Biochemical Pharmacology, Vol. 37, No. 10, pp. 2019–2022, 1988.
Printed in Great Britain.

0006-2952/88 $3.00 + 0.00

INHIBITION OF PYRUVATE OXIDATION IN RAT ISLETS BY α-CYANO-4-HYDROXYCINNAMATE

DIFFERENTIAL EFFECTS ON INSULIN SECRETION AND INOSITOL LIPID METABOLISM

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(*Received* 17 *June* 1987; *accepted* 25 *November* 1987)

Abstract—The oxidation of ^{14}C-pyruvate by isolated rat pancreatic islets was inhibited competitively and in a concentration-dependent manner by α-cyano-4-hydroxycinnamate. A similar, though less marked inhibition was observed of U-^{14}C-glucose oxidation, although oxidation of 1-^{14}C-glucose was slightly enhanced in the presence of the drug. The rate of glycolysis, as estimated by the utilisation of 5-[^{3}H]-glucose and levels of ATP in islets were unaffected by α-cyano-4-hydroxycinnamate. The inhibition of pyruvate oxidation by α-cyano-4-hydroxycinnamate was accompanied by an inhibition of insulin secretion in response to glucose, but not to a combination of Ba^{2+} and theophylline. In contrast, glucose-stimulated inositol lipid breakdown was not affected by the drug. Thus, mitochondrial oxidation of pyruvate appears to be a prerequisite for glucose-stimulated insulin secretion, but not enhanced inositol lipid metabolism.

Pancreatic islets provide one, if not the only, example of a tissue in which hormone secretion can be triggered by nutrient stimuli such as glucose, in addition to agonists which bind to cell surface receptors. Furthermore, the stimulation of insulin release by nutrient secretagogues is accompanied by a marked enhancement in the metabolism of inositol lipids [1]. It is generally accepted that the stimulatory actions of nutrients on pancreatic islets are dependent upon the metabolic oxidation of those nutrients and generation of some as yet unidentified intracellular metabolic signal [2]. Whether such a signal is generated in the cytosol or mitochondrial compartment of the islet cell is unclear. The observation that glyceraldehyde shares the ability of glucose to stimulate secretion implied that a metabolic intermediate of glucose might be involved in stimulus–secretion coupling [3]. Furthermore, the fact that both glucose and glyceraldehyde are able to stimulate inositol lipid metabolism in islets in addition to secretion [4] suggests that these processes may be initiated by a common mechanism.

In the present study, we have investigated the effects upon certain aspects of islet function of α-cyano-4-hydroxycinnamate, a compound which is known to inhibit the transport of pyruvate into mitochondria [5], and resulted in the predicted inhibition of pyruvate oxidation in intact islets. This inhibition of pyruvate oxidation was associated with impaired secretion of insulin, specifically in response to glucose, although the accompanying increase in metabolism of inositol lipids was unaffected. Thus, these respective processes are likely to be initiated and controlled through distinct aspects of nutrient transport and/or metabolism in islet cells.

MATERIALS AND METHODS

Pancreatic islets were isolated from fed adult rats by collagenase digestion [6] and incubated in a bicarbonate-buffered salt solution [7]. Since α-cyano-4-hydroxycinnamate is known to bind strongly to albumin, the latter was omitted from the medium and added immediately prior to termination of the incubations. Insulin release and inositol phosphate production were measured in groups of 15 and 100 islets, as described previously [8]. The oxidation of exogenous nutrients was investigated by incubating groups of 50 islets for 120 min in 100 μl medium containing approx. 0.5 μCi ^{14}C-labelled substrate plus appropriate concentrations of unlabelled substrate. Incubations were carried out in Beckman microfuge tubes placed inside scintillation vials, gassed with O_2/CO_2 (19:1 v/v) and stoppered with rubber "Subaseals". Incubations were terminated by injecting 0.2 ml. 0.1 M HCl into the microfuge tube followed by 0.5 ml hyamine hydroxide into the scintillation vial in order to absorb the generated $^{14}CO_2$. The vials were further incubated at 4°, the microfuge tubes removed from the counting vials and the radioactivity content of the latter counted following the addition of 10 ml Aquasol scintillation fluid. For the measurement of 5-[^{3}H]-glucose utilisation, groups of 50 islets were incubated in 100 μl medium containing 1 μCi 5-[^{3}H]-glucose in microfuge tubes as described above. Incubations were terminated by the injection of 0.2 ml, 0.1 M HCl into the microfuge tube followed by 1 ml, H_2O into the containing scintillation vial. The samples were left to equilibrate at room temperature for 24 hr, then the microfuge tubes were removed and the radioactive content of the vial

counted after the addition of scintillant. Islet ATP content was estimated using a firefly luciferase assay.

myo[2-^{3}H]Inositol, ^{125}I-insulin, D-[U-^{14}C]glucose, D-[1-^{14}C]glucose and 5-[^{3}H]-glucose were supplied by Amersham (U.K.) and [1-^{14}C]pyruvate by New England Nuclear, α-Cyano-4-hydroxycinnamic acid, L-lactate, pyruvate, carbamylcholine, luciferase reagent and hyamine hydroxide were purchased from Sigma (St Louis, MO). In order to enable comparisons to be made between sets of results obtained in separate experiments, the control values from similar experiments were pooled and the test values expressed with respect to the mean control value. Statistical significance was ascribed using Student's *t*-test.

RESULTS

The secretion of insulin from rat pancreatic islets in response to glucose (12 mM) was significantly impaired in the presence of α-cyano-4-hydroxycinnamate (1 mM), whereas secretion evoked by a combination of Ba^{2+} and theophylline (both 5 mM and in the absence of added Ca^{2+}) was unaffected (Table 1). In the case of glucose, this inhibition was most marked at concentrations of the sugar between 10 and 20 mM; higher concentrations of glucose appeared to overcome the effect of the drug (Fig. 1). Furthermore, the inhibition of glucose-induced insulin release by α-cyano-4-hydroxycinnamate was related to concentration of the latter, secretion being reduced to basal levels at a concentration of 10 mM (Fig. 2).

The inhibition of insulin secretion by α-cyano-4-hydroxycinnamate was accompanied by a concentration-related impairment of oxidation of [1-^{14}C]pyruvate (Table 2 and Fig. 3). A double-reciprocal plot (Fig. 4) suggested that this inhibition of pyruvate oxidation by the drug was of a competitive nature, increasing the K_m for pyruvate from approximately 2.5 mM to approximately 6.7 mM, having little or no influence on the V_{max}. α-Cyano-4-hydroxycinnamate also resulted in a less pronounced inhibition of [U-^{14}C]glucose oxidation (Table 2, Fig. 3) although oxidation of glucose labelled in the 1-position, an index of oxidation of the sugar via the pentose phosphate pathway, was modestly, though significantly, enhanced by the drug (Table 2). The rate of utilisation of 5-[^{3}H]glucose, an index of islet glycolysis, was not affected by the presence of α-cyano-4-hydroxycinnamate. Similarly, islet ATP levels were not found to be significantly different in the absence (7.5 ± 0.03 pmol/islet) or presence (8.3 ± 1.4 pmol/islet) of inhibitor. The stimulation of islets with glucose resulted in a marked stimulation of inositol phosphate formation, an index of increased hydrolysis of inositol lipids (Table 3). Incubation in the presence of α-cyano-4-hydroxycinnamate at concentrations of either 1 or 10 mM had no significant effect on either basal or glucose-induced inositol phosphate production (Table 3).

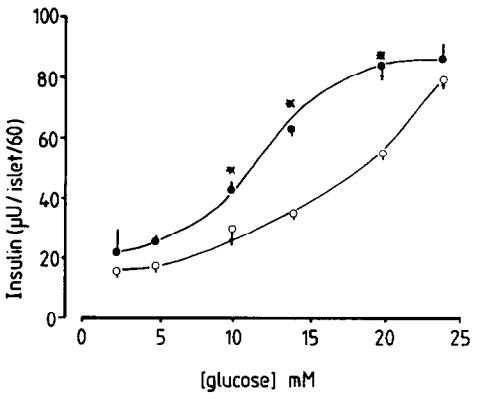

Fig. 1. Glucose-induced insulin secretion in the absence (●) and presence (○) of 1 mM α-cyano-4-hydroxycinnamate. Groups of 15 islets were incubated for 60 min. Each point represents the mean + SEM of six separate determinations. * $P < 0.05$.

DISCUSSION

α-Cyano-4-hydroxycinnamate is a thiol reagent which has been shown to act as a specific inhibitor of pyruvate transport into mitochondria (for review see Ref. 5). This property has permitted the use of this compound for the study of metabolic processes which depend upon the mitochondrial oxidation of pyruvate in both isolated mitochondria and intact cells or tissues. Thus, in the present study, α-cyano-4-hydroxycinnamate has been used in an attempt to investigate to what extent insulin secretion and inositol lipid metabolism in glucose-stimulated islets

Table 1. Effects of 1 mM α-cyano-4-hydroxycinnamate (CNCN) on insulin secretion (IRI; μU/islet/60 min) from rat pancreatic islets

Conditions	IRI	P
1. 2.8 mM Glucose	16 ± 4 (34)	
2. +CNCN	11 ± 1 (6)	NS vs line 1
3. 12 mM Glucose	73 ± 5 (4)	$P < 0.001$ vs line 1
4. +CNCN	53 ± 3 (4)	$P < 0.02$ vs line 3
5. 5 mM BaC12 + 5 mM theophylline	71 ± 12 (4)	$P < 0.01$ vs line 1
6. +CNCN	82 ± 12 (4)	NS vs line 5

The numbers of individual determinations are shown in parentheses. NS = not significant ($P > 0.05$).

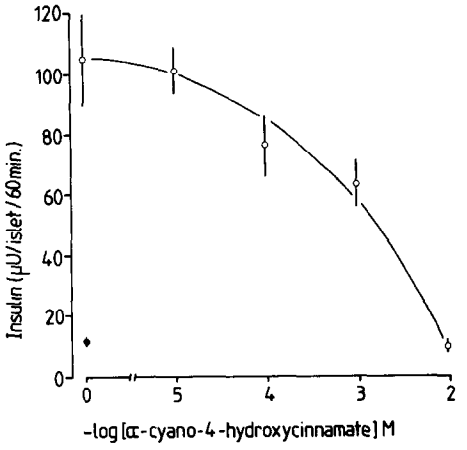

Fig. 2. Effect of α-cyano-4-hydroxycinnamate on insulin secretion in response to 16 mM glucose. The solid circle represents the basal secretory rate (2.8 mM glucose) in the absence of the drug. Each point represents the mean + SEM of four separate determinations.

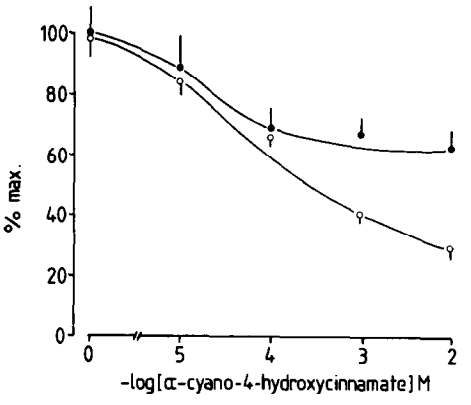

Fig. 3. Effect of α-cyano-4-hydroxycinnamate on the oxidation of glucose (2.8 mM) (●) and pyruvate (1 mM) (○) in rat islets. Groups of 50 islets were incubated for 120 min. Each point represents the mean + SEM of 3–7 separate determinations.

are dependent specifically on the mitochondrial oxidation of glucose.

Inhibition of pyruvate oxidation by the drug was demonstrated in rat islets, kinetic analysis suggesting a competitive form of inhibition. This might explain in part why the inhibition of glucose-induced insulin secretion could be overcome by high concentrations of glucose. In isolated mitochondria, both competitive and non-competitive inhibition have been reported, depending upon the conditions used, although pyruvate transport across the liver cell plasma membrane is competitively inhibited by the drug [9]. In the present studies, it is possible that inhibition by α-cyano-4-hydroxycinnamate of both plasma membrane and mitochondrial pyruvate transport may occur. This might explain in part the fact that the drug results in a considerably greater inhibition of oxidation of exogenous pyruvate than of glucose (see Fig. 3), since only mitochondrial transport of pyruvate would be involved in the latter. An additional explanation is that glucose can be partly oxidised via the non-mitochondrial pentose phosphate pathway. As demonstrated here, the oxidation of 1-^{14}C-labelled glucose, an index of activity of this pathway, was slightly increased, rather than inhibited by the drug. It is possible that in the presence of α-cyano-4-hydroxycinnamate, the activity of the pentose phosphate pathway is enhanced in order to compensate for reduced mitochondrial oxidation of glucose.

The inhibition of pyruvate (and glucose) oxidation in islets by α-cyano-4-hydroxycinnamate was accompanied by an inhibition of insulin secretion in response to glucose. Such a finding is consistent with the idea that increased mitochondrial oxidative flux is necessary, or may even provide a trigger for nutrient-induced insulin secretion [2]. This hypothesis is supported by the insulinotrophic action of α-ketoisocaproate [10], the metabolism of which is thought to be entirely mitochondrial [11]. In this regard, it is of interest to note that high concentrations (10 mM) of α-cyano-4-hydroxycinnamate have been reported to inhibit both the oxidation and insulinotropic action of α-ketoisocaproate [12]. It is interesting to note that insulin release in response to a non-nutrient

Table 2. Effects of 1 mM α-cyano-4-hydroxycinnamate (CNCN) on nutrient oxidation and utilisation in rat pancreatic islets

	pmol/50 islets/120 min	P
1. U-^{14}C-glucose (2.8 mm)	823 ± 75 (6)	
2. +CNCN	557 ± 45 (6)	P < 0.05 vs line 1
3. 1-^{14}C-glucose (2.8 mM)	405 ± 22 (4)	
4. +CNCN	494 ± 26 (4)	P < 0.05 vs line 3
5. 1-^{14}C-pyruvate (1 mM)	2369 ± 195 (7)	
6. +CNCN	969 ± 48 (7)	P < 0.001 vs line 5
7. 5-[^{3}H]-glucose (2.8 mM)	1880 ± 20 (3)	
8. +CNCN	1860 ± 72 (3)	NS vs line 7

The numbers of determinations are shown in parentheses. NS = not significant (P > 0.05).

Table 3. Effects of α-cyano-4-hydroxycinnamate (CNCN) on ^{3}H-inositol phosphate (IP) production in rat pancreatic islets

Conditions	IP (dpm)	P
1. 2.8 mM Glucose	2087 ± 343 (7)	
2. + 1 mM CNCN	2003 ± 142 (3)	NS vs line 1
3. + 10 mM CNCN	2524 ± 139 (3)	NS vs line 1
4. 16 mM Glucose	9122 ± 537 (5)	$P < 0.001$ vs line 1
5. + 1 mM CNCN	9950 ± 1745 (5)	NS vs line 4
6. + 10 mM CNCN	9971 ± 1016 (3)	NS vs line 4

The numbers of determinations are shown in parentheses. NS = not significant ($P > 0.05$).

stimulus (Ba^{2+}/theophylline) was not apparently impaired by the inhibitor of pyruvate oxidation.

In contrast to the observed inhibition of glucose-induced insulin secretion by α-cyano-4-hydroxycinnamate, this compound had no apparent influence, even at high concentrations, on islet inositol lipid metabolism in response to glucose. This differential action of the drug suggests that inositol lipid hydrolysis and insulin secretion may be initiated by distinct signals during stimulation with glucose. Again, the nature of the signal responsible for promoting phosphoinositide hydrolysis is unknown, though the lack of effect of α-cyano-4-hydroxycinnamate suggests that the transport across the plasma membrane and/or cytoplasmic metabolism of glucose might be involved.

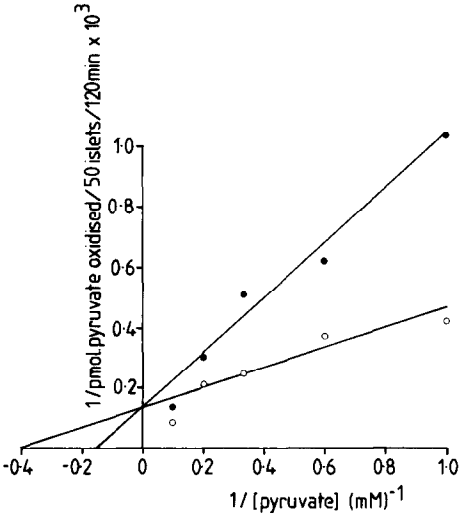

Fig. 4. Double reciprocal plot of 1-^{14}C-pyruvate oxidation in rat islets in the absence (○) and presence (●) of 1 mM α-cyano-4-hydroxycinnamate.

Acknowledgements—This work was supported by the MRC.

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