Endocrine, *vol. 30, no. 2, 207–211, October 2006* 0969–711X/06/30:207–211/$30.00
ENDO (Online) ISSN 1559-0100

Altered K^+ Fluxes and Insulin Release in Pancreatic Islets from ω3 Fatty Acid–Depleted Rats

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A low intake of long-chain polyunsaturated ω3 fatty acid often prevails in Western populations. Its consequences in terms of the control of fuel homeostasis led us to explore functional events in pancreatic islets isolated from either normal or ω3-depleted rats (second generation). In the latter rats, the inflow of K^+ by both ouabain-sensitive and ouabain-resistant modalities was decreased, this coinciding with an impaired insulin secretory response to ouabain. The intravenous injection of a medium-chain triglyceride:fish oil emulsion to ω3-depleted rats 2 h before sacrifice restored a normal value for the inflow of K^+ by the ouabain-sensitive modality, i.e., that linked to the activity of the Na,K-ATPase, but failed to correct the entry of K^+ by the ouabain-resistant modality and the defect of the insulin secretory response to ouabain. In conclusion, an impaired activity of the Na,K-ATPase in insulin-producing cells apparently represents a key determinant of altered islet function in ω3-depleted rats.

Key Words: ω3 fatty acid–depleted rats; pancreatic islets; K^+ fluxes; insulin release.

Introduction

An insufficient intake of long-chain polyunsaturated ω3 fatty acids (ω3), as presently often prevailing in Western populations, results in a perturbation of fuel homeostasis *(1–4)*. The possible participation to such a perturbation of alterations in pancreatic insulin-producing B-cell function led us to examine selected metabolic, cationic, and secretory variables in pancreatic islets isolated from second generation w3-depleted rats *(5,6)*. Considering the key role of K^+ in insulin release *(7,8)*, the first aim of the present study was to investigate possible anomalies of K^+ fluxes in such islets. For such a purpose, pancreatic islets obtained from either normal or ω3-depleted rats were incubated for 10 and 60 min with and without ouabain in the presence of $^{86}Rb^+$, currently used as a radioactive tracer for K^+. Under these experimental conditions, the measurement of $^{86}Rb^+$ net uptake allowed us to characterize both the ouabain-sensitive and ouabain-resistant modalities of K^+ inflow and, by comparison of data collected after 10 and 60 min incubation, the fractional turnover rate as well as steady-state value, at isotopic equilibrium, for the intracellular K^+ pool. Second, the experiments conducted in ω3-depleted rats included, when so required, the intravenous injection 2 h before sacrifice of either an ω3 fatty acid–rich medium-chain triglyceride:fish oil emulsion (MCT:FO) or a control ω3 fatty acid–poor medium-chain triglyceride:olive oil emulsion (MCT:OO). The former emulsion was recently proposed as a tool to induce, within 60 min and for at least 24 h after its bolus intravenous injection to rats or human subjects, a sizable increase of the C20:5ω3 and C22:6ω3 content of cell phospholipids *(9,10)*. Last, the concomitant changes in insulin output evoked by selected secretagogues in the isolated islets were also investigated.

Received August 1, 2006; Revised September 8, 2006; Accepted October 2, 2006.

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Results

$^{86}Rb^+$ Net Uptake

The time course for $^{86}Rb^+$ net uptake by islets incubated in the absence or presence of ouabain (50 μM) is documented in Table 1. In these experiments, groups of 10 islets each were preincubated in the absence or presence of ouabain (100 μM) for 15 min at 37°C in 50 μL of a nonradioactive medium, and then further incubated for 10 or 60 min after addition of 50 μL of medium containing ^{86}RbCl (6.2 μM; 5.0 μCi/mL). The total net uptake and ouabain-sensitive uptake of $^{86}Rb^+$ were significantly lower, after both 10 and 60 min incubation, in islets from ω3-depleted rats not injected with any lipid emulsion (ω3-NI rats) than in islets from control animals. After 10 and 60 min incubation, the values found in the former rats for the ouabain-sensitive uptake of $^{86}Rb^+$ averaged 33.3 ± 12.5% (d.f. = 58; $p < 0.005$) of the corresponding mean values found at the same time in the latter animals (100.0 ± 12.4%; d.f. = 136). Even the

Table 1
$^{86}Rb^+$ Uptake by Pancreatic Islets from Normal and ω3-Depleted Rats

Rats	Control	ω3-NI	ω3-OO	ω3-FO
Primary data: $^{86}Rb^+$ net uptake (pmol/islet)*				
Total				
min 10	117.8 ± 7.4 (33)	69.5 ± 6.6 (15)^{e}	43.8 ± 5.2 (28)^{e}	72.1 ± 7.6 (29)$^{e,\delta}$
min 60	297.2 ± 15.7 (35)	188.3 ± 13.0 (15)^{e}	121.4 ± 8.6 (30)^{e}	232.3 ± 17.0 (28)$^{c,\varepsilon}$
Ouabain-resistant				
min 10	71.9 ± 5.2 (36)	56.2 ± 6.2 (15)	37.1 ± 4.0 (30)^{e}	17.9 ± 3.3 (30)$^{e,\varepsilon}$
min 60	184.0 ± 7.9 (34)	143.1 ± 12.7 (15)^{c}	99.6 ± 8.2 (30)^{e}	82.0 ± 9.2 (29)^{e}
Ouabain-sensitive				
min 10	45.9 ± 8.9 (*67*)	13.2 ± 9.0 (*28*)^{a}	6.7 ± 6.5 (*56*)^{e}	54.2 ± 8.2 (*57*)$^{\varepsilon}$
min 60	113.2 ± 17.7 (*67*)	45.2 ± 18.2 (*28*)^{b}	21.7 ± 11.9 (*58*)^{c}	150.3 ± 15.1 (*55*)$^{\varepsilon}$
Secondary data				
Ouabain-resistant/total uptake (%)	62.2 ± 2.6 (70)	78.4 ± 5.5 (30)^{d}	84.2 ± 5.7 (60)^{e}	34.4 ± 3.8 (59)$^{e,\varepsilon}$
Min 10/min 60 uptake (%)				
total	39.6 ± 2.5 (33)	36.9 ± 3.5 (15)	36.1 ± 4.3 (28)	31.0 ± 3.3 (29)
ouabain-sensitive	40.6 ± 7.9 (*67*)	29.2 ± 19.9 (*28*)	30.8 ± 29.9 (*56*)	36.0 ± 5.4 (*57*)
k (min^{-1})	0.046 ± 0.004	0.041 ± 0.005	0.040 ± 0.006	0.030 ± 0.004
U_{max} (pmol/islet)	317 ± 20	206 ± 20^{d}	134 ± 16^{c}	278 ± 30$^{\varepsilon}$
Inflow–outflow (pmol·min^{-1}/islet)	14.7 ± 1.1	8.5 ± 0.8^{e}	5.3 ± 0.7^{e}	8.4 ± 1.0$^{a\beta}$

*Results are expressed as mean values (± SEM) together with either the number of separate measurements (n) or degree of freedom (italicized numbers) and statistical significance ($^{a}p < 0.05$; $^{b}p < 0.025$; $^{c}p < 0.01$; $^{d,\delta}p < 0.005$; $^{e,\varepsilon}p < 0.001$) of the differences between control and ω3-depleted rats (a to e) or ω3-OO and w3-FO rats (δ, ω).

ouabain-resistant uptake of $^{86}Rb^+$ only represented, in the ω3-NI rats, 78.0 ± 5.4% ($n = 30$; $p < 0.005$) of the corresponding values found after 10 and 60 min incubation in islets from control animals (100.0 ± 4.3%; $n = 70$). A comparable situation prevailed in the islets of ω3-depleted rats injected with the MCT:OO emulsion (ω3-OO rats), the absolute value for the ouabain-sensitive net uptake of $^{86}Rb^+$ not being significantly different, whether after 10 or 60 min incubation, from that found in the ω3-NI rats. The ouabain-resistant uptake of $^{86}Rb^+$ was even somewhat lower ($p < 0.02$ or less) in the former than latter animals. In the ω3-NI or ω3-OO rats, a significant activity of the Na,K-ATPase, as judged from the ouabain-sensitive uptake of $^{86}Rb^+$, could only be documented after 60 min incubation, with an overall mean value of 29.4 ± 10.0 pmol/islet (d.f. = 86; $p < 0.005$).

In the ω3-depleted rats injected with the MCT:FO emulsion (ω3-FO rats), however, the activity of the Na,K-ATPase, as judged by the ouabain-sensitive uptake of $^{86}Rb^+$, was no more significantly different ($p > 0.1$ or more) from that found in control animals, whether after 10 or 60 min incubation. In contrast, the ouabain-resistant uptake of $^{86}Rb^+$ remained much lower ($p < 0.001$) in ω3-FO rats than in control animals.

Although the protein and insulin content of the islets did not differ significantly in the four groups of rats, the ratio between the ouabain-resistant and total uptake of $^{86}Rb^+$ was calculated in each group of rats to provide information on the relative contribution of the two modalities (ouabain-resistant and ouabain-sensitive) of $^{86}Rb^+$ net uptake, independently of any possible difference in islet size between the different types of rats. As indicated in Table 1, such a ratio, as derived from the measurements made after both 10 and 60 min incubation, did not differ significantly ($p > 0.5$) in ω3-NI and ω3-OO rats with an overall mean value of 82.3 ± 4.2%. The latter value was higher ($p < 0.001$) than that found in control animals. On the other hand, the same ratio was lower ($p < 0.001$) in ω3-FO rats than in control animals. This confirms that the respective contributions of the ouabain-resistant and ouabain-sensitive modality of $^{86}Rb^+$ inflow are indeed dissimilar in the different groups of rats.

In these experiments, the absolute values for the net uptake of $^{86}Rb^+$ were always lower ($p < 0.001$) after 10 min than after 60 min incubation. Relative to the latter measurements, those recorded after 10 min incubation were not significantly different from one another in the four groups of rats, whether in the case of the total uptake of $^{86}Rb^+$ or its ouabain-sensitive component (Table 1). Pooling all available data, the 10/60 min ratio averaged 35.9 ± 1.7% ($n = 105$) for the total uptake of $^{86}Rb^+$ and 35.2 ± 8.9% (d.f. = 208) for its ouabain-sensitive component. The lack of proportionality between total $^{86}Rb^+$ uptake and length of incubation ($p < 0.001$ in all cases) is consistent with an exponential pattern for the time-related net uptake of $^{86}Rb^+$, according to the equation $U = U_{max}(1 - e^{-kt})$, in which U represents the uptake of $^{86}Rb^+$ (expressed as pmol/islet) at time t (expressed as min), U_{max} the steady-state equilibrium value for such an uptake, and k the fractional outflow rate for the intracellular pool of $^{86}Rb^+$ (expressed as min^{-1}). For instance, in

Table 2
Effect of a Phorbol Ester (TPA), Cytochalasin B, and Ouabain Upon Glucose-Stimulated Insulin Release

	Rats		
Agent (m*M*)	Control	NI- and OO-ω3	FO-ω3
Nil	100.0 ± 5.5 (20)[a]	100.0 ± 5.4 (48)	100.0 ± 8.1 (15)
TPA (0.001)	171.2 ± 8.5 (20)[d]	215.9 ± 11.5 (32)[d]	147.2 ± 11.1 (15)[c]
Cytochalasin B (0.02)	142.0 ± 7.1 (20)[d]	138.4 ± 16.0 (31)[b]	136.2 ± 9.3 (15)[b]
Ouabain (1.0)	77.6 ± 4.4 (20)[c]	43.9 ± 3.1 (47)[d]	25.8 ± 7.0 (14)[d]

[a]All results are expressed relative to the mean value recorded within the same experiment(s) and in the same type of rats in islets incubated in the sole presence of 8.3 m*M* D-glucose (Nil).

[b]$p < 0.01$; [c]$p < 0.005$ and [d]$p < 0.001$ versus first line (Nil).

the islets from control animals, the experimental values for the total net uptake of $^{86}Rb^+$ were compatible with k and U_{max} values close to 0.046 min^{-1} and 317 pmol/islet, respectively, yielding at isotopic equilibrium an inflow–outflow rate of 14.7 $pmol \cdot min^{-1}$ per islet. The latter value is virtually identical to that reported more than 25 yr ago (14.8 $pmol \cdot min^{-1}$ per islet) and derived from measurements of $^{86}Rb^+$ fractional outflow rate from prelabeled and perifused islets and net uptake of $^{86}Rb^+$ by isolated islets after 90 min of incubation *(7)*.

The fact that the time-course for $^{86}Rb^+$ net uptake was comparable in the four groups of rats suggests, therefore, that differences in the content of polyunsaturated ω3 fatty acids in cell lipids exert no detectable effect on $^{86}Rb^+$ fractional outflow rate, as mediated mainly by K^+ channels. The mean value for k in the four groups of rats averaged 0.039 ± 0.003 min^{-1}. The values for U_{max} and, hence, those for the inflow–outflow rate at isotopic equilibrium were, sizably different, however, in the various groups of rats. They were significantly lower in the ω3-NI and ω3-OO rats than in control animals, the mean values found in the former animals averaging 50.2 ± 6.7% ($n = 4$; $p < 0.005$) of the corresponding values found in control animals. In the ω3-FO rats, the mean values for both U_{max} and inflow–outflow rate were significantly higher than the corresponding values found in ω3-OO rats (Table 1). As expected from the comparable k values found in each group of rats, there was a highly significant correlation ($r = 0.998$; $p < 0.003$) between the calculated values for $^{86}Rb^+$ inflow–outflow rate at isotopic equilibrium and the experimental values for the total net uptake of $^{86}Rb^+$ after 10 min incubation. As is also expected from the exponential time-course for $^{86}Rb^+$ net uptake, the latter values, when expressed as pmol/min, were somewhat lower ($p < 0.001$) than the former ones, with a mean paired ratio of 82.7 ± 1.3% ($n = 4$) between these two variables.

Insulin Release

The perturbation of K^+ fluxes in the islets of ω3-depleted rats coincided with selected anomalies of insulin release.

In the islets from ω3-NI rats, as in those from control animals, the output of insulin progressively increased as a function of the extracellular D-glucose concentration. For instance, in the former rats, it averaged at 8.3 and 16.7 m*M* D-glucose, respectively, 274.8 ± 25.1 and 525.8 ± 26.9% of the mean corresponding value found at 2.8 m*M* D-glucose (100.0 ± 4.4%; $n = 20$ in all cases).

In the present series of experiments, the absolute values for insulin release over 90 min incubation in the sole presence of D-glucose (8.3 m*M*) were not significantly different ($p > 0.3$) in control animals (90.1 ± 4.9 μU/islet; $n = 20$) and ω3-depleted animals (103.2 ± 7.9 μU/islet; $n = 63$). It also failed to differ significantly when comparing either ω3-NI rats to control animals (ω3-NI/control ratio : 117.7 ± 12.9%; d.f. = 56; $p > 0.15$) or ω3-FO to ω3-OO rats (FO/OO ratio: 95.1 ± 10.5%; d.f. = 23; $p > 0.6$). As expected from prior observations *(11–13)* both the phorbol 12-myristate 13-acetate (1.0 μ*M*) and the mould metabolite cytochalasin B (21 μ*M*) augmented glucose-stimulated insulin release in the islets from control animals while, under the present experimental conditions, ouabain (1.0 m*M*) inhibited the secretory response to the hexose. Thus, in the control animals, the output of insulin averaged 90.1 ± 4.9 μU/islet per 90 min in the sole presence of D-glucose, as distinct ($p < 0.001$) from 154.3 ± 7.7 and 127.9 ± 6.4 μU/islet per 90 min ($n = 20$ in all cases) in the concomitant presence of the phorbol ester and cytochalasin B, respectively, and 70.0 ± 4.0 μU/islet per min ($n = 20$; $p < 0.005$) in the presence of ouabain. A comparable situation prevailed in the ω3-depleted rats (Table 2).

Relative to the reference value found within the same experiment(s) in the sole presence of D-glucose, the output of insulin recorded in the concomitant presence of the phorbol ester was significantly higher in the ω3-NI rats than in the control animals (ω3-NI/control ratio : 136.3 ± 10.9%; d.f. = 38; $p < 0.005$) and in the ω3-OO rats than in the ω3-FO rats (OO/FO ratio : 126.9 ± 9.9%; d.f. = 25; $p < 0.02$), the two relevant ratios being quite comparable ($p > 0.5$). Such a situation is similar to that encountered with a num-

ber of other insulin secretagogues *(5,6)*. In the case of cytochalasin B, however, which interferes with a late event in the secretory sequence at the level of the microfilamentous cell web controlling the access of secretory granules to exocytotic sites *(11)*, the enhancement of glucose-stimulated insulin secretion was virtually identical ($p > 0.6$ or more) in all cases (Table 2). Still another pattern was observed in the islets exposed to ouabain. Thus, on the one hand, the output of insulin recorded in the presence of the cardiac glycoside and expressed relative to that found within the same experiment(s) in its absence, was lower ($p < 0.001$) in the ω3-NI and ω3-OO rats than in control animals. In this respect, there was no difference between the ω3-NI and ω3-OO rats, with a NI/OO ratio, as derived from experiments conducted in rats of the same age, averaging 99.6 ± 20.8% (d.f. = 26; $p > 0.98$). On the other hand, in the ω3-FO rats, ouabain decreased glucose-stimulated insulin output to a greater relative extent ($p < 0.02$) than that found in ω3-OO rats (Table 2).

Discussion

The present observations in isolated pancreatic islets from ω3-depleted rats are in agreement with prior findings documenting the modulation of Na,K-ATPase activity by long-chain polyunsaturated ω3 fatty acids in such systems as brain *(14)*, synaptic plasma membrane *(15)*, nerve terminals *(16)*, erythrocytes *(17)*, and aortic smooth muscle cells *(18)*. Specific membrane environments that differ in their fatty acid composition may also affect the ouabain binding site of the Na,K-ATPase *(19)*. Moreover, feeding a diet enriched with fish oil or administration by gavage of liposomes containing C22:6ω3 may increase Na,K-ATPase activity in sciatic nerve and red blood cells of diabetic rats, in which the activity of the enzyme is otherwise impaired *(20–22)*.

The present study indeed documents that the inflow of K^+ by both the ouabain-sensitive and ouabain-resistant modalities is severely decreased in the islets from ω3-depleted rats, relative to the values found in islets from normal rats, this coinciding with an altered insulin secretory response of the islets to ouabain. Moreover, it reveals that, within 120 min after the intravenous injection of the MCT:FO emulsion to ω3-depleted rats, the inflow of K^+ by the ouabain-sensitive modality, but not that by the ouabain-resistant modality, is restored to a normal value.

The former finding extends to pancreatic islet cells the knowledge that the bolus intravenous injection of this MCT:FO emulsion provokes, within 1–2 h, changes in metabolic and functional variables. Within the limits of present knowledge, such changes include enrichment of phospholipids in polyunsaturated long-chain ω3 fatty acids in human platelets and leukocytes *(9)* and rat hepatocytes *(10)*, lowering of the excessive triglyceride content of the liver in ω3-depleted rats *(10)*, and protection of both the heart against the consequence of ex vivo ischemia in the same animals *(23)* and aortic endothelial function against the alteration caused by oxidized low density lipoproteins in either normal or diabetic rats *(24)*.

A participation of fatty acid binding and transporter proteins, indeed present in islet cells *(25,26)*, to the changes in ionic fluxes caused by the prior in vivo injection of the MCT:FO emulsion should not be ignored. However, the clearance of the MCT:FO emulsion only relies on lipoprotein lipase to a very minor extent and may occur mainly by tissue uptake of whole particles followed by intracellular degradation *(27)*.

The secretory data collected in islets exposed to ouabain indicate that an inhibitory component of the insulinotropic action of the cardiac glycoside prevailed in the islets of ω3-depleted rats. For instance, a depletion of endogenous calcium stores in such islets, as could result from the low activity of the Na,K-ATPase, may impede the positive component of the insulinotropic action of ouabain, which is indeed currently ascribed to a rise of the Na^+ content of islet cells with resulting mobilization of Ca^{2+} from intracellular organelles *(28)*. Under these conditions, the further decrease in the K^+ cellular content of islets from ω3-depleted rats, as caused by ouabain, may lead to inhibition of glucose-stimulated insulin release, which is indeed severely impaired in K^+-depleted islets *(8)*. The postulated ouabain-induced further decrease in the islet K^+ content and the resulting inhibition of glucose-stimulated insulin secretion were more pronounced in islets from ω3-FO rats than in those from ω3-OO rats, as expected from the fact that the activity of the Na,K-ATPase is already severely impaired in the latter islets even in the absence of ouabain. It should be stressed, however, that an impaired activity of the Na,K-ATPase does not represent the sole anomaly found in the islets from ω3-depleted rats. Indeed, the metabolism of D-glucose and the fluxes of Ca^{2+}, including both the inflow of Ca^{2+} and the net uptake of $^{45}Ca^{2+}$ at isotopic equilibrium, are also altered in the islets from ω3-depleted rats *(5,6)*.

In conclusion, a perturbation of K^+ fluxes in islet cells may well represent a key determinant of the changes in islet function found in ω3-depleted rats.

Materials and Methods

Pancreatic islets were isolated by the collagenase procedure *(29)* from female fed normal rats (Iffa Credo, L' Arbresle, France) or ω3-depleted rats (second generation) obtained as reported elsewhere *(5)*. Some ω3-depleted rats were injected in a tail vein 120 min before sacrifice with 1.0 mL of either an ω3 fatty acid–rich medium-chain triglyceride:fish oil emulsion (MCT:FO) or a control ω3 fatty acid–poor medium-chain triglyceride:olive oil emulsion (MCT:OO). The composition of these emulsions was previously described *(5)*. These animals are referred to as ω3-FO and

ω3-OO rats, respectively. The ω3-depleted rats not injected with a lipid emulsion before sacrifice are indicated as ω3-NI rats.

The procedures used in this study were approved by the local Animal Experimentation Ethics Committee. The animals were sacrificed under CO_2 anesthesia.

The uptake of $^{86}Rb^+$ was measured by a method previously described and is expressed of K^+, taking into account the $^{86}Rb^+/^{39}K^+$ ratio in the incubation medium *(30)*. Insulin release was measured over 90 min incubation in groups of eight islets each placed in 1.0 mL of a salt-balanced medium containing 1.0 mg/mL bovine serum albumin *(29)*.

All results are presented as mean (± SEM) together with either the number of individual determinations (n) or degree of freedom (d.f.). The statistical significance of differences between mean values was assessed by use of Student's *t* test or, whenever so required, by variance analysis completed by Bonferroni multiple comparison test.

Acknowledgments

This study was supported in part by a grant from the Région de Bruxelles-Capitale (BC-BR 246/2410) to Target Hit s.a. (Brussels, Belgium). The lipid emulsions were provided by B. Braun (Melsungen, Germany). We are indebted to C. Demesmaeker for secretarial help.

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