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ORIGINAL PAPER

Carbamylcholine and ouabain effects on Ca^{2+} handling and insulin release in islets from rats depleted in long-chain polyunsaturated ω3 fatty acids

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Abstract A number of metabolic, ionic and secretory variables were recently found to be affected in pancreatic islets obtained from second generation rats depleted in long-chain polyunsaturated ω3 fatty acids (ω3 rats). The present study further documents three sets of anomalies in such islets. First, after 90 min exposure to D-glucose (8.3 mM), the release of insulin from perifused islets, prelabelled with ^{45}Ca, is lower in ω3 rats than in control animals, despite comparable ^{45}Ca fractional outflow rate. Second, over 15 min exposure to carbamylcholine (0.1 mM), in the presence of D-glucose, the cytosolic concentration of Ca^{2+} is increased to a greater relative extent in dispersed islet cells from ω3 rats, as compared to control animals. This coincides with a greater relative increase in insulin output from perifused islets during the second phase of the secretory response to the cholinergic agent. Last, the increase provoked by ouabain (1.0 mM) in cytosolic Ca^{2+} concentration, ^{45}Ca fractional outflow rate and insulin release are all delayed in the ω3 rats. Taking into account the decreased activity of Na^+, K^+-ATPase in the islets of ω3 rats, these findings are interpreted as reflecting an impaired priming of insulin-producing cells when first exposed for 105 min to a physiological post-prandial concentration of D-glucose.

Keywords Long-chain polyunsaturated ω3 fatty acids · Pancreatic islets · Cytosolic Ca^{2+} concentration · ^{45}Ca handling · insulin release

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Introduction

Recent studies have drawn attention to both the impaired activity of the Na^+, K^+-ATPase in pancreatic islets from rats depleted in long-chain polyunsaturated ω3 fatty acids (ω3 rats), as documented by a decrease in the ouabain-sensitive net uptake of $^{86}Rb^+$, and the resulting alteration in the secretory response of these islets to a number of secretagogues, as measured over 60–90 min incubation [1–4]. In the latter respect, emphasis was placed on an increased responsiveness to most insulinotropic agents, relative to the reference value found in islets exposed to a physiological concentration of D-glucose (8.3 mM).

In the light of these findings, the present study deals specifically with the dynamics of the carbamylcholine and ouabain effects on three interrelated variables, namely the cytosolic concentration of Ca^{2+} in dispersed islet cells $[Ca^{2+}]_i$, the efflux of ^{45}Ca from prelabelled and perifused islets and the release of insulin from the same islets. In all cases, the results obtained in ω3 rats were compared, within the same series of experiments, to those found in control animals.

Results

Cytosolic Ca^{2+}

Over 10 min perifusion in the sole presence of D-glucose (8.3 mM), the cytosolic Ca^{2+} concentration was not significantly different in islet cells from control and ω3-depleted rats, averaging respectively 162.2 ± 6.6 nM ($n = 352$) and 165.3 ± 4.2 nM ($n = 451$) in the first measurements (time zero) and 173.5 ± 9.2 nM ($n = 338$) and 163.9 ± 4.5 nM ($n = 454$) at min 10.

The administration of carbamylcholine (0.1 mM) provoked a rapid increase in $[Ca^{2+}]_i$, peaking within one minute at mean values of 327.4 ± 45.6 ($n = 149$) and 324.7 ± 19.7 nM ($n = 101$) in control and ω3 rats, respectively (Fig. 1). Shortly thereafter, however, the mean value for $[Ca^{2+}]_i$ became significantly higher in ω3 rats than in control animals. Such a difference achieved statistical significance ($P < 0.02$) already 41 s after introduction of carbamylcholine. It persisted up to the end of the experiments (min 25), at which time the $[Ca^{2+}]_i$ averaged 208.0 ± 19.8 mM ($n = 97$) in ω3 rats as distinct ($P < 0.005$) from 124.1 ± 18.2 mM ($n = 85$) in control animals.

A different situation prevailed when the dispersed islet cells were exposed to ouabain (1.0 mM) after min 10 onwards (Fig. 2). The initial increase in $[Ca^{2+}]_i$ caused by the cardiac glycoside occurred earlier and was of greater

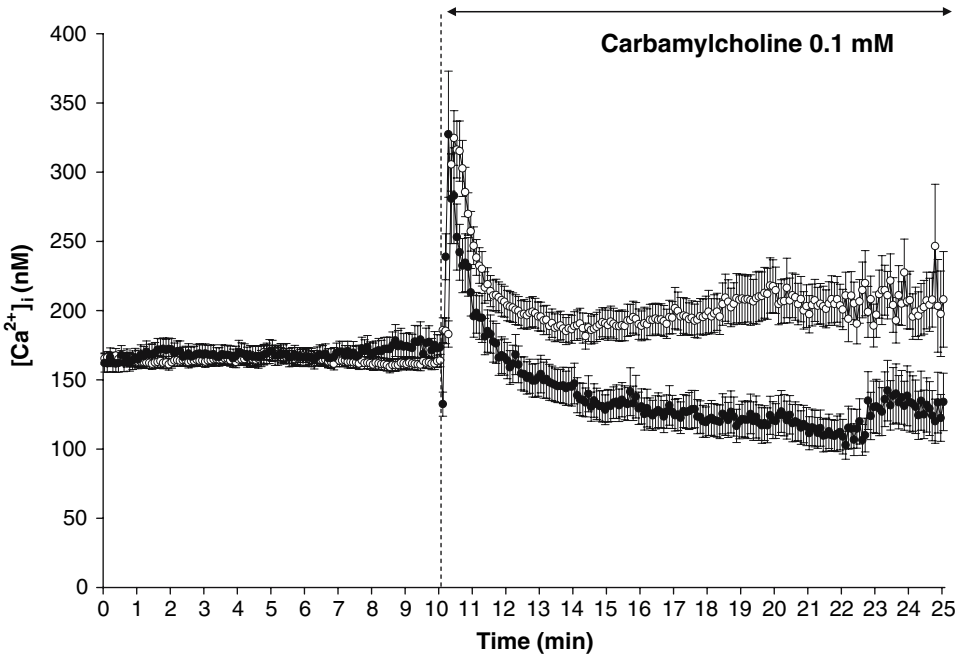

Fig. 1 Time course for the changes in cytosolic Ca^{2+} evoked by carbamylcholine (0.1 mM) in dispersed islet cells from either control animals (closed circles) or ω3-depleted rats (open circles) perifused at 8.3 mM D-glucose. Mean values (±SEM) refer to measurements derived after min 10 from about 105–150 individual cells and collected in 2–3 separate experiments

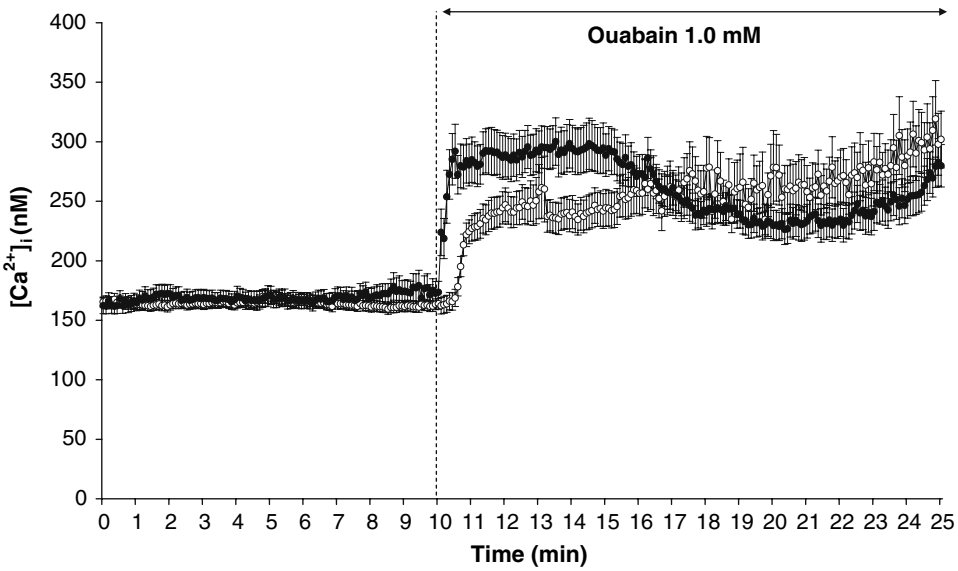

Fig. 2 Time course for the changes in cytosolic Ca^{2+} evoked by ouabain (1.0 mM) in dispersed islet cells from either control animals (closed circles) and ω3-depleted rats (open circles) perifused at 8.3 mM D-glucose. Mean values (±SEM) refer to measurements derived after min 10 from about 140–175 individual cells and collected in 2–3 separate experiments

magnitude in control animals than in ω3 rats. In the control animals, a first peak was reached within 36 s, averaging 292.0 ± 22.7 nM ($n = 141$). At the same time, the cytosolic Ca^{2+} concentration was still close to basal value in the ω3 rats, averaging no more than 168.8 ± 7.2 nM ($n = 175$) and, as such, being significantly lower ($P < 0.001$) than that found in the control animals. Thereafter, the cytosolic Ca^{2+} concentration displayed a multiphasic pattern in control rats, characterized by a decrease after the initial plateau (min 10–15) to reach a nadir value of 229.1 ± 11.6 nM ($n = 141$) about 10 min after introduction of ouabain and by a later reascension to reach a mean reading of 282.7 ± 19.9 nM ($n = 140$) at min 25. The first of the latter two mean values, which were indeed significantly different from one another ($P < 0.025$), was also significantly lower ($P < 0.02$) than that reached 36 s after introduction of ouabain. In the ω3 rats, however, a slow rise in cytosolic Ca^{2+} concentration was observed over the same period, the mean value recorded at the end of the experiments averaging 303.9 ± 28.0 nM ($n = 167$) as distinct ($P < 0.01$) from 224.4 ± 10.0 nM ($n = 175$) after one min exposure to ouabain.

^{45}Ca fractional outflow rate

After 44 min perifusion in the sole presence of D-glucose (8.3 mM), the ^{45}Ca fractional outflow rate (FOR) from the prelabelled islets was not significantly different in ω3 rats ($1.24 \pm 0.07\ 10^{-2} \cdot \text{min}^{-1}$; $n = 16$) and control animals $1.33 \pm 0.07\ 10^{-2} \cdot \text{min}^{-1}$; $n = 12$).

Carbamylcholine (0.1 mM) provoked a rapid peak-shaped increase of ^{45}Ca ouflow (Fig. 3). Relative to the

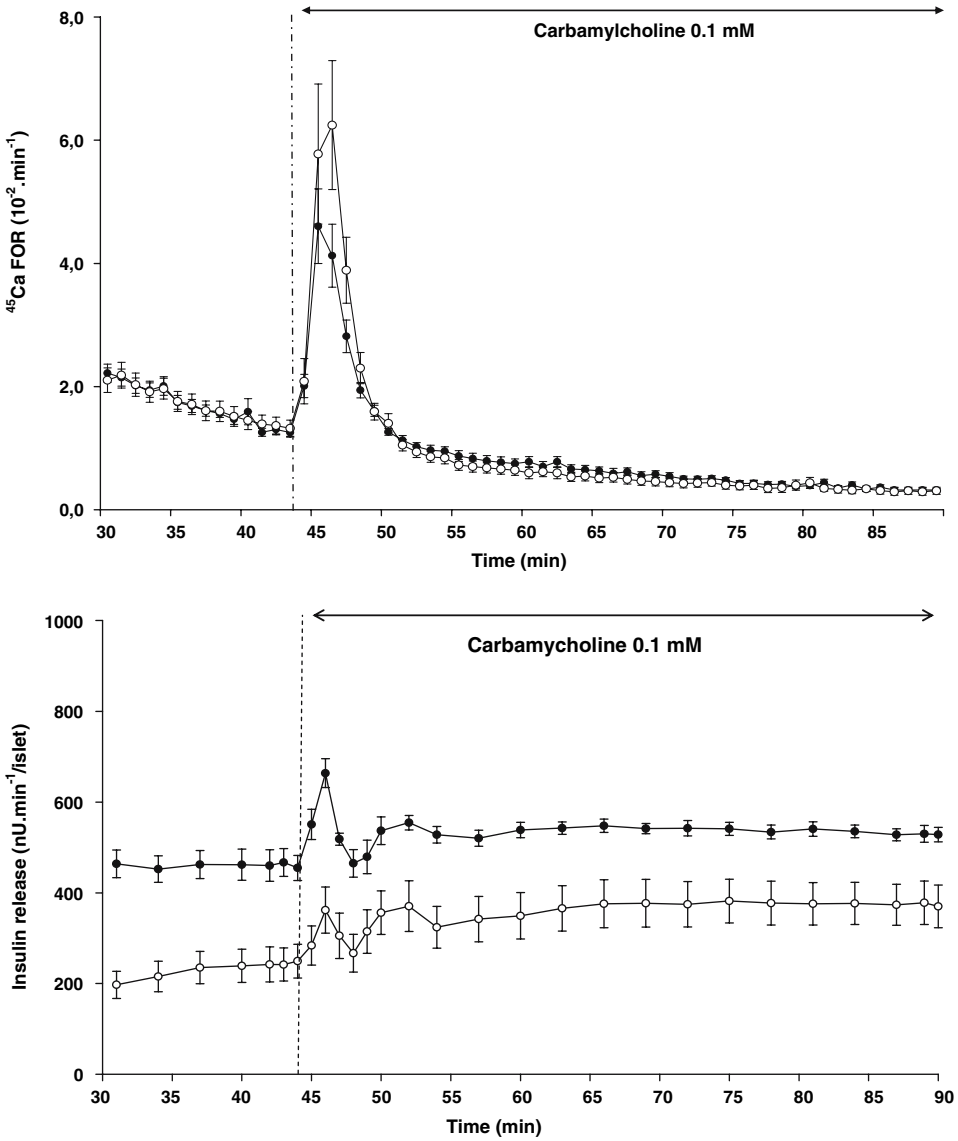

Fig. 3 Time course for the changes in ^{45}Ca fractional outflow rate (upper panel) and insulin release (lower panel) evoked by carbamylcholine (0.1 mM) in prelabelled islets from either control animals (closed circles) or ω3-depleted rats (open circles) perifused at 8.3 mM D-glucose. Mean values (±SEM) refer to 6 individual experiments in control animals, and 7–8 individual experiments in ω3-depleted rats

paired value recorded at min 44, the peak value reached within 2–3 min exposure to the cholinergic agent yielded a maximal increment in ^{45}Ca FOR which was not significantly different in control animals ($n = 6$) versus ω3 rats ($n = 8$), whether expressed in absolute terms (3.35 ± 0.65 versus $5.19 \pm 0.11 \times 10^{-2} \cdot \text{min}^{-1}$) or as the paired min 46–47/44 ratio (357 ± 59 vs. $465 \pm 103\%$). These data indicate that the trend was towards a higher response to carbamylcholine in ω3 rats than in control animals.

Ouabain (1.0 mM) also caused a rapid and monophasic increase in ^{45}Ca efflux, which was less pronounced than that provoked by carbamylcholine (Fig. 4). The peak increment in effluent radioactivity, which was reached at a comparable time in the control animals and ω3 rats, i.e. after 163 ± 10 s exposure to ouabain ($n = 14$), was not significantly different in the latter rats ($1.29 \pm 0.44 \times 10^{-2} \cdot \text{min}^{-1}$; $n = 8$; $P < 0.025$) and in control animals ($1.20 \pm 0.38 \times 10^{-2} \cdot \text{min}^{-1}$; $n = 6$; $P < 0.03$). The cationic response to ouabain was more rapid, however, in control animals than in ω3 rats. Indeed, in the former animals, a sizeable increase in ^{45}Ca FOR ($+0.47 \pm 0.18 \times 10^{-2} \cdot \text{min}^{-1}$; $n = 6$; $P < 0.05$) above the paired basal value recorded at min 44.2 ± 0.2 was already observed within one min exposure to ouabain. In the ω3 rats, however, the paired change in ^{45}Ca FOR after one min exposure to ouabain failed to achieve statistical significance, not exceeding $+0.07 \pm 0.08 \times 10^{-2} \cdot \text{min}^{-1}$ ($n = 8$), i.e. a value significantly lower ($P < 0.05$) than that recorded at the same time in the control animals.

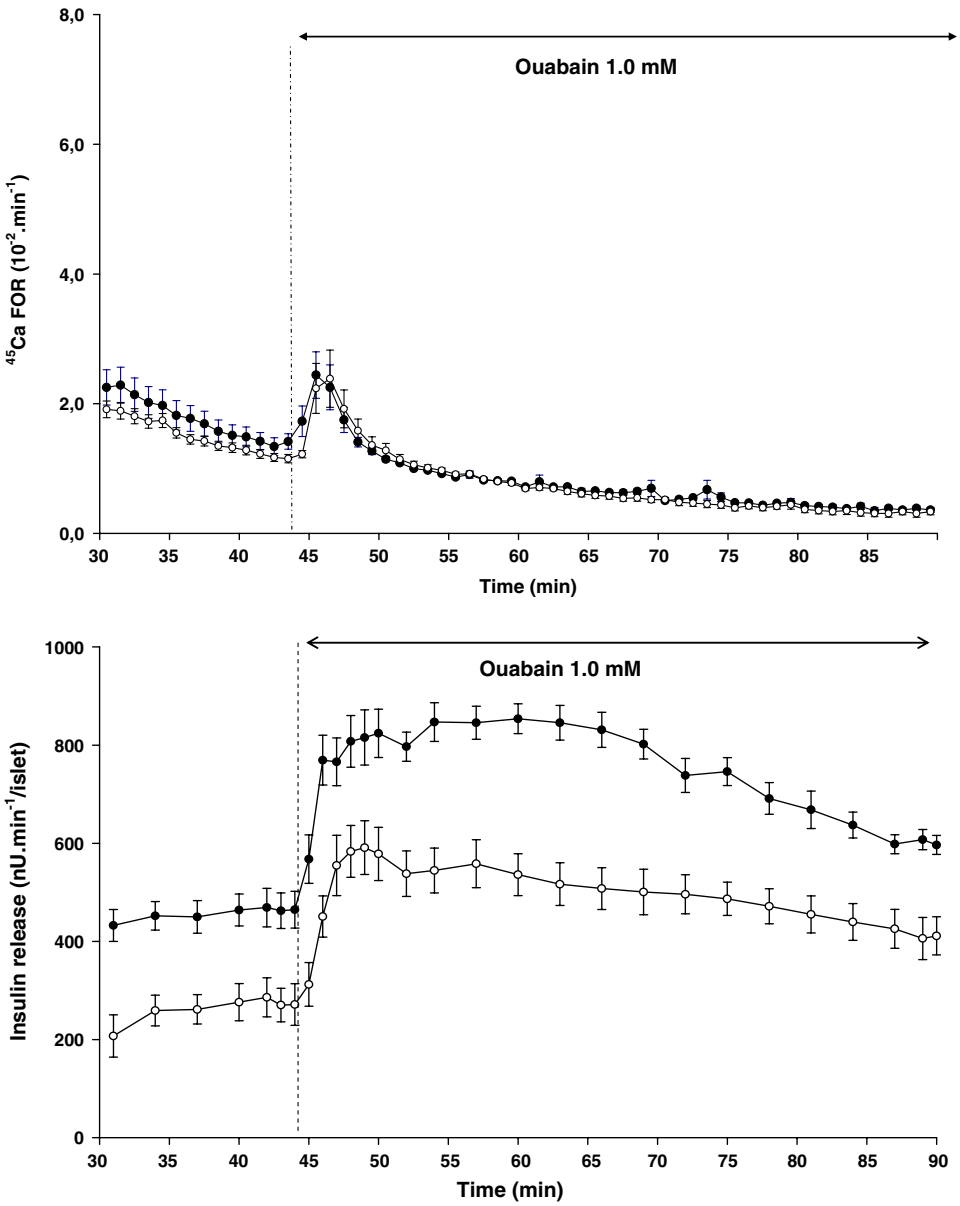

Fig. 4 Time course for the changes in ^{45}Ca fractional outflow rate (upper panel) and insulin release (lower panel) evoked by ouabain (1.0 mM) in prelabelled islets from either control animals (closed circles) or ω3-depleted rats (open circles) perifused at 8.3 mM D-glucose. Mean values (±SEM) refer to 6 individual experiments in both control animals and ω3-depleted rats

Insulin release

The output of insulin from the perifused islets exposed to 8.3 mM D-glucose was lower in ω3 rats than in control animals. For instance, at min 44, it averaged 269 ± 27 nU $\cdot$ min^{-1} $\cdot$ islet^{-1} ($n = 15$) in ω3 rats, as distinct ($P < 0.001$) from 460 ± 22 nU $\cdot$ min^{-1} $\cdot$ islet^{-1} ($n = 12$) in control animals.

In both types of rats, carbamylcholine (0.1 mM) provoked an early peak-shaped increase in insulin output, followed by a later reascension in secretory rate (Fig. 3). The absolute value for the early increment in insulin release (nU $\cdot$ min^{-1} $\cdot$ islet^{-1}) was lower ($P < 0.01$) in ω3 rats ($+112.7 \pm 15.1$; $n = 7$) than in control animals ($+208.9 \pm 24.7$; n = 6). The relative magnitude of such an early secretory response to the cholinergic agent, as judged from the min 46/min 44 ratio in insulin output was virtually identical, however, in ω3 rats ($143.3 \pm 3.5\%$; $n = 7$) and control animals ($147.3 \pm 7.4\%$; $n = 6$). Moreover, the relative magnitude of the later response to carbamylcholine, as judged for instance from the min 90/min 44 ratio in secretory rate, was higher ($P < 0.005$) in ω3 rats ($153.1 \pm 7.6\%$; $n = 7$) than in control animals ($117.8 \pm 6.3\%$; $n = 6$).

The administration of ouabain (1.0 mM) provoked, in both control animals and ω3 rats, a monophasic increase in insulin output (Fig. 4). Two differences in the insulinotropic action of ouabain were observed, however, between these two types of rats. First, in the ω3 rats, the paired min 45 minus min 44 increment in insulin output failed, once again, to achieve statistical significance ($P > 0.2$), not exceeding 34.2 ± 22.9 nU $\cdot$ min^{-1} $\cdot$ islet^{-1} ($n = 8$) whilst, in control animals, the output of insulin at min 45 was already 103.0 ± 31.5 nU $\cdot$ min^{-1} $\cdot$ islet^{-1} ($n = 6$; $P < 0.025$) higher than the paired value recorded at min 44. Second, whereas the highest value for insulin release reached at min 49 yielded, in absolute terms, comparable increments ($P > 0.2$) in ω3 rats ($+302.6 \pm 24.9$ nU $\cdot$ min^{-1} $\cdot$ islet^{-1}; $n = 8$) and control animals ($+350.6 \pm 29.6$ nU $\cdot$ min^{-1} $\cdot$ islet^{-1}; $n = 6$), the mean paired min 49/min 44 ratio in secretory rate was again higher in ω3 rats than in control animals. Likewise, the relative magnitude of the late secretory response to ouabain, as judged from the mean paired min 90/min 44 ratio in insulin release, was also higher in ω3 rats than in control animals. As judged by the latter two sets of data, the output of insulin recorded in the presence of ouabain between min 49 and 90, expressed relative to the paired reference value (min 44) represented in the ω3 rats $118.7 \pm 6.2\%$ ($n = 16$; $P < 0.05$) of the mean corresponding values found in control animals ($100.0 \pm 5.7\%$; $n = 12$).

Discussion

The present findings provide information on both the handling of Ca^{2+} and release of insulin in three distinct situations, namely that prevailing in islet cells exposed to 8.3 mM D-glucose in the absence of any other insulin secretagogues and those found, at the same hexose concentration, in the concomitant presence of either carbamylcholine or ouabain.

In the first of these situations, our measurements of cytosolic Ca^{2+} concentration in dispersed islet cells exposed to 8.3 mM D-glucose are in fair agreement with secretory data previously collected in freshly isolated islets obtained from control and ω3-depleted rats. Indeed, in these previous experiments, the absolute value for insulin release over 90 min incubation in the sole presence of D-glucose (8.3 mM) was not significantly different [4] or slightly higher [3] in ω3 rats than in control animals.

A different situation prevailed, however, in the perifused islets examined after 60 min preincubation in the presence of 8.3 mM D-glucose. In the latter case, the output of insulin recorded in the sole presence of the hexose (8.3 mM) was indeed significantly lower in the islets from ω3 than in those obtained from control animals (Fig. 2). Nevertheless, the fractional outflow rate of ^{45}Ca recorded in the sole presence of D-glucose was not significantly different in perifused islets from control and ω3-depleted rats. The latter finding again differs from that found in freshly isolated islets, in which case the fractional outflow rate of ^{45}Ca was much lower in islets from ω3-depleted rats than in islets obtained from control rats [3].

The contrasting situation found in terms of both insulin release and ^{45}Ca outflow, when comparing freshly isolated islets to those first preincubated for 60 min in the presence of 8.3 mM D-glucose, strongly suggests that the priming action normally resulting from such a preincubation was severely impaired in the islets from ω3-depleted rats. Such a priming action normally results in a higher rate of insulin secretion from islets subsequently exposed to an insulinotropic concentration of D-glucose and is thought to be attributable, in part at least, to such processes as activation of protein kinase C [5, 6] and an increase in the K^+ content of insulin-secreting cells [7]. Two factors may well account for the impairment of this priming process in the islets from ω3 rats. First, the metabolism of D-glucose is less efficient in the latter islets than in those obtained from control rats [1]. Second, a marked decrease in the activity of the Na^+, K^+-ATPase, as recently documented in the islets from ω3-depleted rats [4] may oppose the intracellular accumulation of K^+ ions otherwise occurring in islets from normal rats preincubated at an insulinotropic concentration of the hexose.

Likewise, in the range of D-glucose concentrations between 7.0 and 8.3 mM, the ^{45}Ca FOR of islets obtained from normal rats is lower than that recorded either in the absence of any exogenous nutrient or at higher concentrations of the hexose [8]. Hence, the present results suggest that the priming of the islets resulting from their preincubation at 8.3 mM D-glucose after the isolation of the islets conducted in the absence of exogenous nutrient allowed, in islets from control rats, the hexose, when present at 8.3 mM, to provoke the expected decrease in ^{45}Ca FOR, whilst such was apparently not the case in the islets from ω3 rats.

In fair agreement with previous findings, however, the net uptake of ^{45}Ca after 60 min preincubation, as reflected by the total amount of the radioactive tracer both release by the islets between the 31st and 90th min of perifusion and recovered in the islets at the latter time, was higher in the ω3 rats than in the control animals. The value found in the latter animals indeed only represented 53.9 ± 3.5 % ($n = 12$) of that recorded in the ω3 rats (100.0 ± 11.0%; $n = 16$). Likewise, in a prior study conducted in freshly isolated islets [3], the net uptake of ^{45}Ca after 60 min incubation in the presence of 8.3 mM D-glucose only represented, in the control animals, 63.8 ± 4.6 % ($n = 27$) of the mean value recorded in ω3 rats (100.0 ± 10.6%; $n = 20$). Even if one ignores the SEM's on the mean reference value found in ω3 rats, the mean results obtained in control animals in the present and prior experiments are not significantly different ($P > 0.15$) from one another. The inflow rate of Ca^{2+} into the islet being lower in ω3 rats than in control animals [4], the higher net uptake of ^{45}Ca found after 60 min (pre)incubation in the former rats may well reflect a prior depletion of intracellular calcium pools, as conceivably attributable to their mobilization by an elevated intracellular Na^+ content, itself resulting from the low Na^+, K^+-ATPase activity in the islets of ω3 rats.

The findings recorded in islets exposed to carbamylcholine document (i) a higher cytosolic Ca^{2+} concentration in ω3 rats than in control animals, (ii) a trend towards a higher increment in ^{45}Ca FOR in response to administration of the cholinergic agent to prelabelled perifused islets, prepared from ω3 rats, as compared to control animals, and (iii) a greater secretory responsiveness to the cholinergic agent, in ω3 rats than in control animals, as judged from increase in secretory rate recorded during sustained exposure for 45 min to carbamylcholine and expressed relative to the reference value found prior to its administration to the perifused islets. These converging findings are reminiscent of those collected in prior investigations, in which several nutrient and non-nutrient insulin secretagogues tested over 60–90 min incubation in the presence of 8.3 mM D-glucose, were also found to augment insulin release to a greater relative extent in ω3 rats than in control animals.

Last, the present results concerning the cationic and secretory effects of ouabain reveal two major differences between control animals and ω3 rats. First, as judged from the time course of the changes evoked by ouabain in $[Ca^{2+}]_i$, ^{45}Ca FOR or insulin release, a less rapid response to the cardiac glycoside was always observed when comparing ω3 rats to control animals. This situation is consistent with the low activity of the Na^+, K^+-ATPase in the islets of the former rats. Second, the later changes caused by ouabain in both $[Ca^{2+}]_i$ and insulin output were also different in control animals and ω3 rats. The multiphasic pattern of $[Ca^{2+}]_i$ otherwise recorded over 15 min exposure to ouabain in control animals was suppressed in the ω3 rats. Moreover, a striking difference was found when comparing the response to either carbamylcholine or ouabain in ω3 rats versus control animals. As judged from the paired difference in the mean value for $[Ca^{2+}]_i$ found during the 15 min period of exposure to these insulin secretagogues and the mean reference value recorded, in the same cells, during the initial 10 min period conducted in the sole presence of D-glucose (8.3 mM), carbamylcholine increased to a greater extent $[Ca^{2+}]_i$ in ω3 rats than in control animals. Indeed, such a paired difference averaged, in relative terms, 25.3 ± 4.3% ($n = 105$) in ω3 rats, as distinct ($P < 0.02$) from only 7.8 ± 6.1% ($n = 86$) in control rats. In the case of ouabain, however, the relative incremental value found in ω3 rats (40.2 ± 5.8%; $n = 198$) was virtually identical to that recorded in control animals (42.6 ± 4.9%; $n = 141$). This coincides with the fact that, at variance with the situation found in islets exposed to carbamylcholine or other insulin secretagogues, no increased secretory responsiveness to ouabain is observed in islets from ω3 rats versus control animals, when exposed for 90 min to the cardiac glycoside in the presence of 8.3 mM D-glucose [4]. The latter situation was tentatively ascribed to the fact that the activity of Na^+, K^+-ATPase is already low in islets of ω3 rats, even in the absence of ouabain.

Nevertheless, the present results document that, in ω3 rats, ouabain is still able to provoke an increase in $[Ca^{2+}]_i$.

Moreover, in the experiments conducted in perifused islets, the relative magnitude of the ouabain-induced increment in insulin output was higher in ω3 rats than in control animals, despite the fact that the cardiac glycoside increased to the same relative extent the fractional outflow rate of ^{45}Ca from the prelabelled perifused islets. Once again, this apparent discrepancy between the secretory data previously collected in freshly isolated islets incubated for 90 min in the absence or presence of ouabain and those here obtained in perifused islets may well be accounted for by the prolonged exposure of the latter islets to 8.3 mM

D-glucose during both the 60 min preincubation and the 45 min of perifusion preceding the introduction of the cardiac glycoside. The relative magnitude of the ouabain-induced changes in insulin release are indeed tightly dependent on their nutrient supply, as previously documented in normal islets exposed to increasing concentrations of D-glucose [9]. Under these conditions, a dual effect of ouabain upon insulin release was observed, eventually resulting in stimulation of insulin release at low concentrations of the hexose and inhibition of insulin secretion at high concentration of the sugar [9, 10]. Hence, taking into account the postulated impairment of the priming action of D-glucose in the islets of ω3 rats, as opposed to control animals, it could indeed be expected that the former islets would display a higher secretory responsiveness to ouabain than the latter ones in the present perifusion experiments.

In any case, these experiments reveal that, after prolonged exposure of the islets to a physiological concentration of D-glucose, the absolute values for insulin release both before and after introduction of ouabain are lower in ω3 rats than in control animals. Within the limits of present knowledge, two factors may well account for such a difference, namely the impairment of D-glucose metabolism in the islets of ω3 rats [1] and their decreased Na^+, K^+-ATPase activity. As recently proposed, the latter anomaly may unfavourably affect insulin release by lowering the K^+ content of islet cells [4].

In conclusion, the results of the present investigations reinforce the concept of a multifactorial dysregulation of the process of stimulus-secretion coupling in insulin-producing cells from rats depleted in long-chain polyunsaturated ω3 fatty acids [1]. Within limits, such a perturbation may be directly ascribed to a decreased content of islet cell phospholipids in such ω3 fatty acids. It was indeed recently documented that the intravenous injection of an ω3 fatty acid-rich medium-chain triglyceride:fish oil emulsion to ω3-depleted rats 60–120 min before sacrifice corrects some, but not all, of the defects otherwise found in the islets of these ω3-depleted rats, with emphasis on the restoration of a close-to-normal activity of the Na^+, K^+-ATPase [4].

Materials and methods

Female normal rats (Iffa Credo, L'Arbresle, France) and ω3-depleted rats of comparable age were given free access to food and water up to the time of sacrifice. The procedure used to prepare second generation rats depleted in long-chain polyunsaturated ω3 fatty acids (ω3 rats), the diets offered to control and ω3 rats, and the metabolic and hormonal status of these animals were all described in a recent publication [1].

Likewise, the methods used to measure the cytosolic free Ca^{2+} concentration in dispersed islet cells [3] and both ^{45}Ca fractional outflow rate [11] and insulin release [12] from prelabelled perifused pancreatic islets were previously described in the cited references. In the present experiments, the labelling of the islets by ^{45}Ca was conducted over 60 min preincubation in the presence of 8.3 mM D-glucose.

All results are presented as mean values (±SEM) together with the number of separate determinations (n). The statistical significance of differences between mean values was assessed by use of Student's t-test.

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