Biochem. J. (1985) **227**, 995–1002
Printed in Great Britain

Participation of endogenous fatty acids in the secretory activity of the pancreatic B-cell

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(*Received 7 November 1984/27 December 1984; accepted 29 January 1985*)

The pancreatic B-cell may represent a fuel-sensor organ, the release of insulin evoked by nutrient secretagogues being attributable to an increased oxidation of exogenous and/or endogenous substrates. The participation of endogenous fatty acids in the secretory response of isolated rat pancreatic islets was investigated. Methyl palmoxirate (McN-3716, 0.1 mM), an inhibitor of long-chain-fatty-acid oxidation, suppressed the oxidation of exogenous [U-^{14}C]palmitate and inhibited $^{14}CO_2$ output from islets prelabelled with [U-^{14}C]palmitate. Methyl palmoxirate failed to affect the oxidation of exogenous D-[U-^{14}C]glucose or L-[U-^{14}C]glutamine, the production of NH_4^+ and the output of $^{14}CO_2$ from islets prelabelled with L-[U-^{14}C]glutamine. In the absence of exogenous nutrient and after a lag period of about 60 min, methyl palmoxirate decreased O_2 uptake to 69% of the control value. Methyl palmoxirate inhibited insulin release evoked by D-glucose, D-glyceraldehyde, 2-oxoisohexanoate, L-leucine, 2-aminobicyclo[2.2.1]heptane-2-carboxylate or 3-phenylpyruvate. However, methyl palmoxirate failed to affect insulin release when the oxidation of endogenous fatty acids was already suppressed, e.g. in the presence of pyruvate or L-glutamine. These findings support the view that insulin release evoked by nutrient secretagogues tightly depends on the overall rate of nutrient oxidation, including that of endogenous fatty acids.

The pancreatic B-cell may be viewed as a fuel-sensor organ (Malaisse *et al.*, 1979*c*; Malaisse, 1983*a*). Indeed, the capacity of nutrient secretagogues to stimulate insulin release is thought to reflect their capacity to increase oxidative fluxes in the pancreatic B-cell. Such an increase may result from a mere Mass Action phenomenon and/or the activation of key regulatory enzymes (Malaisse, 1983*a*). In this respect, insulin release appears to depend on the integrated oxidation rate of both exogenous and endogenous nutrients in the islet cells (Malaisse, 1983*a*). Endogenous fatty acids and amino acids were claimed to represent the two major substrates utilized by the islets to cover their basal energy expenditure (Malaisse *et al.*, 1983*a*; Malaisse, 1983*b*). If so, an inhibition of endogenous fatty acid oxidation should alter the secretory response of the pancreatic B-cell to exogenous nutrients. In the present work, already reported in abstract form elsewhere (Malaisse-Lagae *et al.*, 1984), a specific inhibitor of the carnitine-dependent oxidation of long-chain fatty acids, methyl palmoxirate (Tutwiler *et al.*, 1978, 1979; Tutwiler & Dellevigne, 1979; Tutwiler & Ryzlak, 1980), was used to assess the relevance of endogenous fatty acid oxidation to the secretory behaviour of isolated rat pancreatic islets.

Materials and methods

Methyl palmoxirate (McN-3716) was kindly given by Dr. G. F. Tutwiler (McNeil Laboratories, Fort Washington, PA, U.S.A.) solubilized in dimethyl sulphoxide at an initial concentration of 25 mM, and eventually diluted in a bicarbonate-buffered incubation medium (Malaisse *et al.*, 1970) at a final concentration of 0.1 mM. Control media contained the same amount of the solvent (4 μl/ml), at which concentration dimethyl sulphoxide fails to affect metabolic and secretory variables in pancreatic islets (Levy *et al.*, 1976).

All experiments were performed with pancreatic islets isolated from fed albino rats (Lacy & Kostianovsky, 1967). The methods used to mea-

sure the release of insulin (Malaisse *et al.*, 1970), the oxidation of exogenous nutrients (Carpinelli *et al.*, 1980), the output of NH_4^+ (Malaisse *et al.*, 1981) and the production of $^{14}CO_2$ from islets prelabelled with either [U-^{14}C]palmitate (Sener *et al.*, 1978; Malaisse *et al.*, 1979*b*) or L-[U-^{14}C]glutamine (Malaisse *et al.*, 1981) were all described previously.

The respiratory rate of the isolated islets was assayed with the aid of Cartesian divers (Holter & Linderström-Lang, 1943). The adaptation of the technique for measurements of pancreatic-islet respiration has been described in detail previously (Hellerström, 1967). In the present study, groups of four to eight isolated islets were incubated in a Krebs–Ringer/Hepes medium (composition: Na^+, 143.6mM; K^+, 5.9mM; Ca^{2+}, 2.5mM; Mg^{2+}, 1.2mM; Cl^-, 146.5mM; PO_4^{3-}, 1.2mM; SO_4^{2-}, 1.2mM; Hepes, 10.0mM; pH7.4) with or without addition of 0.1mM-methyl palmoxirate (McN-3716). The medium volume in each experiment was 1.0μl, placed as a small drop in the diver neck. The gas phase consisted of ambient air and the incubation temperature was 37°C. The CO_2 evolved was trapped in alkali and the islet oxygen consumption computed from the change in equilibrium pressure of the diver over an incubation period of more than 100min.

All results are expressed as the mean (±S.E.M.), together with the number of individual observations (*n*) or degree of freedom (d.f.) and statistical significance of differences between mean control and test values, as assessed by Student's *t* test.

Results

Metabolism of fatty acids

McN-3716, at an 0.1mM concentration, virtually abolished the oxidation of exogenous [U-^{14}C]-palmitate (0.31mM) by islets incubated in the presence of 8.3mM-D-glucose (Table 1).

When islets were preincubated for 120min in the presence of D-glucose (8.3mM) and [U-^{14}C]palmitate (0.32mM) and then incubated for 120min in the absence of exogenous palmitate, the $^{14}CO_2$ output from the prelabelled islets was also inhibited by McN-3716 (Table 2). The inhibitory action of McN-3716 was apparently less marked, however, than that seen with exogenous [U-^{14}C]-palmitate. Thus, after correction for the mean readings collected within the same experiment(s) in the presence of respiratory poisons, the relative magnitude of the inhibitory action of McN-3716 on $^{14}CO_2$ output from the prelabelled islets averaged 40.5±3.9 and 33.4±5.4% in the absence and presence of D-glucose (16.7mM), respectively. D-Glucose itself significantly decreases $^{14}CO_2$ output from the prelabelled islets ($P<0.001$). Moreover, in the presence of D-glucose, the McN-3716-induced decrease in $^{14}CO_2$ output was reduced to 67.9±12.9% of the paired value found in the absence of D-glucose.

There was an apparent discrepancy between the partial inhibition by McN-3716 of $^{14}CO_2$ output from islets prelabelled with [U-^{14}C]palmitate (Table 2) and the virtually complete suppression by the drug of exogenous [U-^{14}C]palmitate oxidation (Table 1). Such a difference led us to perform the following series of experiments. Pancreatic islets were preincubated for 120min in the presence of D-glucose (8.3mM) and [U-^{14}C]palmitate (0.31mM), with or without McN-3716 (0.1mM). At the end of this preincubation, the radioactive content of the islets was not significantly affected by McN-3716. Expressed as palmitate residues with the same specific radioactivity as that of exogenous [U-^{14}C]palmitate, it averaged 2.64±0.12 and 2.83±0.14pmol/islet in the absence and presence of McN-3716 respectively ($n=32$ in both cases, $P>0.3$). The islets were then incubated for 120min in the absence of exogenous palmitate. In the islets prelabelled in the absence of McN-3716, the results were comparable with those already presented in Table 2. Thus, during the final incubation, McN-3716 caused a partial decrease in $^{14}CO_2$ output. After correction for the readings obtained in the presence of respiratory poisons, the inhibitory action of McN-3716 (0.1mM) in the presence of D-

Table 1. *Effect of McN-3716 upon the oxidation of exogenous nutrients*
Values are means±S.E.M.

Nutrient(s) (mM)	McN-3716 ...	Oxidation (pmol of labelled substrate oxidized/120min per islet)	
		Absent	0.1mM
[U-^{14}C]Palmitate (0.3), D-[U-^{12}C]glucose (8.3)		1.05±0.07 (13)	0.09±0.02 (14)
D-[U-^{14}C]Glucose (5.6)		20.1±1.7 (20)	21.0±2.0 (20)
D-[U-^{14}C]Glucose (16.7)		61.7±5.6 (20)	64.1±6.0 (20)
L-[U-^{14}C]Glutamine (1.0)		16.9±1.9 (11)	14.8±2.2 (11)
L-[U-^{14}C]Glutamine (1.0), D-[U-^{12}C]glucose (16.7)		16.4±2.3 (10)	16.0±2.4 (11)

glucose (8.3mM) averaged 40.5 ± 6.7% (Table 3). A vastly different picture was seen in the islets prelabelled with [U-^{14}C]palmitate in the presence of McN-3716. In such islets, the output of $^{14}CO_2$, in the absence of glucose, averaged only 7.6 ± 0.4% of the control value found in islets preincubated in the absence of McN-3716 (Table 3). This behaviour is reminiscent of that characterizing the oxidation of exogenous [U-^{14}C]palmitate, as already documented in Table 1. It should be underlined that the dramatic inhibition of $^{14}CO_2$ output from islets preincubated with McN-3716 occurred despite their normal radioactive content and despite the absence of McN-3716 during the final incubation, indicating that the inhibitory action of the drug was not rapidly reversible. This is supported by the observation that the output of $^{14}CO_2$, in the presence of D-glucose, was not significantly lower ($P > 0.1$) in islets exposed to McN-3716 during both the preincubation and incubation periods than in islets exposed to the drug solely during preincubation (Table 3). In this series of experiments, D-glucose (8.3mM) slightly reduced $^{14}CO_2$ output, whether in islets preincubated with or without McN-3716. After correction for the readings obtained with respiratory poisons, the values recorded in the presence of D-glucose averaged 88.2 ± 6.1% ($n = 15$) of their corresponding mean basal values. In the islets prelabelled in the presence of McN-3716, the production of $^{14}CO_2$ was significantly decreased ($P < 0.005$) by the combination of D-glucose (8.3mM) and McN-3716 (0.1mM) relative to the basal value. Thus, after correction for data obtained with respiratory poisons, the decrease in $^{14}CO_2$ output attributable to the latter combination averaged 31.7 ± 4.4% (Table 3).

Table 2. *Effect of McN-3716 on $^{14}CO_2$ output from islets prelabelled with [U-^{14}C]palmitate*

Mean values (±S.E.M.) are expressed as a percentage of the mean control value found within the same experiment(s) in the absence of any agent (first line). Such a control value corresponded to a $^{14}CO_2$ output representing 6.95 ± 0.18% of the ^{14}C content of the islets. After 120min preincubation in the presence of D-glucose (8.3mM) and [U-^{14}C]palmitate (320μM), the ^{14}C content of the islets corresponded to 1.93 ± 0.10pmol of [U-^{14}C]palmitate per islet ($n = 77$).

Agent(s) (mM)	$^{14}CO_2$ output/ ^{14}C content (%)
None	100.0 ± 2.6 (19)
McN-3716 (0.1)	68.2 ± 3.1 (20)
D-Glucose (16.7)	78.7 ± 2.2 (9)
D-Glucose (16.7) + McN-3716 (0.1)	56.9 ± 3.5 (9)
Antimycin A (0.01) + rotenone (0.01) + KCN (3.0)	21.4 ± 3.2 (20)

Metabolism of other nutrients

McN-3716 failed to affect the oxidation of exogenous D-[U-^{14}C]glucose or L-[U-^{14}C]glutamine (Table 1).

Two approaches were used to assess a possible interference of McN-3716 with the metabolism of endogenous amino acids. In the first approach, the islets were preincubated for 30min with L-[U-^{14}C]-glutamine (1.0M) and then incubated for 30min in the absence of exogenous glutamine. The output of $^{14}CO_2$ from the islets prelabelled with L-[U-^{14}C]-glutamine was not affected by McN-3716 when the islets were incubated in the absence of exogenous nutrient (Table 4). When the islets were incubated in the presence of D-glucose (16.7mM), the $^{14}CO_2$ was higher ($P < 0.001$) than the basal value, in good agreement with a previous observation (Malaisse *et al.*, 1983*a*). In the presence of D-glucose, the output of $^{14}CO_2$ was slightly decreased by McN-3716 ($P < 0.01$), but remained higher than

Table 3. *Effect of McN-3716 during preincubation and/or incubation on $^{14}CO_2$ output from islets preincubated for 120min in the presence of D-glucose (8.3mM) and [U-^{14}C]palmitate (0.31mM) and incubated for 120min in the absence of exogenous palmitate*

The output of $^{14}CO_2$ during the final incubation is expressed as a percentage of the ^{14}C content of the islets.

Preincubation [McN-3716] (mM)	Incubation in the presence of: D-Glucose (mM)	McN-3716 (mM)	Respiratory poisons (mM)	$^{14}CO_2$ output/ ^{14}C content (%)
—	—	—	—	6.72 ± 0.23 (8)
—	8.3	—	—	6.05 ± 0.35 (8)
—	8.3	0.1	—	3.95 ± 0.35 (8)
—	—	—	Antimycin A (0.01), rotenone (0.01), KCN (3.0)	0.86 ± 0.12 (8)
0.1	—	—	—	0.51 ± 0.03 (8)
0.1	8.3	—	—	0.46 ± 0.05 (7)
0.1	8.3	0.1	—	0.38 ± 0.02 (7)
0.1	—	—	Antimycin A (0.01), rotenone (0.01), KCN (3.0)	0.10 ± 0.01 (8)

the basal value ($P < 0.05$). Even after correction for the readings collected in the presence of respiratory poisons, the inhibitory action of McN-3716 upon $^{14}CO_2$ output from the islets preincubated in the presence of L-[U-^{14}C]glutamine (1.0 mM) and incubated in the presence of D-glucose (16.7 mM) averaged no more than $15.5 \pm 5.1\%$ (d.f. = 22).

In the second approach, the output of NH_4^+ was measured over 90 min incubation in the presence or absence of D-glucose (8.3 mM) and/or McN-3716 (Table 5). D-Glucose decreased ($P < 0.001$) NH_4^+ output significantly, whether in the absence or presence of McN-3716. The latter drug failed to affect NH_4^+ output significantly whether in the absence or presence of D-glucose.

Table 4. *Effect of McN-3716 on $^{14}CO_2$ output from islets prelabelled with L-[U-^{14}C]glutamine*

Mean values (±S.E.M.) are expressed as a percentage of the mean control value found in the absence of any agent (first line). Such a control value corresponded to a $^{14}CO_2$ output representing $24.2 \pm 0.7\%$ of the ^{14}C content of the islets. After 30 min preincubation in the presence of L-[U-^{14}C]glutamine (1.0 mM), the ^{14}C content of the islets corresponded to 9.1 ± 0.3 pmol of L-[U-^{14}C]glutamine per islet ($n = 60$).

Agent(s) (mM)	$^{14}CO_2$ output/ ^{14}C content (%)
None	100.0 ± 2.8 (12)
McN-3716 (0.1)	101.4 ± 2.7 (12)
D-Glucose (16.7)	124.9 ± 3.8 (12)
D-Glucose (16.7) + McN-3716 (0.1)	109.8 ± 3.7 (12)
Antimycin A (0.01) + rotenone (0.01) + KCN (5.0)	21.2 ± 1.1 (12)

Table 5. *Effect of McN-3716 upon NH_4^+ output*

[D-Glucose] (mM)	[McN-3716] (mM)	NH_4^+ output (pmol/120 min per islet)
—	—	95.8 ± 3.4 (11)
—	0.1	94.1 ± 4.2 (12)
8.3	—	74.9 ± 3.0 (12)
8.3	0.1	67.8 ± 2.0 (11)

Oxygen uptake

When islets were incubated in the absence of exogenous nutrient, the respiratory rate during the first 60 min of incubation remained fairly constant, and was not different ($P > 0.9$) in the presence or absence of McN-3716 (0.1 mM) respectively (Table 6). After about 60 min of incubation, the rate of O_2 consumption decreased below its initial value in the islets exposed to McN-3716 (Fig. 1). As judged from the records of O_2 uptake as a function of time, such a decrease in O_2 consumption was initiated at a later time ($P < 0.025$), i.e. at about 95 min of incubation, in the control islets not exposed to the drug (Table 6). Thus, between 60 and 95 min of incubation, the mean respiratory rate remained close to its initial value ($P > 0.6$) in control islets, but was significantly lower than its initial value ($P < 0.05$) in the islets exposed to McN-3716 (Table 6). Over this period (60–95 min), the rate of oxygen consumption in the presence of McN-3716 averaged $69.4 \pm 13.9\%$ ($n = 8$) of the mean control value found over the same time interval in the absence of the drug.

Insulin release

McN-3716 (0.1 mM) failed to affect significantly the basal release of insulin (Tables 7 and 8), but inhibited insulin secretion evoked by D-glucose in the 5.6–11.1 mM range. At a higher glucose concentration (16.7 mM), however, the release of insulin in the presence of McN-3716 was not significantly lower than that recorded in the absence of the drug (Table 7). When the islets were removed from rats fasted for 48 h, instead of fed animals as in all other experiments, D-glucose (16.7 mM) augmented insulin release from a basal value of 15.4 ± 2.9 to 106.5 ± 7.0 μunits/90 min per islet ($n = 30$ in both cases) and McN-3716 (0.1 mM) decreased glucose-stimulated insulin release ($P < 0.005$) to 80.3 ± 4.7 μunits/90 min per islet ($n = 30$).

Table 6. *Effect of McN-3716 on O_2 uptake by islets deprived of exogenous nutrient*

Mean values (±S.E.M.) for O_2 uptake were measured during the initial period (0–60 min) and late period (60–95 min) of incubation. Also shown is the time at which a sizeable decrease in O_2 uptake below the initial value was first observed.

	Control	McN-3716 (0.1 mM)
Initial respiratory rate (nl of O_2/60 min per islet)	7.6 ± 0.9 (7)	7.7 ± 0.9 (8)
Length of the period of linear O_2 uptake (min)	95 ± 10 (7)	60 ± 9 (8)
Respiratory rate between 60 and 95 min (nl of O_2/60 min per islet)	6.9 ± 1.1 (7)	4.8 ± 1.0 (8)

Experiments designed to explore the dose–response relationship and time course for the inhibitory action of McN-3716 on insulin release evoked by D-glucose revealed the following features. First, the concentration of McN-3716 used throughout this work, i.e. 0.1 mM, apparently caused a close-to-maximal inhibitory effect. Thus, over 90 min incubation in the presence of 8.3 mM-D-glucose and relative to the mean control value (79.2 ± 5.2 μunits/90 min per islet; $n = 20$), the secretory rate averaged 96.4 ± 6.8%, 66.6 ± 6.2% and 54.0 ± 2.4% in the presence of 1.0 μM-, 10.0 μM- and 0.1 mM-McN-3716 respectively ($n = 19$–20 in all cases). Secondly, when islets were incubated for 30, 60 and 90 min in the presence of 8.3 mM-D-glucose with or without McN-3716 (0.1 mM), a significant decrease in insulin output was only seen between 61 and 90 min of incubation. Thus, relative to the mean control value seen over the same period (43.5 ± 5.0 μunits/30 min per islet; $n = 25$), the secretory rate in the presence of McN-3716 averaged 84.3 ± 10.9% ($P > 0.1$) and 34.5 ± 9.7% ($P < 0.001$) from 31 to 60 min and from 61 to 90 min of incubation respectively ($n = 12$–14).

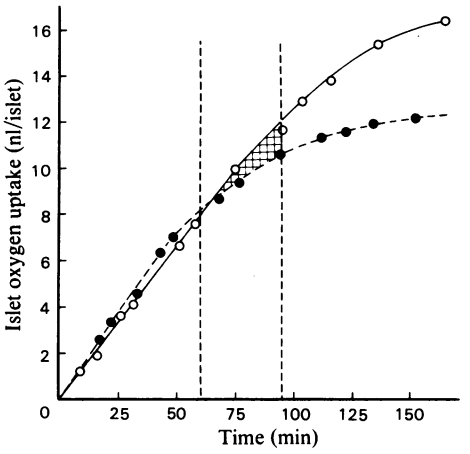

Fig. 1. *Kinetics of islet oxygen uptake in the absence (○) or presence (●) of 0.1 mM-methyl palmoxirate (McN-3716)*
In each of the two experiments shown in the graph, five to seven collagenase-isolated islets were incubated in 1 μl of Krebs–Ringer/Hepes buffer placed in a Cartesian diver according to a technique described in detail previously (Hellerström, 1967; Hellerström *et al.*, 1979). The equilibrium pressures of the divers were measured at 37°C and the lines describing the oxygen uptake fitted to the plot. The cross-hatched area between the two broken vertical lines represents the difference in oxygen consumption between 60 and 95 min after the start of the experiment. Whereas the respiratory rate of the control islets is approximately linear up to 95 min, there is a retardation after about 60 min in the presence of McN-3716.

McN-3716 inhibited insulin release evoked by other nutrient secretagogues, such as D-glyceraldehyde, 2-oxoisohexanoate, L-leucine, 2-aminobicyclo[2.2.1]heptane-2-carboxylate or 3-phenylpyruvate (Table 8). McN-3716 also impaired insulin release evoked, in the absence of exogenous nutrient, by the combination of a tumour-promoting phorbol ester and a hypoglycaemic sulphonylurea. The magnitude of the inhibitory action of McN-3716 was not identical in all cases. For instance, the secretory response to L-leucine was less affected, in relative terms, than that evoked by either its non-metabolized analogue, 2-aminobicyclo[2.2.1]heptane-1-carboxylate, or its deamination product, 2-oxoisohexanoate.

McN-3716 failed to inhibit insulin release under three experimental conditions. First, in the presence of D-glucose (8.3 mM), the pyruvate-induced increment in insulin release was not significantly different ($P > 0.5$) in the absence (+68.0 ± 10.8 μunits/90 min per islet; d.f. = 52) and presence (+60.0 ± 5.6 μunits/90 min per islet; d.f. = 52) of McN-3716 respectively. This contrasts

Table 7. *Effect of McN-3716 on glucose-induced insulin release*
Mean values (±S.E.M.) are expressed as μunits/90 min per islet and are shown together with the number of individual observations (in parentheses) and statistical significance of the differences between control and test values (P).

[D-Glucose] (mM)	McN-3716 ...	Insulin release (μunits/90 min per islet) Absent	0.1 mM	P
—		14.7 ± 2.0 (76)	9.4 ± 2.8 (27)	>0.1
5.6		22.8 ± 3.4 (27)	11.4 ± 4.0 (27)	<0.05
7.0		43.9 ± 4.4 (18)	17.3 ± 2.5 (18)	<0.001
8.3		82.1 ± 6.4 (36)	42.8 ± 2.9 (36)	<0.001
11.1		157.0 ± 9.2 (18)	110.4 ± 7.4 (16)	<0.001
16.7		265.1 ± 9.3 (44)	244.4 ± 10.3 (45)	>0.1

Table 8. *Effect of McN-3716 upon insulin release evoked by different secretagogues*
The presentation is the same as in Table 7. In the presence of D-glyceraldehyde, the incubation time was reduced to 60min, and the results are expressed as μunits/60min per islet. Abbreviations used: BCH, 2-aminobicyclo[2.2.1]-heptane-2-carboxylate; TPA, 12-*O*-tetradecanoylphorbol 13-acetate.

Secretagogues (mM)	McN-3716 ...	Insulin release (μunits/90min per islet) Absent	0.1mM	*P*
—		10.6±2.7 (17)	9.7±2.4 (18)	>0.8
D-Glyceraldehyde (10.0) (see above)		33.6±2.1 (23)	15.1±1.3 (24)	<0.001
D-Glucose (8.3)		82.1±6.4 (36)	42.8±2.9 (36)	<0.001
D-Glucose (8.3)+pyruvate (30.0)		150.1±8.4 (18)	102.8±5.5 (18)	<0.001
D-Glucose (8.3)+L-glutamine (10.0)		79.1±6.1 (10)	73.8±6.4 (10)	>0.5
2-Oxoisohexanoate (10.0)		102.1±3.7 (16)	49.5±3.7 (18)	<0.001
L-Leucine (20.0)		60.1±3.5 (36)	49.9±1.8 (35)	<0.02
L-Leucine (10.0)+L-glutamine (10.0)		166.6±16.4 (19)	184.2±9.1 (19)	>0.3
BCH (20.0)		44.1±2.2 (29)	26.3±2.5 (24)	<0.001
3-Phenylpyruvate (10.0)		34.8±3.2 (36)	22.3±2.9 (36)	<0.01
TPA (0.002)+gliclazide (0.08)		243.7±6.5 (24)	184.6±4.3 (22)	<0.001
Ba^{2+} (2.0)+theophylline (1.4)+no Ca^{2+}		102.7±7.7 (20)	101.0±6.8 (19)	>0.8

with the fact that, in the absence of McN-3716 when the release of insulin evoked by D-glucose is decreased by lowering the glucose concentration below 8.3mM, the magnitude of the enhancing action of pyruvate on insulin release also diminishes (Sener *et al.*, 1978). Secondly, in the presence of L-glutamine, McN-3716 also failed to affect insulin release. L-Glutamine was tested in the presence of either L-leucine (10.0mM), in which case L-glutamine markedly enhances insulin release, or D-glucose (8.3mM), in which case the amino acid exerts little effect on secretory rate (Malaisse *et al.*, 1980*b*; Sener & Malaisse, 1981). In the latter case, the protective action of L-glutamine was quite obvious, since McN-3716 severely inhibited insulin release from islets exposed to the same concentration of D-glucose (8.3mM) in the absence of L-glutamine. Last, the release of insulin evoked, in the absence of Ca^{2+}, by the combination of Ba^{2+} and theophylline, was not affected by McN-3716.

Discussion

The present data are compatible with the view that McN-3716 acts as a rather specific inhibitor of carnitine-dependent long-chain-fatty-acid oxidation (Tutwiler & Dellevigne, 1979; Tutwiler & Ryzlak, 1980; Tutwiler *et al.*, 1978, 1979). Indeed, McN-3716 severely inhibited the oxidation of exogenous palmitate, but failed to affect the oxidation of exogenous glucose and glutamine or the production of NH_4^+ by rat pancreatic islets. The drug exerted a minor inhibitory effect upon the production of $^{14}CO_2$ from islets prelabelled with L-[U-^{14}C]glutamine, and this only in the presence of glucose.

The inhibition of [U-^{14}C]palmitate oxidation by McN-3716 was virtually complete when the islets were simultaneously exposed to both the exogenous labelled nutrient and the drug. In this case also, the inhibitory effect of the drug was not rapidly reversible, a severe decrease in $^{14}CO_2$ output being still observed in islets preincubated with both [U-^{14}C]palmitate and McN-3716 but incubated in the absence of these two agents. A different picture was found in islets preincubated with [U-^{14}C]palmitate in the absence of McN-3716. In the latter case, when the islets were incubated with the drug, the output of $^{14}CO_2$ was only partially decreased. Since McN-3716 is supposed to primarily inhibit carnitine acyltransferase I (Tutwiler & Dellevigne, 1979; Tutwiler & Ryzlak, 1980), it is understandable that the drug failed to affect the oxidation of labelled fatty acyl derivatives already located in the mitochondria and/or their metabolites.

The latter view is supported by the finding that McN-3716 inhibited O_2 uptake after a lag period of about 60min. As judged from the respiratory data collected between 60 and 95min of incubation, the oxidation of endogenous fatty acids could account for approx. 30% of the basal respiratory rate. Such a value is in fair agreement with previous and indirect estimations, from which it was concluded that the oxidation of endogenous fatty acids accounts for about 40% of basal respiratory rate (Malaisse *et al.*, 1981, 1982; Malaisse-Lagae *et al.*, 1982).

The fact that McN-3716 failed to affect D-[U-

^{14}C]glucose oxidation although inhibiting the oxidation of fatty acids could suggest that, in the islets, the rate of glycolysis is not tightly modulated by the oxidation of fatty acids, a situation at variance with that encountered in other tissues (Garland *et al.*, 1963). It should be stressed, however, that the present experiments were restricted to investigating the role of endogenous, and not exogenous, fatty acid oxidation on islet metabolism. Incidentally, McN-3716 failed to augment, and actually inhibited, insulin release evoked by D-glucose (16.7mM) in islets removed from 48h-fasted rats. This is at variance with a recent report in which another inhibitor of fatty acid oxidation, 2-bromostearate, was claimed to partially restore the secretory responsiveness to glucose in islets removed from fasted rats (Bedoya *et al.*, 1984).

In considering the influence of McN-3716 on the secretory behaviour of the islets, it is important to keep in mind that the drug may not completely or immediately abolish the oxidation of endogenous fatty acids and their metabolites.

McN-3716 inhibited insulin release evoked by several nutrient secretagogues, such as D-glucose, D-glyceraldehyde, L-leucine, 2-aminobicyclo-[2.2.1]heptane-2-carboxylate, 2-oxoisohexanoate and 3-phenylpyruvate. McN-3716 also inhibited insulin release evoked by non-nutrient secretagogues, such as the association of a tumour-promoting phorbol ester and a hypoglycaemic sulphonylurea (Malaisse *et al.*, 1983*b*). Four series of observations support the view that the inhibitory action of McN-3716 on insulin release was attributable to a specific alteration of fatty acid oxidation rather than some untoward side-effect of the drug.

First, and most importantly, McN-3716 failed to affect insulin release in the presence of L-glutamine, which itself abolishes the oxidation of endogenous fatty acids in the islets (Malaisse *et al.*, 1980*b*). Likewise, McN-3716 failed to affect the increment in secretion rate evoked by pyruvate, which is also a potent inhibitor of endogenous fatty acid oxidation in pancreatic islets (Sener *et al.*, 1978).

Secondly, the relative extent of the inhibitory action of McN-3716 on insulin release evoked by distinct nutrient secretagogues was inversely related to the magnitude of the sparing action of these nutrients on endogenous fatty acid oxidation. Thus the inhibition of insulin release was, in relative terms, most obvious at low glucose concentrations, in the presence of 2-oxoisohexanoate or when either 2-aminobicyclo[2.2.1]heptane-2-carboxylate or 3-phenylpyruvate was used to facilitate the catabolism of endogenous amino acids (Malaisse *et al.*, 1983*c*; Sener *et al.*, 1981). Under all these conditions the oxidation of endogenous fatty acids is little or not affected (Malaisse *et al.*, 1979*a*; Hutton *et al.*, 1979; Malaisse-Lagae *et al.*, 1982; Malaisse *et al.*, 1983*c*). The inhibitory action of McN-3716 was less marked, in relative terms, at high glucose concentrations or in the presence of L-leucine, in which cases the exogenous secretagogues exert an obvious sparing action on the utilization of endogenous fatty acids (Malaisse *et al.*, 1979*a*, 1980*a*).

Thirdly, there was a close parallelism between the time course for the inhibitory action of McN-3716 on insulin release and O_2 uptake respectively, both variables being only affected after a lag period of about 60min.

Lastly, McN-3716 inhibited insulin release evoked by the association of a tumour-promoting phorbol ester and a hypoglycaemic sulphonylurea, although failing to affect significantly the release of insulin evoked, in the absence of Ca^{2+}, by the association of Ba^{2+} and theophylline. The latter finding again indicates that McN-3716 does not invariably inhibit insulin secretion. The divergent effect of the drug on the secretory response to these distinct combinations of non-nutrient secretagogues should be considered in the light of the following considerations. The release of insulin evoked by the combination of 12-*O*-tetradecanoylphorbol 13-acetate and gliclazide represents a sustained phenomenon, depending on the integrity of oxidative events (Malaisse *et al.*, 1983*b*). Hence it may be expected that McN-3716, by causing long-term inhibition of fatty acid oxidation, impairs insulin release during prolonged exposure of the islets to the combination of 12-*O*-tetradecanoylphorbol 13-acetate and gliclazide. In contrast, however, the release of insulin evoked, in the absence of Ca^{2+}, by Ba^{2+} and theophylline is characterized by a large initial stimulation of insulin release, which then progressively decreases during prolonged exposure to these secretagogues (Somers *et al.*, 1976). It is conceivable, therefore, that the early, and predominant, secretory response to Ba^{2+} and theophylline occurred at a time when the supply of fatty acids to the mitochondria did not yet represent a major limiting factor in the oxidation of endogenous nutrients in the islet cells.

The finding that alteration in the oxidation of endogenous fatty acids modulates the magnitude of the secretory response to various secretagogues *in vitro* is compatible with the knowledge that, *in vivo*, circulating fatty acids may either directly stimulate insulin secretion (Crespin *et al.*, 1969) or modulate the secretory response to various secretagogues (Balasse & Ooms, 1973). It should be underlined, however, that the contribution of circulating fatty acids to the fuel metabolism of islet cells *in vivo* remains to be assessed.

In conclusion, the present data afford direct

support to the view that insulin release evoked, in the pancreatic B-cell, by nutrient secretagogues tightly depends on the overall rate of nutrient oxidation, including that of endogenous fatty acids.

We thank M. Mahy, A. Tinant, J. Schoonheydt and M. Urbain for technical assistance to C. Demesmaeker for secretarial help. This work was supported in part by grants from the Belgian Foundation for Scientific Medical Research (3-4528-79 and 3-4541-84), Belgian Ministry of Scientific Policy (80/85-16) and Swedish Medical Research Council (12X-109).

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