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ACUTE STIMULATION OF PANCREATIC ISLETS BY INHIBITORS OF LACTIC ACID TRANSPORT

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Abstract—The transport of L-lactate into rat pancreatic islets and RINm5F insulinoma cells was inhibited by α-cyano-4-hydroxycinnamate, α-fluorocinnamate, quercetin and by *p*-chloromercuribenzenesulphonic acid. The addition of each of these compounds to perifused islets resulted in an immediate, marked stimulation of insulin release. Enhanced insulin secretion was accompanied by a similarly rapid and pronounced increase in the rate of $^{45}Ca^{2+}$ efflux from pre-loaded, perifused islets. In general, these stimulatory effects were most pronounced in the presence of a threshold concentration of glucose (5 mM) in the perifusion medium. In islets pre-loaded with $^{86}Rb^{+}$, the addition of α-fluorocinnamate or quercetin caused a modest diminution in efflux rate whilst enhanced rates of $^{86}Rb^{+}$ outflow were apparent in the presence of 5 mM glucose. It is suggested that these inhibitors of lactic acid transport stimulate the β-cell, at least in part, by increasing the intracellular : extracellular lactate gradient, thereby promoting the electrogenic efflux of endogenous lactate from the cell.

The secretion of insulin in response to nutrients is dependent upon the metabolism of the nutrient within the pancreatic β-cell [1, 2]. It is likely that increased oxidative metabolism provides, in the form of ATP, an energy supply to support enhanced secretory and synthetic activity. In addition, it has been suggested that an increase in ATP/ADP ratio in nutrient-stimulated β-cells [3] could provide a "metabolic signal", coupling metabolism, via modulation of a nucleotide-sensitive K^+ channel [4, 5], to cell membrane depolarization and hence stimulated insulin release.

In the case of glucose, whilst a significant proportion of its metabolism can be accounted for by complete oxidation to CO_2, approximately 50% of glucose converted to triose phosphates can be recovered as lactate, released into the incubation medium [6, 7]. The extrusion of this lactate from the β-cell, in common with several other mammalian cell types, presumably involves a transport system [8]. This lactate $^{-}H^{+}$ co-transporter is inhibited by several compounds including cinnamate derivatives, the bioflavenoid quercetin and the sulphydryl reagent *p*-chloromercuribenzenesulphonic acid (pCMBS) [8–11].

In order to assess the importance of lactate formation and efflux in the pancreatic β-cell, we have examined the acute effects of several of these inhibitors on islet function.

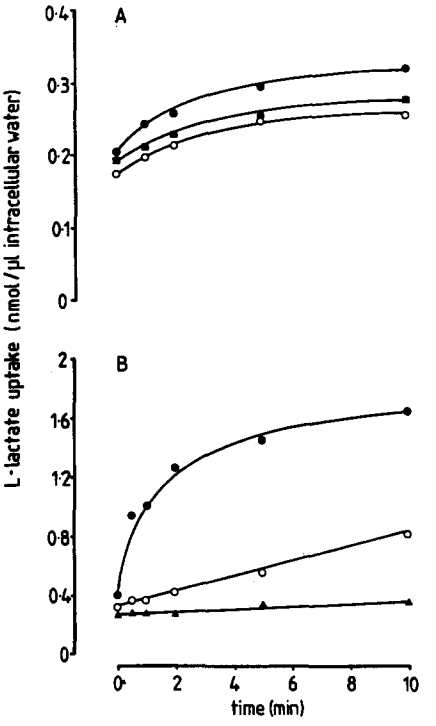

Fig. 1. Inhibition of 1 mM L-lactate transport in rat pancreatic islets (A) and in RINm5F cells (B) by 5 mM α-fluorocinnamate (■), 10 mM α-cyano-4-hydroxycinnamate (○) and by 0.25 mM pCMBS (▲).

MATERIALS AND METHODS

Pancreatic islets were isolated from fed adult rats by collagenase digestion [12] and incubated in a bicarbonate-buffered medium supplemented with 20 mM HEPES and gassed with O_2:CO_2 (95:5, v/v), RINm5F cells were a kind gift from Dr M. J. Dunne, and were cultured essentially as described elsewhere [13]. Albumin was omitted from the media since this is known to bind certain of the test agents. Insulin secretion, $^{45}Ca^{2+}$ and $^{86}Rb^{+}$ fractional outflow rates were measured essentially as described previously [14].

Lactate transport activity was measured either in rat islets or the cloned β-cell line RINm5F, using a silicone oil filtration technique. $HClO_4$ (50 μL) (20%, w/v) was placed in a small polythene

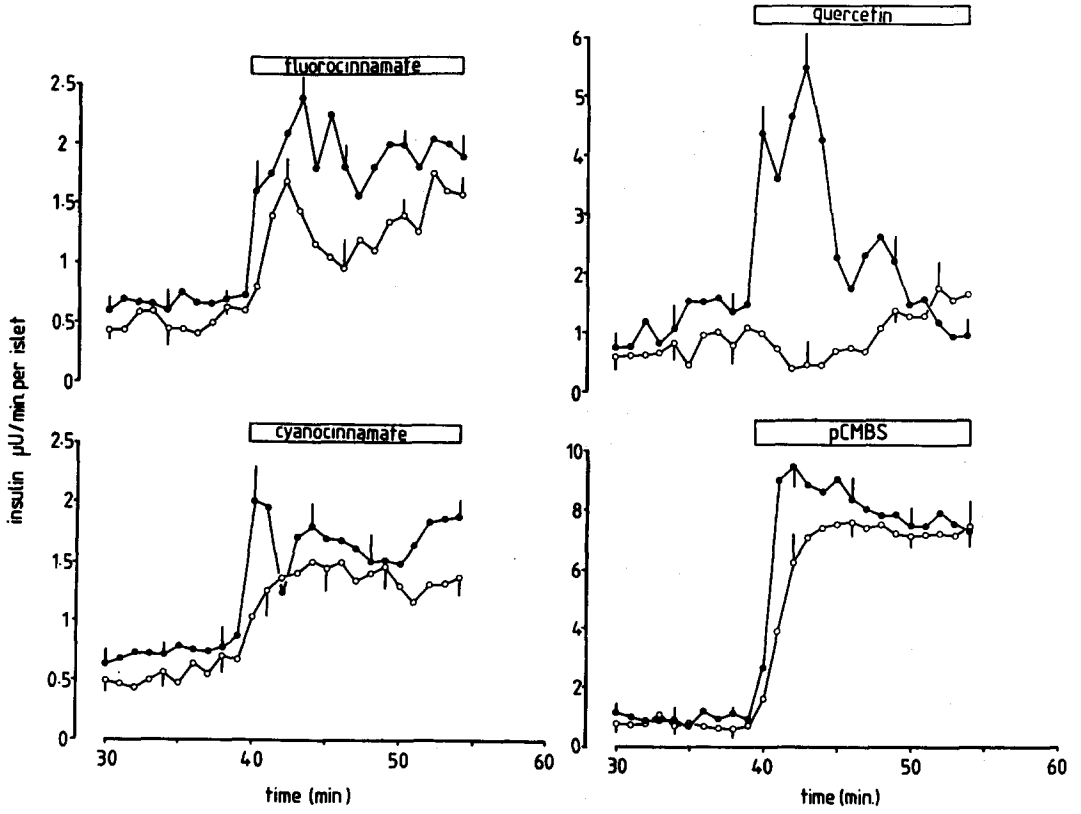

Fig. 2. Effects of α-fluorocinnamate (5 mM), α-cyano-4-hydroxycinnamate (10 mM), quercetin (0.25 mM) and pCMBS (0.25 mM) on insulin secretion from rat pancreatic islets. Groups of 100 islets were perifused in the absence (○) or presence (●) of 5 mM glucose and exposed to the test agent during the period designated by the horizontal bars. Each point represents the mean ± SE of 3–5 determinations.

microfuge tube, and 50 μL oil consisting of equal volumes of silicone oil MS550, (sp.g. 1.07) and dinonyl phthalate (sp.g. 0.98) layered on top of this. Incubation medium (90 μL), containing either 100 islets or approximately 10^6 RIN cells, either in the presence or absence of inhibitor, was subsequently layered upon the oil. Incubations were carried out at room temperature and were initiated by the addition of 10 μL medium containing 10 mM L-[U-^{14}C]lactate (2 μCi/mL) and ^3H_2O (20 μCi/mL) for the estimation of intracellular volume. In some experiments, the extracellular compartment was estimated by the use of [^{14}C]inulin; this value was subtracted from that for the total cell pellet. For the islet experiments, 10-fold higher specific activities were employed owing to the smaller amounts of tissue used. Incubations were terminated by centrifugation for 1 min, the islets or cells being sedimented through the oil layer into the $HClO_4$. The tube was rapidly frozen in liquid N_2, the tip cut off with a sharp blade and placed in 8 mL scintillant. The ^{14}C and ^3H_2O content of the cell pellet were counted by dual channel liquid scintillation counting, and the results expressed as intracellular concentration of lactate.

$^{45}CaCl_2$, $^{86}RbCl$, [^{125}I]insulin, L-[U-^{14}C]lactate, [^{14}C]inulin and ^3H_2O were obtained from Amersham International (Amersham, U.K.). Silicone oil MS 550 and dinonyl phthalate were from BDH Chemicals (Poole, U.K.), α-cyano-4-hydroxycinnamate, α-fluorocinnamate, quercetin, *p*-chloromercuribenzenesulphonic acid (pCMBS) and all other chemicals were purchased from the Sigma Chemical Co. (Poole, U.K.).

RESULTS

Figure 1 shows the time courses of L-lactate transport in pancreatic islets and RIN cells. In both cases, the uptake of 1 mM lactate was inhibited by either 5 mM α-fluorocinnamate or 10 mM α-cyano-4-hydroxycinnamate, and by 0.25 mM pCMBS in RIN cells. L-Lactate uptake in RIN cells was also inhibited by approximately 50% by 0.25 mM quercetin after 5 min (not shown). The intracellular lactate concentration at equilibrium was considerably lower in islets than in RINm5F cells; this was reflected by a smaller degree of inhibition of lactate transport in islets by the cinnamate derivatives. The reason for this difference between rat islet and RIN cells is at present unknown but could reflect differences in intracellular pH in islet and RIN cells. The apparent residual "uptake" at time zero probably represents a combination of the delay inherent in centrifugation together with a rapid lactate binding component [9].

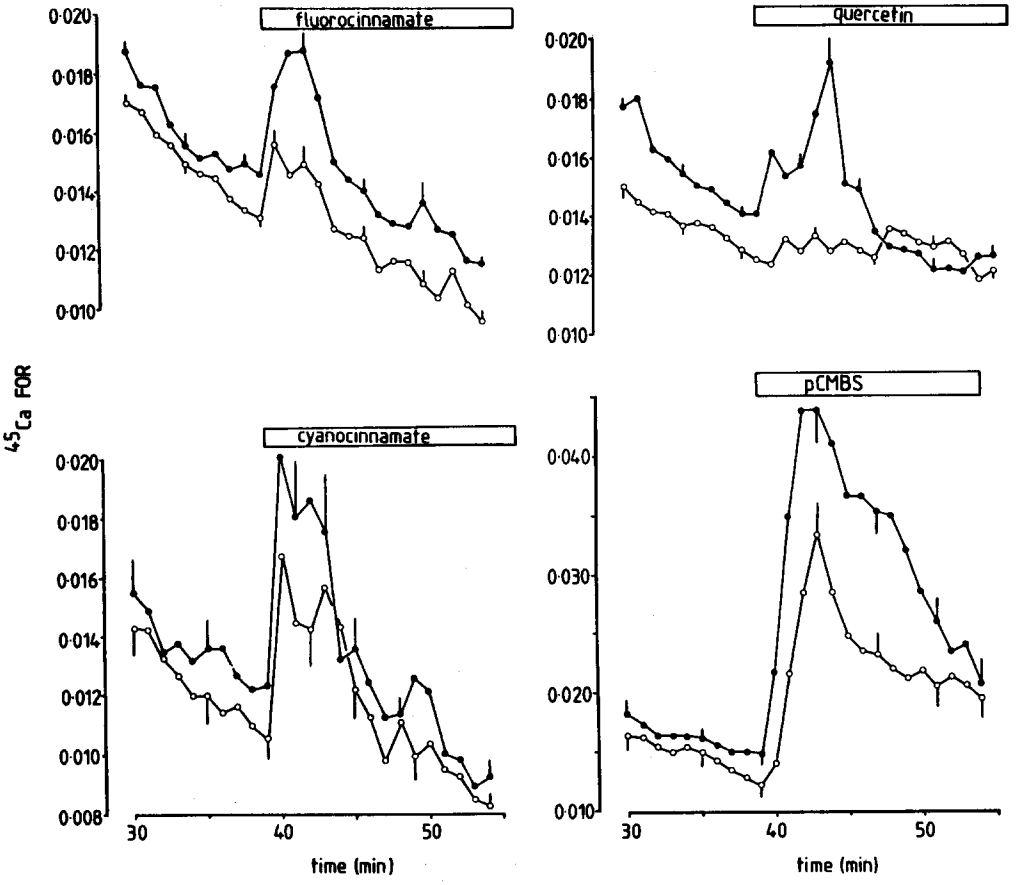

Fig. 3. Effects of α-fluorocinnamate (5 mM), α-cyano-4-hydroxycinnamate (10 mM), quercetin (0.25 mM) and pCMBS (0.25 mM) on the fractional outflow rate (FOR) of $^{45}Ca^{2+}$ from pre-loaded perifused pancreatic islets. Groups of 100 islets were perifused in the absence (○) or presence (●) of 5 mM glucose and exposed to the test agent during the period designated by the horizontal bars. Each point represents the mean ± SE of 3–5 determinations.

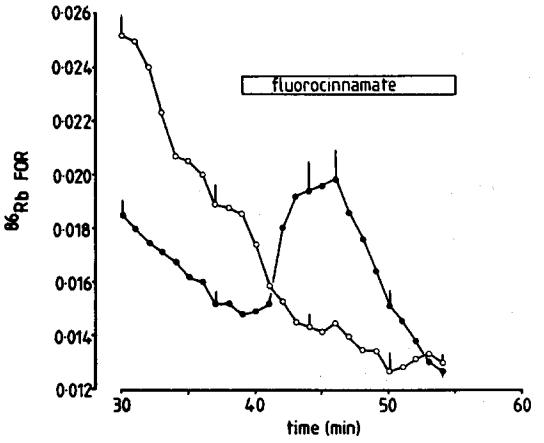

Fig. 4. Effects of α-fluorocinnamate (5 mM) on the fractional outflow rate (FOR) of $^{86}Rb^{+}$ from pre-loaded, perifused pancreatic islets. Groups of 100 islets were perifused in the absence (○) or presence (●) of 5 mM glucose and exposed to the test agent during the period designated by the horizontal bar. Each point represents the mean ± SE of 3 determinations.

The addition of lactate transport inhibitors to perifused pancreatic islets results in an immediate, pronounced stimulation of insulin release (Fig. 2). This effect was particularly pronounced and prolonged in the case of pCMBS.

The insulinotropic effects of the above agents were associated with corresponding increases in the rate of $^{45}Ca^{2+}$ efflux from pre-loaded, perifused islets (Fig. 3), suggesting enhanced Ca^{2+} entry into the islet cells. In general, the stimulatory effects of the cinnamate derivatives and pCMBS were more pronounced in the presence of a threshold concentration of glucose (5 mM); in the case of quercetin, this effect was dependent upon the presence of glucose.

The effect of α-fluorocinnamate on $^{86}Rb^{+}$ outflow rate is shown in Fig. 4. In the absence of glucose, α-fluorocinnamate or quercetin (not shown) appeared to cause a modest diminution in $^{86}Rb^{+}$ efflux; in contrast, in the presence of 5 mM glucose, addition of lactate transport inhibitor resulted in a marked rise in the rate of outflow.

DISCUSSION

It has been previously reported by ourselves and

others that a significant proportion of glucose metabolized by pancreatic islets can be accounted for by the production of lactate [7, 14] which can be recovered from the incubation medium [14]. Furthermore, the formation of endogenous lactate is dependent upon the ambient glucose concentration [7, 14]. It would thus be predicted that the β-cell would be equipped with one or more mechanisms for the extrusion of lactate from the cell, in common with numerous other cells which produce significant quantities of lactate [8]. This transport of lactate in such tissues occurs, at least in part, via a lactate$^-$/H^+ co-transporter which is competitively inhibited by certain cinnamate derivatives and non-competitively by the bioflavenoid quercetin and the sulphydryl group reagent pCMBS [8]. The existence of such a carrier in islet cells and in the cloned β-cell line RINm5F is suggested by the results shown in Fig. 1.

In order to assess the significance of endogenous lactate production and transport in pancreatic islet cells, we have studied the acute effects of these inhibitors on several aspects of islet function. All of these compounds shared the ability to cause a rapid and pronounced stimulation of insulin release and $^{45}Ca^{2+}$ efflux rate. Although the mechanism underlying these effects is uncertain, it was possible that reduced potassium permeability and subsequent depolarization of the β-cell plasma membrane might be involved. Whilst in the absence of glucose, a modest diminution in $^{86}Rb^+$ outflow rate was observed, in the presence of the sugar a marked increase in $^{86}Rb^+$ permeability was apparent, possibly as a result of Ca^{2+}-activated K^+ channel activity [15] since it coincided with an apparent increase in $^{40}Ca^{2+}$ entry.

We propose, as an alternative, that the activation of islet cells by inhibitors of lactate transport may be linked to a rise in the intracellular/extracellular lactate ratio which is likely to arise from blockade of the lactate transporter [16]. A rapid, relative increase in intracellular lactate concentration would be predicted to favour the electrogenic (as opposed to electroneutral, carrier-mediated) efflux of lactate from the cell, thus leading to depolarization of the plasma membrane and consequently cell activation. In this respect, addition of a lactate transport inhibitor might be expected to mimic a rise in glucose concentration which would also lead to increased intracellular lactate levels. These experiments are reminiscent of a previous study where the relative intracellular lactate concentration was raised by exposure of perifused islets to lactate followed by rapid withdrawal of the latter from the perifusate, a manipulation which also resulted in a marked stimulation of islet cell activity [14].

Such a mechanism would, in addition, explain why the stimulatory effects of the lactate transport inhibitors were more pronounced in the presence of glucose, when the islet cell capacity to generate lactate would be higher. This distinction was especially evident in the case of quercetin. Additional actions of these compounds, independent of lactate transport and perhaps including effects on potassium channel activity, cannot of course be ruled out, and it should be borne in mind that pCMBS in particular is likely to have numerous additional non-specific effects. Clearly, a number of differences were observed between the inhibitors investigated. It is possible that such variation could be determined by factors such as nature of inhibition (kinetics, competitive vs non-competitive), permeability of the plasma membrane to the inhibitor and non-selectivity of these compounds.

In summary, the present findings are consistent with a proposed relationship between β-cell activity and lactate output [6, 7, 13], and support our previous suggestion that the conversion of glucose to lactate, and the electrogenic efflux of the latter from the β-cell, could be an important determinant of β-cell membrane depolarization by the sugar [14, 17].

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