Increased insulin secretion and normalization of glucose tolerance by cholinergic agonism in high fat-fed mice

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Increased insulinotropic activity by the cholinergic agonist carbachol exists in insulin-resistant high fat-fed C57BL/6J mice. We examined the efficiency and potency of carbachol to potentiate glucose-stimulated insulin secretion and to improve glucose tolerance in these animals. Intravenous administration of carbachol (at 15 and 50 nmol/kg) markedly potentiated glucose (1 g/kg)-stimulated insulin secretion in mice fed both a control and a high-fat diet (for 12 wk), with a higher relative potentiation in high fat-fed mice measured as increased (1–5 min) acute insulin response and area under the 50-min insulin curve. Concomitantly, glucose tolerance was improved by carbachol. In fact, carbachol normalized glucose-stimulated insulin secretion and glucose tolerance in mice subjected to a high-fat diet. Carbachol (>100 nmol/l) also potentiated glucose-stimulated insulin secretion from isolated islets with higher efficiency in high fat-fed mice. In contrast, binding of the muscarinic receptor antagonist [N-methyl-3H]scopolamine to isolated muscarinic receptors and the contractile action of carbachol on ileum muscle strips were not different between the two groups. We conclude that carbachol normalizes glucose tolerance in insulin resistance.

Acetylcholine receptors; carbachol; glucose intolerance; C57BL/6J mice

The pancreatic islets are innervated by postganglionic cholinergic nerve fibers emanating from nerve cell bodies in the pancreatic ganglia; the preganglionic fibers emanate from the vagus nerves (1, 8, 40). Activation of the vagus nerves, therefore, causes a local islet release of the classical neurotransmitter acetylcholine as well as various neuropeptides (1, 8). Activation of the vagus nerves as well as cholinergic, muscarinic receptors stimulates insulin secretion, as has been demonstrated both in vivo and in vitro in a number of different species (1, 3, 8, 18, 20, 23, 29). It has been demonstrated that this cholinergically induced insulinotropic action is mediated by the muscarinic M3 receptors and not by the muscarinic M1 or M2 receptors (17, 23, 44). Subsequent cellular signals include the phospholipase C-signaling pathway involving formation of diacylglycerol with activation of protein kinase C (PKC) as well as formation of inositol 1,4,5-trisphosphate with liberation of calcium from intracellular stores (1). It is thought that a main physiological function of the cholinergic system in relation to insulin secretion is to contribute to the rapid insulin release seen during the cephalic phase after food intake (10, 36, 39), which is of importance for normal glucose tolerance (28, 42).

Insulin resistance in high fat diet-treated C57BL/6J mice is accompanied by hyperinsulinemia in combination with glucose intolerance and defective insulin secretion (5, 27, 41). We showed recently that although glucose-stimulated insulin secretion is impaired in this model, the insulinotropic action of cholinergic activation by carbachol is exaggerated (7, 35). Similarly, in the preobese ob/ob mouse, carbachol exerts potentiated insulinotropic action when compared with littermates (12), and the hyperinsulinemia in adult obese ob/ob mice is sensitive to reduction of the cholinergic tonus by the muscarinic antagonist methylatropine (4) and to vagotomy (14). Furthermore, in mildly diabetic rats, increased insulinotropic sensitivity to activation of PKC exists (31) and in the diabetic obese fa/fa rats, the hyperinsulinemia is partly dependent on vagus activity (32). Moreover, in obese humans, a potentiated insulin secretory response to cholinergic stimulation by pyridostigmine has been demonstrated (13). These studies therefore collectively suggest that increased islet sensitivity to cholinergic agonism exists in insulin resistance. This, in turn, may be an important mechanism underlying the compensatory hypersecretion of insulin and hyperinsulinemia, which are well-known phenomena in insulin resistance (9, 22, 26). These results may also suggest that pharmacological stimulation of insulin secretion by cholinergic agonists may be a target for treatment of early stages of diabetes. However, exploration of such an idea requires knowledge of the mechanisms underlying the potentiated insulinotropic action to muscarinic receptor activation in insulin resistance, which at present is very limited. It is also unknown whether the potentiated insulin secretion after administration of cholinergic agonists improves the glucose tolerance in insulin resistance.

In this study, therefore, we have compared the action of carbachol on glucose tolerance and glucose-stimulated insulin secretion in C57BL/6J mice fed a normal vs. a high-fat diet. The experiments on insulin secretion were performed both in vivo and in isolated islets to establish the efficiency and potency of the compound under these two conditions. In comparison with our recent reports (7, 35) on the exaggerated insulin response to carbachol in high fat-fed insulin-resistant mice, the present study also examined the impact of carbachol on glucose-stimulated insulin secretion and glucose tolerance, the dose-response relationships were established, and studies were also undertaken in iso-
lated islets in vitro. Furthermore, we have in the present study also examined the binding of the muscarinic receptor antagonist [N-methyl-\(^{14}\)H]scopolamine ([\(^{14}\)H]NMS) to islets isolated from these two groups of C57BL/6J mice, to explore whether the increased insulinotropic action of carbachol is a result of increased receptor binding. Finally, we have also examined the contractility action of carbachol on ileum muscle strips obtained from control diet- and high fat diet-fed mice to establish whether the increased sensitivity for carbachol, which is evident for its action to stimulate insulin secretion, is restricted to islets or is a more general phenomenon.

**METHODS**

Animals and diets. Female mice of the C57BL/6J strain (Bomholtgaard Breeding and Research Centre, Ry, Denmark) received either a high-fat diet or a standard rodent Chow diet (Research Diets, New Brunswick, NJ) for 12 wk, starting at the age of 4 wk. On a calorific basis, the high-fat diet consisted of 16.4% protein, 25.6% carbohydrates, and 58.0% fat (a total energy content of 23.4 kJ/g), whereas the control diet consisted of 25.8% protein, 62.8% carbohydrates, and 11.4% fat (a total energy content of 12.6 kJ/g). The mice had free access to food and water. Four to five mice were kept per cage in a temperature-controlled (22 ± 1°C) room with a 12:12-h light-dark cycle with lights on at 6 AM. The study was approved by the Animal Ethics Committee at Lund University.

Insulin secretion experiments in vivo. After 12 wk on the respective diets, nonfasted animals (n = 86) were anesthetized with an intraperitoneal injection of midazolam (Dormicum, Hoffmann-La Roche, Basel, Switzerland; 0.4 mg/mouse) and a combination of fluanisone (0.9 mg/mouse) and fentanyl (Hypnorm, Janssen, Beerse, Belgium; 0.02 mg/mouse). Thereafter, a blood sample was taken from the retroluberal, intraorbital, capillary plexus in heparinized tubes, and then 1 g glucose [British Drug Houses (BDH), Poole, UK; (1 g/kg)] was injected rapidly intravenously either alone or together with carbachol (carbamylcholine chloride; BDH) at different dose levels. The volume load was 10 µl/g body wt. Blood samples were taken immediately before the intravenous injection and 1, 5, 20, and 50 min after. Repeated experiments in our laboratory have shown that the insulin response to carbachol in anesthetized mice peaks at 1 min after the intravenous injection (unpublished observations). After centrifugation, plasma was stored at −20°C until assayed.

Insulin secretion experiments in vitro. At 2–3 wk after the in vivo studies, pancreatic islets were isolated with the collagenase isolation technique. In brief, the pancreas was retrogradely filled with 3 ml of Hanks’ balanced salt solution (Sigma), supplemented with 0.4 mg/ml of collagenase P (activity 1.86 U/mg; Boehringer-Mannheim, Mannheim, Germany). The pancreas was subsequently removed and incubated for 20 min at 37°C. After being rinsed, the islets were handpicked under a stereomicroscope and incubated over-night in RPMI 1640 medium (glucose 11.1 mmol/l) supplemented with 10% fetal calf serum, 2.05 mmol/l L-glutamine (GIBCO BRL, Paisley, Scotland), 100 IU/ml penicillin, and 100 µg/ml streptomycin (Biological Industry, Beit Haemek, Israel) at 37°C in humidified air equilibrated with 5% CO₂. After the overnight incubation, the islets were washed three times and then preincubated for 60 min at 37°C in a HEPES medium (pH 7.36) supplemented with 0.1% human serum albumin (Sigma) and 3.3 mmol/l glucose. The medium consisted of (in mmol/l): 125 NaCl, 5.9 KCl, 1.2 MgCl₂, 1.28 CaCl₂ (all Sigma), and 25 HEPES (Boehringer-Mannheim). After the preincubation, groups of three islets were transferred into separate chambers containing 200 µl of the medium supplemented with glucose at various concentrations. After incubation at 37°C for 60 min, 25 µl of the medium were collected from each chamber and stored at −20°C until analysis.

Islet binding studies. Islets were isolated as described in insulin secretion experiments in vitro by the collagenase isolation technique and handpicked under a stereomicroscope. Islets were then homogenized for 30 s (Ultra-Torrax homogenizer) in 10 ml of medium consisting of 50 mmol/l NaHPO₄/Na₂HPO₄ and 2 mmol/l MgCl₂ with the addition of bovine serum albumin (1 mg/ml), bacitracin (0.2 mg/ml), aprotinin (500 kallikrein-inhibitor units/ml; all Sigma), and Pefabloc (Boehringer-Mannheim, 0.1 mmol/l), pH 7.4. The homogenate was then centrifuged (50,000 g) for 20 min at 2°C. The pellet was resuspended in 20 ml of the medium with the additions as above; the membranes suspended were used for binding assay. The membranes (from 30 islets/tube) were incubated in the medium for 2 h at 25°C in the presence of the muscarinic receptor antagonist [\(^{3}H\)NMS (specific activity, 80 Ci/mmol; New England Nuclear, Boston, MA) in a final volume of 1 ml. After the 2-h incubation, free and bound radioactivity was separated by filtration over Whatman GF/B filters that were washed with 3 × 5 ml of ice cold phosphate assay buffer.

Ileum contractions. The C57BL/6J mice were killed by means of cervical dislocation, the terminal 15 cm of ileum were removed, and 1.5- to 2.0-cm lengths were prepared and mounted in 10-ml organ baths containing calcium-deficient medium of the following composition (in mm; all Sigma): 137 NaCl, 2.68 KCl, 0.9 CaCl₂, 1.05 MgCl₂, 11.9 NaHCO₃, 0.42 NaH₂PO₄, and 5.5 glucose. The organ bath was maintained at 37°C and gassed with 95% O₂-5% CO₂. The mechanical activity of the muscle was measured by a HSE 351 isometric transducer connected via a HSE bridge amplifier to a potentiometric pen recorder (Hugo Sachs Elektronik). Resting tension was 1 g, and the tissue was left to equilibrate for 1 h. Dose-response curves to added carbachol were constructed sequentially with 30-s contact time with a drug concentration being added every 3 min. Contractions were measured as millimeter responses.

Analysis. Plasma insulin was measured radioimmunochemically with a guinea pig anti-rat insulin antibody, 125I-labeled human insulin as tracer and, as standard, rat insulin (Linco Research, St. Charles, MO). The separation of free and bound radioactivity was performed by use of an anti-IgG (goat anti-guinea pig) antibody (Linco). The sensitivity of the assay is 12 pmol/l, and the coefficient of variation is <3%. Plasma glucose levels were measured by the glucose oxidase method.

Calculations and statistics. The results are expressed as means ± SE. The acute insulin response (AIR) to glucose with or without carbachol was calculated as the mean of suprabasal 1- and 5-min postchallenge plasma insulin levels. Area under the curve (AUC) was calculated for suprabasal plasma insulin (AUC insulin) and plasma glucose (AUC glucose) levels, respectively, for the entire 50-min study period with the trapezoid rule. To determine the degree of significance, Student’s t-test for unpaired data was used. When comparing the dose-response relationships, ANOVA with Bonferroni post hoc analysis for multiple comparisons was undertaken. Pearson’s product-moment correlation was used to estimate linear relationships between variables. A P value < 0.05 was considered significant.
RESULTS

Body weight and baseline insulin and glucose. Throughout the study, mice given a high-fat diet had higher body weight than control diet-fed mice and they also had increased plasma glucose and insulin levels, confirming that high-fat diet induces insulin resistance in this strain of mice. The body weight and baseline (nonfasting) values of insulin and glucose at 4, 8, and 12 wk on the respective diets are shown in Fig. 1. The results in all time points after the start of the high-fat diet are statistically significant between the two groups (P < 0.05 or less).

Insulin secretion and glucose tolerance after intravenous glucose with or without carbachol. After a period of 12 wk on the respective diets (age 16 wk), the animals underwent an in vivo experiment with the intravenous challenge of glucose (1 g/kg) alone or together with different concentrations of carbachol (ranging from 1.5 to 50 nmol/kg). Figure 2 shows the plasma insulin levels in the two groups of animals in this experimental series. The intravenous injection of glucose elicited a rapid insulin response in both groups of mice with a maximal increase in plasma insulin already at 1 min after injection. The peak insulin level after glucose administration was significantly potentiated by carbachol at the two highest doses (15 and 50 nmol/kg) in both normal fed (P < 0.017 for 15 nmol/kg; P < 0.001 for 50 nmol/kg) and high fat-fed animals (P < 0.001 for both doses). In contrast, at 1.5 and 5 nmol/kg, carbachol did not significantly affect the peak plasma insulin levels. Figure 3 shows the corresponding plasma glucose levels. Plasma glucose levels peaked at 1 min.
after injection. In control diet-fed mice, plasma glucose levels thereafter declined to reach baseline values within the 50-min study period. However, in high fat diet-fed animals, plasma glucose levels were still elevated above the preinjection levels at 50 min after glucose administration ($P < 0.001$). At this time point, high fat diet-fed animals injected with glucose and carbachol at 50 nmol/kg had lower plasma glucose than animals injected with glucose alone ($P = 0.029$), whereas high fat diet-fed mice injected with carbachol at lower dose levels had a 50-min plasma glucose level not significantly different from that in glucose-injected controls.

Figure 4 shows the calculated AIR, $\text{AUC}_{\text{insulin}}$, and $\text{AUC}_{\text{glucose}}$ as a function of dose of carbachol in mice fed a high-fat diet vs. those fed a control diet, whereas $\text{AUC}_{\text{glucose}}$ was 43% higher ($P < 0.001$) as a sign of the impairment of glucose-stimulated insulin secretion and glucose tolerance that accompanies insulin resistance. AIR and $\text{AUC}_{\text{insulin}}$ were potentiated by carbachol at dose levels of 15 and 50 nmol/kg in both groups of animals ($P < 0.05$), whereas at the two lower dose levels (1.5 and 5 nmol/kg), carbachol had no significant effect. Furthermore, $\text{AUC}_{\text{glucose}}$ was reduced by carbachol at 15 and 50 nmol/kg in both groups ($P < 0.05$).

After administration of carbachol at 15 and 50 nmol/kg to mice fed a high-fat diet, $\text{AUC}_{\text{glucose}}$ did not differ significantly from the $\text{AUC}_{\text{glucose}}$ in control diet-fed mice.
animals given glucose alone, i.e., carbachol normalized glucose tolerance.

Figure 5 shows the AIR and AUC_glucose in relative response to glucose alone after administration of carbachol, expressed as percentage of the response to glucose alone in the two groups of mice. At 50 nmol/kg, carbachol increased AIR in high fat diet-fed mice by 479 ± 86% vs. by only 237 ± 36% in normal diet-fed mice (P = 0.019), and the corresponding values at 15 nmol/kg carbachol were 392 ± 64% in high fat-fed animals vs. 127 ± 39% in mice fed a normal diet (P = 0.002). Moreover, the AUC_glucose was reduced by carbachol at 50 nmol/kg by 38 ± 8% in high fat diet-treated animals vs. 18 ± 6% in normal animals (P = 0.026). Also, when given at 15 nmol/kg, carbachol reduced AUC.glucose by 36 ± 6% in high fat diet-fed animals and by 16 ± 5% in control diet-fed animals (P = 0.012).

Relation between plasma insulin and plasma glucose. Across all animals and when results were included from all time points (n = 430), plasma glucose correlated significantly with plasma insulin (r = 0.58, P < 0.001) and the correlation was stronger when plasma insulin levels were transformed logarithmically (r = 0.71, P < 0.001). This correlation was evident in both control diet-fed and high fat-fed mice and in mice injected both with glucose alone and with carbachol at the different dose levels (Table 1). The slope of the regression differed between high fat-fed and control diet-fed mice, however. Thus, in animals injected with glucose alone, the slope of the regression was lower in high fat-fed than in control diet-fed mice (P < 0.001) as a sign of impaired glucose sensitivity to increase plasma insulin in mice fed a high-fat diet. Furthermore, the interception of the regression with the y-axis was higher in high fat-fed than in control diet-fed mice (P < 0.001). Carbachol administration at 15 and 50 nmol/kg increased the slope of the regression in both high fat-fed and control diet-fed animals (P < 0.001) without significantly affecting the interception of the regression with the y-axis. In fact, the slope of the regression after carbachol administration in high fat diet-fed mice did not differ significantly from that in control mice given glucose alone, indicating that carbachol normalized the relation between glucose and plasma insulin in insulin-resistant C57BL/6J mice.

Relation between insulin secretion and glucose tolerance. Figure 6 shows the ratio between AIR and AUC_glucose, which is a measure of the relation between insulin secretion and glucose tolerance, in the two groups after administration of the various doses of carbachol. It is seen that after administration of glucose alone or glucose with the low doses of carbachol, animals fed a control diet had a higher AIR-to-AUC_glucose ratio than animals fed a high-fat diet (P = 0.020), indicating insufficient insulin secretion in relation to the ambient glucose tolerance in high fat diet-fed animals. However, at the two highest doses of carbachol (15 and 50 nmol/kg), the AIR-to-AUC_glucose ratio did not differ significantly between animals given control diet and animals given high-fat diet. This indicates a normalization of the relation between insu-
Insulin secretion and glucose tolerance by carbachol in high fat diet-fed animals.

Insulin secretion in isolated islets. Figure 7 shows that carbachol-stimulated insulin secretion from islets isolated both from high fat diet- and control diet-fed mice. The effect of carbachol was dose dependent with a maximal effect obtained at 1 µmol/l (control diet-fed mice) and 10 µmol/l (high fat-fed mice). Furthermore, the insulinotropic action of carbachol was glucose dependent because the effect was only minimal at 5.6 mmol/l glucose but marked at 11.1 mmol/l glucose. At dose levels >1 µmol/l, carbachol significantly stimulated insulin secretion more markedly in islets from high fat diet-fed mice than in islets from control diet-fed mice (P < 0.05 or less). At very high-dose levels (>100 µmol/l), the insulinotropic action of carbachol was lower than at lower dose levels, but still with a higher efficiency in islets isolated from high fat-fed mice.

Carbachol binding to isolated islets. Figure 8 shows the Scatchard plot of the binding of [3H]NMS to islets from mice of the two groups, each point consisting of 30 islets. It is seen that the binding kinetics were not different for the two groups of animals (n = 3 for each group). The dissociation constant (K_d) for binding was 0.17 ± 0.02 nmol/l in mice fed a control diet vs. 0.22 ± 0.04 nmol/l in mice fed a high-fat diet, and the corresponding values for maximum binding capacity (B_max) were 0.46 ± 0.04 and 0.47 ± 0.07 fmol/30 islets, respectively.

Ileum contractions. When carbachol was added to the ileum from the mice, a contraction was induced. There was no difference, however, in the contractile response to carbachol between control diet- and high fat diet-fed mice (Fig. 9).

DISCUSSION

In this study, we confirm that mice of the C57BL/6J strain fed a high-fat diet develop slight hyperglycemia and hyperinsulinemia in association with the increased body weight as a sign of insulin resistance (7, 35, 41). It is known that these mice exhibit impaired insulin secretion after glucose administration as a sign of islet dysfunction (7, 27, 41). We previously showed that the administration of the muscarinic agonist carbachol induces an exaggerated insulin secretory response under baseline conditions, i.e., without any concomitant administration of another insulin secretagogue, in these insulin-resistant mice (7, 35). This suggested that although the insulin secretory sensitivity to glucose is impaired, the response to cholinergic agonism is potentiated. In this study, we have examined the influence of...
carbachol on glucose-stimulated insulin secretion and glucose tolerance in mice fed a high-fat diet. We also characterized the dose-response relationships and examined the direct in vitro influence of carbachol on isolated islets. We showed that carbachol also potentiates glucose-induced insulin secretion in the mice fed a high-fat diet and a control diet. Because the glucose-induced insulin secretion per se was lower in mice fed a high-fat vs. a control diet and the resulting insulin secretory response to glucose plus carbachol was the same in the two groups of animals, the net effect of carbachol to augment glucose-stimulated insulin secretion was more marked in the mice fed a high-fat diet. This was particularly evident when calculating AIR, i.e., the insulin response during the first 5 min, which represents the first phase insulin secretion. Hence, also with respect to glucose-stimulated insulin secretion, an exaggerated insulinotropic response to carbachol is evident after high-fat diet in the C57BL/6J mice. This further supports the notion that insulin resistance is accompanied by an adaptively increased cholinergic sensitivity in the islets, which previously has been observed in obese and preobese ob/ob mice (4, 12). Also in obese, insulin-resistant humans, an exaggerated insulin response to cholinergic activity has been documented (13). The hyperinsulinemia in insulin resistance might therefore be dependent on an increased cholinergic sensitivity in the islets.

It is necessary to comment on a few aspects of the design of the present study. First, we used the increase in plasma insulin levels as a determinant for insulin secretion. Although this is a valid parameter used in a number of studies, a drawback is that also possible differences in insulin clearance between the two groups of animals might contribute to the parameter. This might be important because it is known that insulin resistance per se might be accompanied by reduced insulin clearance (19, 43). However, we also showed that carbachol induced a more marked insulin secretion in islets isolated from high fat-fed mice vs. from control mice. Therefore, although reduced insulin clearance might contribute to the raised plasma insulin levels after carbachol in high fat-fed mice, the exaggerated insulin response to carbachol also represents an exaggerated insulin secretion. Second, we used the cholinergic agonist carbachol to activate the cholinergic receptors, and when given centrally (in the cerebroventricular system), it is known that carbachol induces a "stress" response involving hyperglycemia and hyperglycagonemia with unchanged insulin levels (45). However, we do not consider such an action to contribute to the observed effects on plasma insulin and glucose in our experiments, because carbachol, due to its polar structure, does not pass the blood-brain barrier when given peripherally (37). Furthermore, we have previously shown that methylatropine, which also does not pass the blood-brain barrier, inhibits carbachol-stimulated insulin secretion in mice (3). This suggests that the effects of carbachol in our present study are exerted peripherally. Third, even though the effects of carbachol are peripherally mediated, it is possible that the drug also exerts indirect, islet-independent, peripheral actions. For example, carbachol has been shown to stimulate epinephrine release from the adrenals (24) and secretion of glucagon-like peptide-1 (GLP-1) from the gut enteroglucagon cells (11), which might contribute to the insulinotropic action of the drug. The possibility that the stimulatory action of epinephrine (the β-adrenoreceptor activation) contributes to the insulinotropic action of carbachol is, however, unlikely, because we have shown that propranolol, inhibiting β-adrenoreceptor-activated insulin secretion, does not inhibit carbachol-stimulated insulin secretion in mice (3). Whether GLP-1, and possibly also gastric inhibitory polypeptide (GIP), contribute to the insulinotropic action, however, remains to be studied in more detail. Nevertheless, the in vitro results, showing that carbachol potently stimulates insulin secretion from isolated islets, suggest that the insulinotropic action of the drug as observed in the present study is most likely caused by a direct islet action. Finally, although induction of anesthesia and the experimental procedure might have induced a stress response in the animals, the stress level is remarkable low in this type of experiments. For example, we have previously shown that anesthesia reduces the circulating levels of catecholamines by ~80% when compared with studies in nonanesthetized animals (2, 15); hence, our experimental model provides a good technique associated with only a low degree of stress. The potential influence of the unavoidable stress on the metabolic status of the animals has not been established in detail. However, we have shown that circulating glucose does not increase after saline injection in anesthetized mice, which suggests that the model is not associated with significant metabolic responses associated with stress (15).

In this study, we characterized the relationship between dose of carbachol and effects on glucose-stimulated insulin secretion and glucose tolerance in the two groups of mice. We showed that a threshold for a
potentiated glucose-stimulated insulin secretion by carbachol in mice exists at a dose between 5 and 15 nmol/kg and that this threshold was similar in mice fed a high-fat diet and in those fed a control diet. Therefore, the increased insulinotropic response to carbachol in high fat-fed animals is reflected as increased efficiency rather than increased potency. To examine whether this increased efficiency is also evident in islets, we performed an in vitro study. The results show that carbachol dose and glucose dependently stimulated insulin secretion from islets isolated from control diet- and high fat diet-fed mice. Furthermore, the pattern of the effects of carbachol also shows that in vitro it is the efficiency rather than the potency that is increased. In both groups, a “bell-shaped” dose-response relationship existed between dose of carbachol and insulin secretion, with diminished action at high-dose levels. This is a well-known phenomenon for cholinergic agonism in different in vitro systems (21, 25, 30, 33, 34, 38). We also show that the binding of carbachol to islets isolated from the control diet-fed and high fat diet-fed animals was the same. These results together indicate that the increased activity of carbachol to stimulate insulin secretion in insulin-resistant mice is not dependent on increased receptor sensitivity but rather on increased intrinsic activity subsequent to acetylcholine-receptor activation in islets. This may be due to increased efficiency in the signaling pathways activated by these receptors.

Our study on the contractile response to carbachol in the ileum preparations from the two groups of mice showed no difference between control diet-fed- and high fat-fed mice. This shows that the increased responsiveness to carbachol in insulin secretion is not reflective of a generalized increased responsiveness to muscarinic agonism after high-fat feeding. It is also important to acknowledge that the high fat-fed mice had a reduced insulin secretory response to glucose per se, which was evident both by the lower AIR and AUC$_{insulin}$, as well as by the lower slope between the regression between plasma levels of glucose and insulin. Hence, an exaggerated signaling is probably not a general phenomenon in the islets, but specific to certain secretagogues. In this context, it should be mentioned that we have previously shown that the insulinotropic response also to cholecystokinin and to the gluco-incretin hormone GLP-1 are exaggerated in C57BL/6J mice (35), suggesting a generalized increased responsiveness to nonglucose insulin secretagogues.

The relation between circulating glucose and insulin was linearly correlated both in control diet-fed and in high fat-fed mice, and the relation was improved by logarithmic transformation of the insulin data. However, as is shown in detail in Table 1, the slope of the relation was lower in high fat-fed animals than in control diet-fed animals, illustrating the insensitivity for glucose for the insulin secretion in insulin resistance. By challenging the mice with intravenous carbachol, we increased the slope of the relation between glucose and insulin. Of importance is that the slope was the same in control diet-fed animals given glucose as in high fat-fed mice given glucose + carbachol, suggesting that carbachol normalizes the insulin-glucose relation in insulin-resistant mice.

The mice fed the high-fat diet had a markedly impaired glucose tolerance, as judged by the marked increase in AUC$_{glucose}$ after the glucose administration and the finding that plasma glucose levels after glucose administration were still elevated after 50 min. The potentiated glucose-stimulated insulin secretion resulted in reduction in AUC$_{glucose}$, as a sign of improvement of glucose tolerance. In fact, the AUC$_{glucose}$ in high fat-fed animals given glucose plus carbachol (15 or 50 nmol/kg) was the same as in control diet-fed animals given glucose alone, and, furthermore, the ratio between AIR to AUC$_{insulin}$ was not different between the two groups of animals given carbachol at the higher dose levels. This further suggests that carbachol has the ability through its potentiated action on insulin secretion to normalize glucose tolerance in high-fat mice. Still, however, the 50-min glucose levels were slightly higher than baseline in high fat diet-fed mice given carbachol at the highest dose level, suggesting that an even more efficient insulinotropic signal is required for complete normalization of the glucose intolerance. This is, however, not possible with carbachol due to its muscarinic receptor subtype nonspecific action. Although not studied directly in the present study, the improved glucose tolerance after carbachol administration is probably mediated by the increased plasma insulin levels rather than by any direct action of carbachol on peripheral insulin sensitivity, because a recent study in rats showed that administration of acetylcholine did not affect insulin sensitivity during a euglycemic clamp study (46).

In conclusion, the study has presented evidence that endogenous administration of the muscarinic agonist carbachol elicits a more marked insulin secretory response in high fat-fed insulin-resistant mice than in control mice, with improved glucose tolerance as a result. The exaggerated insulin response is exerted mainly through increased efficiency rather than increased potency. Furthermore, the study also shows that islet binding of muscarinic antagonist is not altered in mice fed a high-fat diet and that the ileum contraction activity of carbachol is not different between the groups. The study thus suggests that insulin resistance is associated with increased islet, but not general, muscarinic sensitivity. This may be a mechanism underlying the hyperinsulinemia in insulin resistance. It may also be suggested that development of islet-specific muscarinic agonists, with lesser general muscarinic activity, might be a feasible target to improve failure of insulin secretion in insulin resistance during the development of type 2 diabetes. The rapid potentiation of the immediate (first 5 min) glucose-stimulated insulin secretion is in this context of great interest, because impairment of first phase insulin secretion is an important islet defect in glucose intolerance and type 2 diabetes (6, 16).

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