests that this receptor is unlikely to mediate the response, since it is activated by both saturates and mono-unsaturates and it appears to require a free carboxy group [22] such that it is unlikely to be readily activated by methylated fatty acids. A second receptor, GPR120, may be preferentially activated by long-chain unsaturated fatty acids and has been proposed as the target mediating cytoprotection by these molecules in the intestinal L-cell line, STC-1 [23]. However, this receptor was reportedly absent from MIN6 β-cells and, as in the case of GPR40, there is evidence that GPR120 may not be activated efficiently by methylated fatty acids [24]. Nevertheless, in contrast with the data from MIN6 cells, we find that GPR120 is expressed in rat β-cell lines by RT–PCR (reverse transcription–PCR) analysis and, in view of this, it may be premature to exclude this molecule as a possible mediator of cytoprotection (especially in the light of its proposed role in STC-1 cells). Unfortunately, there are no reports of selective synthetic agonists that can differentiate between GPR40 and GPR120 (note that the low-molecular–mass ligand, GW9508, is agonistic at both receptors [25]) and the availability of a GPR120-selective ligand would be of great benefit to the understanding of its functional role.

Mechanisms involved in mediating cytoprotection by long-chain MUFAs

One important feature of the protective response to mono-unsaturates that is especially relevant to discussions on possible cell-surface receptor involvement is the time course of their effects. In this context, a very surprising feature of the response was revealed by the observation that addition of palmitoleate to cells that are already beginning to show signs of apoptosis after prior incubation with palmitate can lead to cell death arrest [9,13]. This effect is observed as late as 10 h after initial exposure to palmitate and implies that the anti-apoptotic mechanism elicited on addition of palmitoleate must operate very quickly. This, in turn, implies that altered gene transcription is unlikely to play a primary role in initiating cytoprotection (although altered transcription could contribute to later events) and that a much more rapid effector process is activated. Receptor-mediated activation of a critical signal-transduction pathway might fulfil this role, and one obvious possibility is that a rise in cAMP could be involved since certain other cytoprotective agents {e.g. GLP-1 (glucagon-like peptide 1) [26]} probably exert at least part of their influence by raising the cAMP levels [27]. However, the effects of palmitoleate do not appear to involve any change in β-cell cAMP levels [10]. Rather, we have observed that treatment of β-cells with palmitoleate causes a marked and very rapid attenuation of caspase 3/7 activity [13]. Since these caspase enzymes mediate the effector phase of apoptosis, this mechanism could be responsible for initiating the cytoprotective response. Caspase 3/7 activity is increased very dramatically during treatment of β-cells with long-chain saturated fatty acids (e.g. palmitate) and the rate of increase in activity is halted on addition of palmitoleate (although it should be noted that palmitoleate does not reverse the extent of activation already achieved by palmitate at the time of addition of the mono-unsaturate; it acts to prevent any further increase in activity). If the two are added simultaneously, then no activation of caspase 3/7 is detected, since the pro-apoptotic action of palmitate is entirely prevented by the mono-unsaturate. These results imply that palmitoleate promotes the rapid induction of an anti-apoptotic signalling mechanism in β-cells, which culminates in the attenuation of caspase 3/7 activation. The precise nature of this signalling mechanism remains to be established, but it does not appear to involve 'classical' anti-apoptotic pathways dependent on molecules such as PI3K (phosphoinositide 3-kinase). This conclusion arises from the observation that cytoprotection is not altered by inhibitors of PI3K kinase and that exposure of cells to palmitoleate does not lead to rapid phosphorylation of downstream targets of PI3K, such as Akt (also called protein kinase B) (S. Dhayal and N.G. Morgan, unpublished work).

In contrast with the situation with PI3K, we have observed increased phosphorylation of ERK (extracellular-signal-regulated kinase) in β-cells after the addition of palmitoleate but, despite this, selective inhibitors of ERK fail to influence the cytoprotective actions of palmitoleate, suggesting that ERK activation is not critical to this response. The mechanism by which ERK is activated in response to palmitoleate is not known, but it is interesting to note that agonists of GPR120 can promote ERK phosphorylation [23].

An important possibility that must be considered when investigating the mechanism by which palmitoleate attenuates caspase 3/7 activation in β-cells is the involvement of a direct, non-specific inhibition of the enzyme (without the intervention of any signalling intermediates). In an attempt to exclude this possibility, we studied the sensitivity of β-cells to induction of apoptosis by an agent, etoposide, which is likely to act by a mechanism that is entirely different from that induced by saturated fatty acids (and involves inhibition of DNA topoisomerase activity). It was found that the rate of increase in cell death during exposure to etoposide was not altered by palmitoleate and that the mono-unsaturate caused only a very modest reduction in caspase 3/7 activity under these conditions [13]. Therefore it seems reasonable to conclude that palmitoleate does not directly inhibit caspase 3/7 activity in β-cells but, rather, it mediates its effects indirectly via other pathways. Identification of these signalling steps would represent a considerable advance and may open the way for selective targeting to be employed as a means to reduce β-cell apoptosis either *in vivo* or *in vitro* (e.g. during isolation and incubation of islets prior to their use in transplantation).

Taken together, the studies outlined in the present study reveal that pancreatic β-cells are equipped with an anti-apoptotic mechanism that is activated selectively by certain long-chain MUFAs. This mechanism displays considerable structural specificity and it appears to regulate a signalling

pathway that controls the activity of effector caspase enzymes in the cells. Further clarification of the molecular details of this mechanism is now of paramount importance in order to establish the basis of the differential effects of saturated versus mono-unsaturated non-esterified fatty acids on the viability of pancreatic β-cells.

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