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In vivo and in vitro increased pancreatic beta-cell sensitivity to glucose in normal rats submitted to a 48-h hyperglycaemic period

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Summary. We investigated the importance of the level and the duration of glucose stimulation on the in vivo and in vitro insulin response to glucose in normal rats previously submitted to hyperglycaemia. Rats were made hyperglycaemic by a 48-h glucose infusion. Glucose-induced insulin secretion was investigated in vivo by a 20-min hyperglycaemic clamp and in vitro by the isolated perfused pancreas technique, 3 h after the end of the in vivo glucose infusion. In glucose-infused rats, as compared to controls, in vivo incremental plasma insulin values above baseline integrated over the 20-min hyperglycaemic clamp (ΔI) were five times higher during 8 mmol/l glucose clamp, only two times higher in 11 mmol/l glucose clamp and no different in 16.5 mmol/l. Compared to the controls, in vitro incremental plasma insulin concentration above baseline integrated over a 20-min period (ΔI) in glucose-infused rats was 16 times higher in response to 2.8 mmol/l glucose, two times higher in response to 5.5 mmol/l, similar in response to 8.3 mmol/l and significantly lower in response to 16.5 mmol/l. In conclusion, our data suggest that a 48-h hyperglycaemic period results in an increased response of the pancreatic beta cell to low glucose. The response is immediately maximal and can not be increased with higher glucose concentrations. This situation could explain the apparent minimal effect of high concentrations on in vitro insulin secretion in previously hyperglycaemic rats and may provide insights into the sequence of events leading to the impairment of beta-cell function in Type 2 (non-insulin-dependent) diabetes mellitus.

Key words: Glucose infusion, in vivo insulin secretion, in vitro insulin secretion, beta-cell sensitivity.

The deteriorating effect of chronic hyperglycaemia on glucose-induced insulin secretion in diabetic subjects or in animal models of Type 2 (non-insulin-dependent) diabetes mellitus is supported by several lines of direct and indirect evidence [1].

The effect of hyperglycaemia on insulin secretion in non-diabetic human subjects or animals appears more complex and is still a matter of controversy. In healthy subjects, enhanced beta-cell function has been demonstrated after a 20–24-h period of glucose-infusion [2, 3] or a 3-day consumption of a high carbohydrate diet [4]. In normal rats, prolonged exposure (48–96-h) of pancreatic beta-cells to high glucose levels in vivo was repeatedly found to profoundly impair the further in vitro insulin secretory response to glucose, as measured by the isolated perfused pancreas technique [5–12]. By contrast, a 2–4-day hyperglycaemia dramatically and durably potentiated the subsequent in vivo insulin secretion in response to glucose, when measured by intravenous glucose tolerance tests (IVGTT) [9, 13].

The differential effect of prolonged hyperglycaemia on the further insulin secretion according to whether it was tested in vivo or in vitro could be at least partly explained by the duration and magnitude of glucose stimulation which are different in vivo and in vitro. During IVGTT, the glucose stimulation is very short and the plasma glucose concentration rises modestly especially in previously hyperglycaemic rats (maximal level of glycaemia in previously hyperglycaemic rats: 6–7 mmol/l, [9]). When insulin release is studied by the isolated perfused pancreas technique, the duration of the stimulation with glucose is usually at least 20 min and glucose is perfused at a concentration of 16–17 mmol/l. Therefore, an impairment of the in vivo insulin response to a high concentration of glucose in hyperglycaemic rats could be missed, whereas an increase in the beta-cell response to low glucose concentrations in vitro could not be detected.

For these reasons, it is important to compare in vivo and in vitro insulin secretion under similar conditions.

In the present study, we investigated the role of the level of glucose stimulation on the in vivo and in vitro insulin response to glucose in rats previously submitted to a

48-h hyperglycaemic period. In this way, unrestrained healthy rats were continuously infused with glucose over 48 h and the further insulin secretory response to glucose was investigated either in vivo by 20-min glucose clamps (8, 11, 16.5 mmol/l), or in vitro by the isolated perfused pancreas technique using various concentrations of glucose (2.8, 5.5, 8 and 16.5 mmol/l).

Materials and methods

Animals

Three-month-old female Wistar rats weighing about 250 g given free access to water and standard laboratory chow pellets UAR 113 (Usine d'Alimentation Rationnelle, Villemoisson sur Orge, France) were used. They were rendered hyperglycaemic for 48 h by continuous glucose infusion under unrestrained conditions as described previously [13, 14]. These rats were called HG rats. Control rats were catheterized but not infused. Indeed, previous experiments showed that the infusion per se did not result in detectable changes in glycaemic control and insulin secretion [9]. The infusion period started on day 2 after surgery and lasted 2 days. Glucose (30% weight/volume, Chaix and Du Marais, Paris, France) was infused at an initial rate of 50 µl/min to produce hyperglycaemia around 20–25 mmol/l. When necessary, the initial infusion rate was slightly modulated to maintain glycaemia in the desired range throughout the infusion period. When glycaemia was not maintained in the desired range, the experiment was discontinued and the data omitted.

During the glucose infusion period, plasma glucose and insulin concentrations were measured five times per day in glucose-infused rats on blood samples collected from the tail vessels. In control rats, these parameters were measured only twice per day because they remained stable. At the end of the infusion period, plasma glucose and insulin concentrations were measured at 30-min intervals for 3 h i.e. until in vivo glucose clamp studies were performed or the pancreas was removed and perfused in vitro.

Hyperglycaemic clamp studies

Hyperglycaemic clamps were performed in control and glucose-infused rats 3 h after the end of the infusion. Rats were anaesthetized with pentobarbital (Clin-Midy, Paris, France) anaesthesia (50 g/kg body weight, i.p.). Three series of hyperglycaemic clamps were performed corresponding to three plasma glucose concentrations (8, 11 and 16.5 mmol/l). For each rat, only one glucose concentration was used. Glucose was infused via a saphenous vein by a butterfly needle. In 8 and 11 mmol/l glucose clamps, a 5 (8 mmol/l) and 10% (11 mmol/l) solution of glucose was infused at an initial flow rate of 80 µl/min and 64 µl/min, respectively. In 16.5 mmol/l glucose clamps, a 15% solution of glucose was used and the initial flow rate was 40 µl/min. In each clamp, blood was sampled at 2-min intervals to determine blood glucose concentration and to adjust the rate of glucose infusion to maintain the blood glucose concentration at the level desired (i.e. 8, 11 or 16.5 mmol/l) for 20 min. For each rat, only one concentration was used.

Isolated perfused pancreas technique

In vitro insulin secretion was investigated 3 h after the end of the infusion period. The rats were anaesthetized with pentobarbital (Clin-Midy; 50 g/kg body weight, i.p.). Isolation and perfusion of the pancreas were performed as described previously [9]. The pancreata were first perfused with a Krebs'-Ringer bicarbonate buffer (KRB) containing 2.8 mmol/l of D-glucose for a 20-min equilibration period. The stimulatory effect of glucose was then investigated for 20 min. Four concentrations of glucose were used, corresponding to four groups of rats: 2.8, 5.5, 8 and 16.5 mmol/l. The stimulatory period was followed by a 10-min perfusion period with KRB-2.8 mmol/l glucose. In 5.5, 8 and 16.5 mmol/l glucose perfusion studies, the effluent of the last 10 min of the equilibration period was collected at 1-min intervals for determination of the basal rate of insulin release. When a 2.8 mmol/l glucose perfusion was used, 2.8 mmol/l glucose-KRB was replaced by a glucose-free KRB solution during the last 10 min of the equilibration period. This constituted the basal insulin release.

Analytical methods

Plasma glucose was determined using a glucose analyser (Beckman, Fullerton, Calif., USA). Plasma and pancreatic immunoreactive insulin (IRI) concentration was estimated with purified rat insulin as standard (Novo, Copenhagen, Denmark), antibody to mixed (porcine and bovine) insulin and porcine monoiodinated ^{125}I-insulin. Charcoal was used to separate free from bound hormone. This method allows the determination of 19.5 pmol/l with a coefficient of variation within and between assays of 6%.

Calculations

Insulin response during hyperglycaemic clamps or in vitro perfusion of pancreas was calculated, for each glucose concentration, as the incremental plasma insulin concentration or insulin concentration in the effluent above baseline integrated over a 20-min period (ΔI). ΔI was used to compare the insulin response to different glucose concentrations in control and HG rats.

Statistical analysis

Results are expressed as mean ± SEM. The significance of differences between means was evaluated by a one-way analysis of variance (ANOVA).

Results

Plasma glucose and insulin concentrations during and after glucose infusion

Sustained and steady hyperglycaemia of about 25 mmol/l was maintained in rats throughout the period of glucose infusion (6.5 ± 0.1 mmol/l in control rats). Elevated hyperglycaemia was related to high plasma insulin levels in glucose-infused rats throughout the infusion period. The mean plasma insulin was about nine times higher in glucose-infused (HG) rats than in control (2.68 ± 0.09 nmol/l in HG rats vs 0.36 ± 0.06 nmol/l in control rats; $n = 7$, $p < 0.001$) (Fig. 1).

In HG rats, the plasma glucose concentration dramatically decreased when glucose was no longer infused. Plasma glucose concentration was similar to controls 30 min after the infusion was discontinued. It continued to decline so that 3 h after the end of the infusion, it was significantly lower than in controls (4.0 ± 0.2 mmol/l in HG rats vs 6.7 ± 0.2 mmol/l in control rats; $n = 7$, $p < 0.01$). Concomitantly, the plasma insulin concentration decreased rapidly in HG rats, but to a lesser extent than in the controls (3 h after the end of glucose-infusion, the values were 0.8 ± 0.09 nmol/l in HG rats vs 0.32 ± 0.04 nmol/l in control rats; $n = 7, p < 0.01$) (Fig. 1).

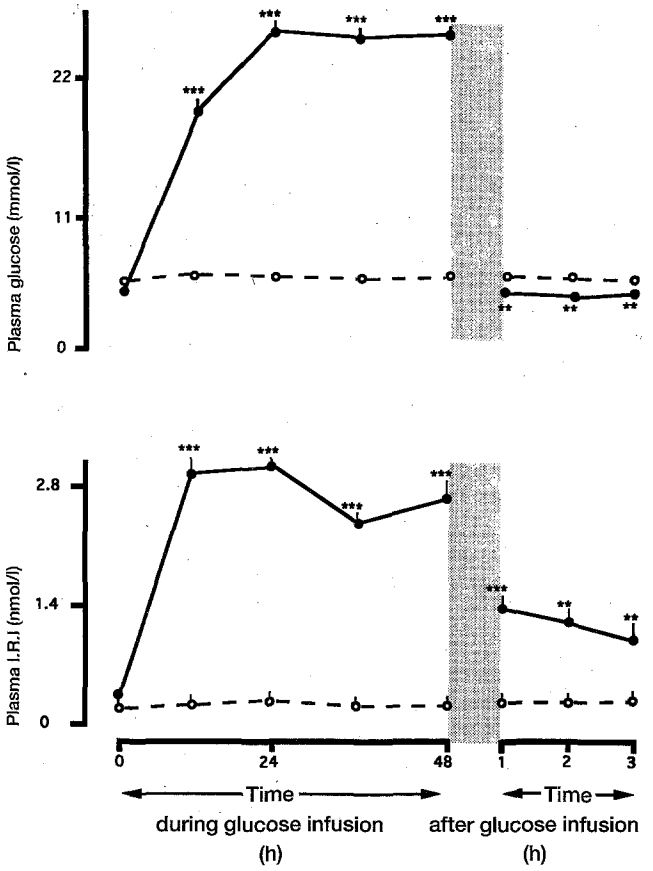

Fig. 1. Plasma glucose and immunoreactive insulin (I.R.I.) concentrations in glucose infused rats (●) during and after the end of the infusion and during the same period in control rats (○). Values are means ± SEM of 39 control and glucose-infused rats. ** $p < 0.01$, *** $p < 0.001$, significantly different from controls

Insulin secretion during hyperglycaemic clamps

As a general rule, glucose infusion provoked a rapid increase in plasma glucose concentration in HG as well as in control rats, whatever the level of the hyperglycaemic clamp. In all cases, the hyperglycaemic plateau was reached no later than 5 min after the beginning of glucose infusion (Fig. 2). In each series of hyperglycaemic clamps (8, 11 and 16.5 mmol/l), when glycaemia stabilized, it was similar in both the control and HG rats (Fig. 2).

As judged from the time course of plasma insulin concentration and the ΔI, insulin secretion in response to hyperglycaemia was very different between the two groups of rats according to the level of hyperglycaemia.

Glucose at 8 mmol/l failed to significantly increase insulin secretion in control rats, whereas it induced a dramatic and sustained rise in plasma insulin concentration in HG rats. This situation was reflected in the mean incremental plasma insulin concentration above baseline (ΔI) which was eight times higher in HG rats (ΔI = 13.8 ± 3.3 nmol/l; $n = 7$) than in controls (ΔI = 1.8 ± 0.2 nmol/l; $n = 7, p < 0.001$) (Fig. 3).

Insulin secretion was powerfully stimulated during 11 mmol/l glucose clamps in both groups of rats. In controls, the insulin response, as judged by the ΔI, was six times higher than during 8 mmol/l glucose clamps. In HG

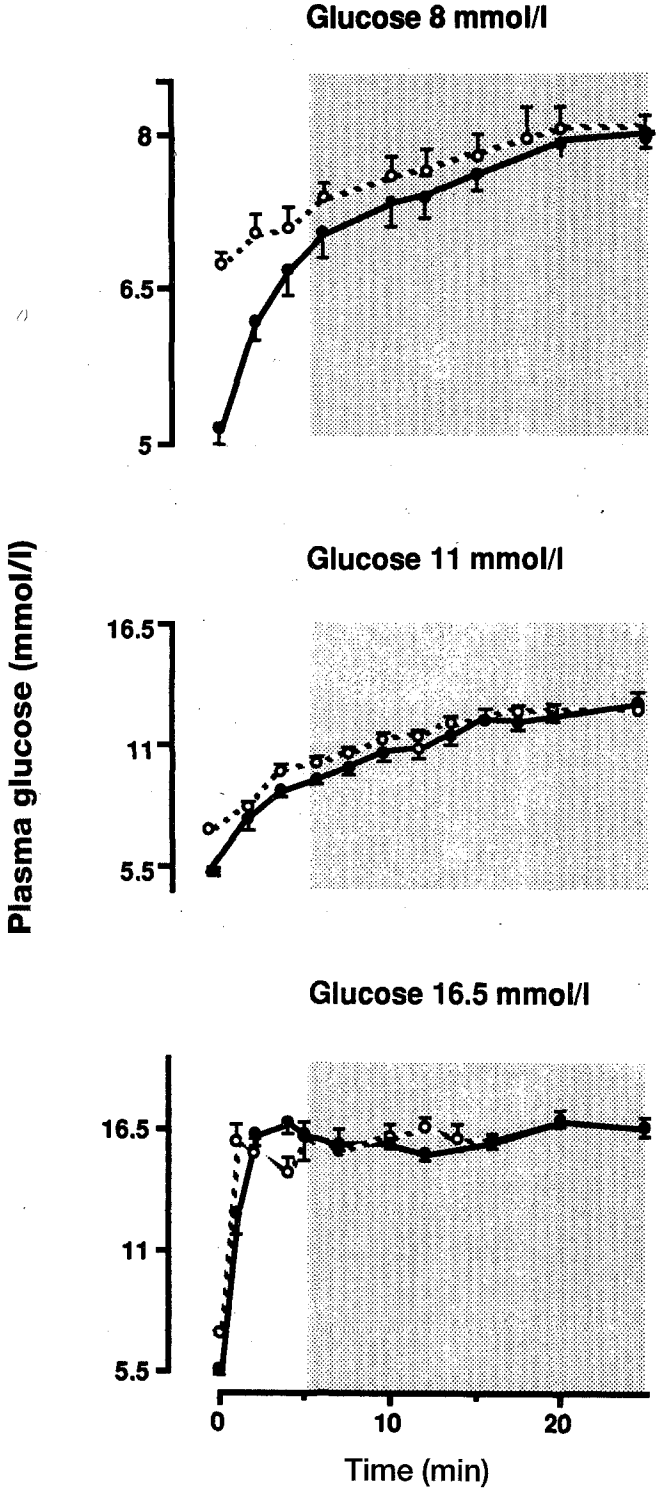

Fig. 2. Time-course of plasma glucose concentrations during the different hyperglycaemic clamps (8, 11, 16.5 mmol/l) performed 3 h after the end of the glucose infusion in control rats (○) and in glucose-infused rats (●). Values are means ± SEM of seven control rats and glucose-infused rats for each hyperglycaemic clamp

rats, the difference was much less pronounced since it was multiplied by 1.6 when compared to 8 mmol/l clamps. Thus, ΔI was only doubled in HG rats compared to control rats (ΔI = 22.8 ± 3.7 nmol/l in HG rats vs ΔI = 11.7 ± 1.5 nmol/l in control rats; $n = 7, p < 0.05$) (Fig. 3).

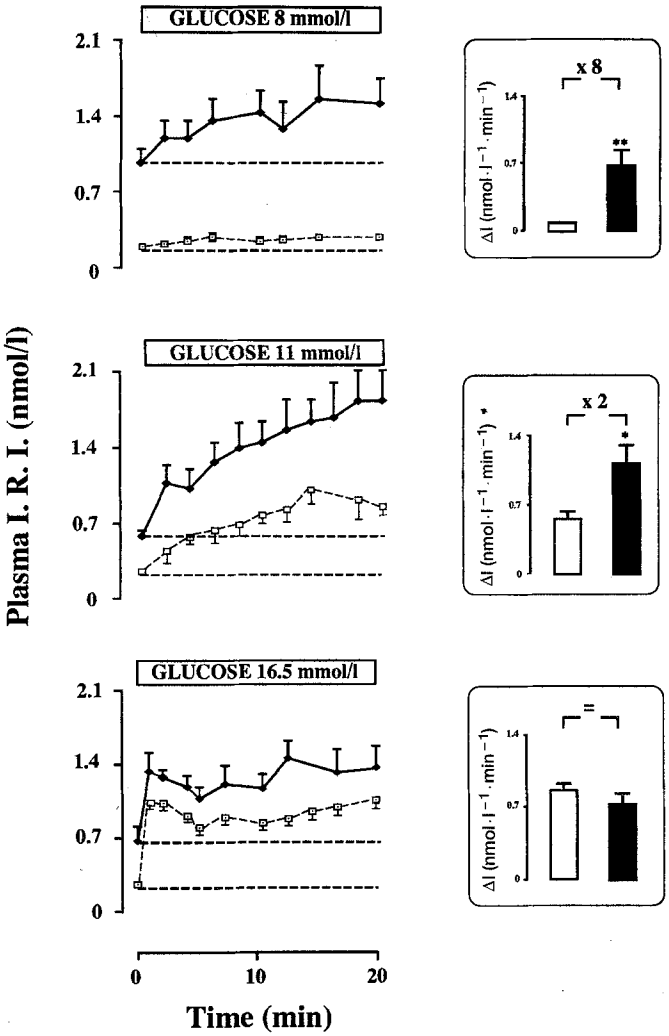

Fig. 3. Plasma immunoreactive insulin (I.R.I.) and incremental plasma insulin values above baseline integrated over the 20-min hyperglycaemic clamp (ΔI) during hyperglycaemic clamps in control rats (□) and glucose-infused rats (■), 3 h after the end of glucose infusion. Values are means ± SEM of seven control rats and glucose-infused rats. * $p < 0.05$, ** $p < 0.01$, significantly different from controls

When glycaemia was clamped at 16.5 mmol/l, insulin secretion was the highest in control rats (Fig. 3). The ΔI (17.7 ± 6.6 nmol/l; $n = 7$) was 10 and 1.5 times higher than during 8 mmol/l and 11 mmol/l glucose clamps, respectively. By contrast, in HG rats, the insulin response to 16.5 mmol/l (ΔI = 14.9 ± 2.1 nmol/l; $n = 7$) was not higher than during 8 mmol/l and 11 mmol/l glucose clamps (Fig. 3). Therefore, insulin secretion in response to 16.5 mmol/l was no longer different in control and HG rats (ΔI = 17.7 ± 2.6 nmol/l in control rats vs ΔI = 14.9 ± 2.1 nmol/l in HG rats; $n = 7$) (Fig. 3).

Insulin release from isolated perfused pancreata

In control rats, in vitro insulin release was well correlated to the concentration of glucose infused ($r^2 = 0.66$; $p < 0.001$, $n = 7$). Insulin release was almost absent in response to 2.8 mmol/l and very low in response to 5.5 mmol/l glucose (Fig. 4). Perfusion with 8 mmol/l and 16.5 mmol/l glucose resulted in a dramatic increase in insulin release which showed a normal biphasic pattern (Fig. 5). The response was the highest with 16.5 mmol/l glucose (Table 1).

In HG rats, insulin release was different in many ways compared to controls: 1) during the pre-stimulatory period, the rate of insulin release was already significantly greater in the HG group than in controls. The values were on average 3.41 ± 0.36 nmol/l in HG rats (vs 0.14 ± 0.02 nmol/l in control rats; $n = 21$, $p < 0.001$) when the medium contained 2.8 mmol/l glucose and 3.18 ± 0.26 nmol/l in HG rats (vs 0.11 ± 0.04 nmol/l in control rats; $n = 7$, $p < 0.001$) when the medium was glucose free (Figs. 4 and 5). 2) Insulin secretion during the stimulatory period was not related to the glucose concentration: the lower glucose concentration induced a high insulin response. No significant increase was observed from 2.8 to 16.5 mmol/l, as reflected in the evolution of the ΔI in function of the glucose concentration in both groups of rats (Table 1, Figs. 4 and 5). Compared to control rats, incremental insulin concentration in the effluent above baseline integrated over a 20-min period (ΔI) was 16 times higher in response to 2.8 mmol/l, 2-times increased in response to 5.5 mmol/l, similar in response to 8 mmol/l and significantly lower in response to 16.5 mmol/l (Figs. 4 and 5).

Discussion

The time-course of plasma glucose and insulin concentrations during and after the glucose infusion was very similar in this study to those previously reported [9, 13] and the data have been already largely discussed in these papers. However, special attention must be paid to the persistence of sustained hyperinsulinaemia despite low plasma glucose levels when the infusion was disrupted. This suggests an increased beta-cell sensitivity to glucose in vivo, although it cannot be excluded that hypoglycaemia is the result of increased peripheral insulin action.

The greater sensitivity of beta cells in vivo is also suggested by the in vivo hyperglycaemic clamp study. It is noteworthy that a low (8 mmol/l) glucose concentration

Table 1. Time course of in vitro incremental insulin concentration in the effluent above baseline integrated over a period of 20 min (ΔI) in response to various glucose concentrations in control- and glucose-infused rats, 3 h after the infusion

	ΔI (nmol · l⁻¹ · min⁻¹)	
Glucose concentration	Control (7)	Glucose infused (7)
2.8 mmol/l	0.07 ± 0.02	1.13 ± 0.14[b]
5.5 mmol/l	0.40 ± 0.09[c]	0.88 ± 0.16[a]
8 mmol/l	1.98 ± 0.06[c, d]	2.26 ± 0.89
16.5 mmol/l	3.60 ± 0.08[c, e, f]	1.90 ± 0.60[a]

Values are means ± SEM. Number of experiments in parentheses. [a] $p < 0.05$, [b] $p < 0.01$: glucose-infused rats vs control rats; [c] $p < 0.01$: 5.5, 8 mmol/l and 16.5 mmol/l glucose vs 2.8 mmol/l glucose; [d] $p < 0.05$, [e] $p < 0.001$: 8 mmol/l and 16.5 mmol/l glucose vs 5.5 mmol/l glucose; [f] $p < 0.001$: 8 mmol/l glucose vs 16.5 mmol/l glucose

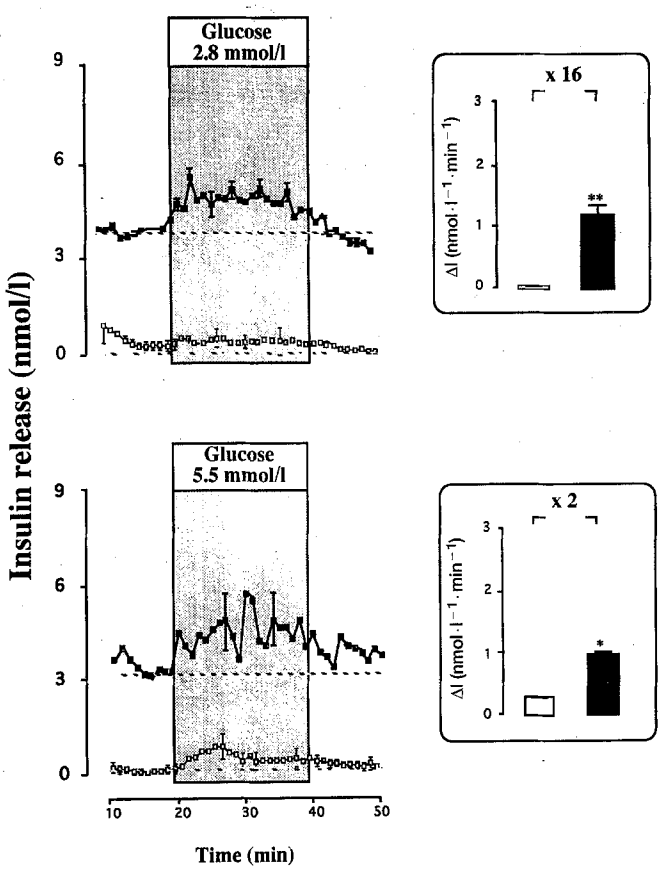

Fig. 4. Effect of 2.8 and 5.5 mmol/l glucose on in vitro insulin release and incremental insulin concentration in the effluent above baseline integrated over a period of 20 min (ΔI) in control rats (□) and glucose-infused rats (■), 3 h after the end of glucose infusion. Baseline was calculated as the insulin released during the final 10 min of the pre-stimulation period in the medium containing 2.8 mmol/l glucose when glucose 5.5 mmol/l perfused previously or in a glucose free medium when the response to glucose 2.8 mmol/l was studied. Values are means ± SEM of seven control rats and glucose-infused rats. * $p < 0.05$, ** $p < 0.01$, significantly different from controls

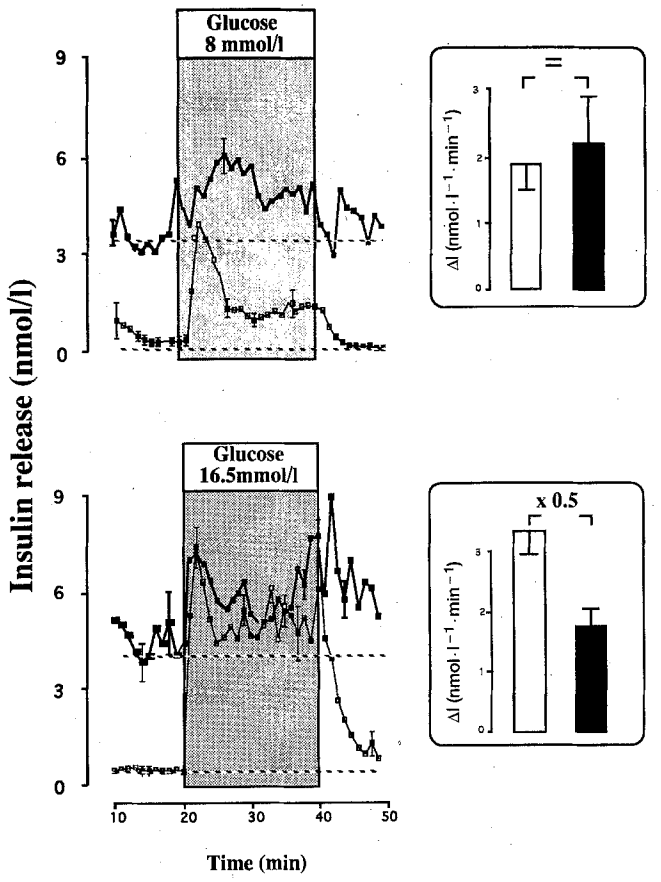

Fig. 5. Effect of 8 and 16.5 mmol/l glucose on in vitro insulin release and incremental insulin concentration in the effluent above baseline integrated over a period of 20 min (ΔI) in control rats (□) and glucose-infused rats (■), 3 h after the end of glucose infusion. Baseline was calculated as the insulin released during the final 10 min of the pre-stimulation period in the medium containing 2.8 mmol/l glucose. Values are means ± SEM of seven control rats and glucose-infused rats

which, as expected, induced no insulin response in control rats had a potent insulin secretory effect in HG rats. Moreover, glucose induced insulin secretion to submaximal glucose levels (11 mmol/l glucose clamps) remained higher in HG rats than in controls. In a previous study [9], we investigated in vivo insulin secretion in hyperglycaemic rats by performing IVGTTs. During these tests, plasma glucose levels peaked at only 7 mmol/l, a non-stimulatory concentration for insulin secretion. Nevertheless, the plasma insulin concentration dramatically increased, thus suggesting increased islet sensitivity to glucose; however, this increase was transient. The present data strengthen this conclusion and provide further information, i. e. when glucose concentration is clamped at a low value, a sustained and stable insulin response can be obtained with priming hyperglycaemia. However, the potentiating effect of previous hyperglycaemia on insulin secretion was no longer observed when high glucose concentrations were used: the highest insulin secretion, which was obtained with 16.5 mmol/l glucose in control rats, was similar in both groups.

Altogether these observations suggest that the potentiating effect of a 48-h hyperglycaemic period on in vivo insulin secretion in normal rats is characterized by an increase in sensitivity to glucose demonstrated by a high insulin response to low glucose.

These results are to be compared with those obtained in healthy human subjects. When hyperglycaemia was induced for 20 h in non-diabetic humans by a continuous glucose infusion, it resulted in an increase in the slope of glucose potentiation of arginine [15]. The authors of this study interpreted these data as enhanced beta-cell sensitivity to glucose. More direct evidence of improved beta-cell function by priming hyperglycaemia was recently provided by Kahn et al. [3] who showed that a 24-h glucose infusion, resulting in both hyperglycaemia and hyperinsulinemia, led to an increase in the insulin response, as assessed by IVGTT. In non-diabetic elderly subjects, insulin secretion was also primed by previous mild and discontinuous hyperglycaemia induced by a 3-day high-carbohydrate diet [4]. Therefore, it seems also unlikely that short-term exposure of beta cells to high glucose levels could

provoke in vivo severe islet dysfunction in healthy human subjects.

In many respects, the data obtained in vitro with the isolated pancreas technique are similar to those obtained in vivo. Very low concentrations of glucose, which were ineffective (2.8 mmol/l) or poorly effective (5.5 mmol/l) in eliciting a significant increase in insulin release in control rats, induced an impressive response in HG rats; the highest response being obtained with the lowest glucose concentration. However, when stimulatory concentrations of glucose were used, glucose-induced insulin release in HG rats, as judged by the ΔI, was not higher (8 mmol/l) and was even lower (16.5 mmol/l) than in controls. These data confirm the results obtained in vivo: priming hyperglycaemia provokes a high insulin response of the beta cell to low glucose. However, the incremental insulin response was less important in HG rats than in controls in response to high glucose concentrations (16.5 mmol/l). This is in agreement with the data of Timmers et al. [16] who showed a leftward shift of the glucose dose-response curve and decreased maximum response to high glucose in isolated islets from rats submitted to a 48-h glucose infusion. In previous studies [9, 11, 13, 17], showing in vitro beta-cell dysfunction, insulin release by the isolated perfused pancreas was always evaluated in response to high glucose concentrations (16.5 mmol/l) which can explain the apparent lack of effect of glucose on in vitro insulin secretion in previously hyperglycaemic rats.

The present data cannot entirely explain the differential effect of a 48-h hyperglycaemic period on the subsequent in vivo and in vitro insulin secretion that we and others reported previously [9, 18–20]. In particular, the in vivo insulin response to a low glucose concentration (8 mmol/l) was much higher in HG rats than in controls whereas this difference disappeared in vitro. Moreover, in vivo, ΔI was as high in HG rats as in controls in response to 16.5 mmol/l glucose whereas, in vitro, it was lower in the former than in the latter group. Extrapancreatic factors, especially those involved in the central nervous system control of insulin secretion, which are probably modified by hyperglycaemia, can interfere in vivo with hyperglycaemia to modulate insulin secretion. Interestingly, Styrud et al. [21] recently showed that a continuous 48-h glucose infusion in rats caused a persistent redistribution of the blood flow within the pancreas, a fraction of the pancreatic blood flow being diverted through the islets. Glucose specifically increases pancreatic islet blood flow [22] and this effect is, at least partly, under the control of the parasympathetic nervous system [23]. A modification of the pancreatic islet blood flow could, therefore, be one of the links between prolonged hyperglycaemia, alteration of nervous control of insulin secretion and increased glucose-induced insulin release in HG rats. To support this hypothesis, in the obese fa/fa Zucker rat, both in the basal state and under glucose stimulation, the pancreatic islet blood flow is much higher than in control rats and this high blood flow is involved in the basal hyperinsulinaemia and in the elevated insulin response to glucose observed in these rats. Vagotomy resulted both in decreased pancreatic islet blood flow and in in vivo insulin secretion [24].

We cannot exclude that the beta-cell response to low glucose seen in HG rats, rather than being an indication of improved beta-cell function, could be the initial step in a cascade of events leading to beta-cell unresponsiveness. In the study of Timmers et al. [16], the initial in vitro hypersensitivity to glucose of isolated islets from hyperglycaemic non-diabetic rats, was followed by an impairment of insulin release. Hansen and Bodkin [25] observed that during development of Type 2 diabetes induced in the Rhesus monkey, the first phase of the alteration of pancreatic islet function was an increase in the insulin response to glucose, whereas glucose homeostasis remained normal. A similar situation is seen in spontaneous models of Type 2 diabetes. Particularly in the db/db mouse, there is hyperinsulinaemia and increased glucose-induced insulin secretion both in vivo and in vitro before detectable impairment of glucose homeostasis. Afterwards, plasma insulin decreases to reach values lower than normal and glucose-stimulated insulin release is profoundly impaired. Concomitantly, diabetes develops [26].

In conclusion, our data suggest that a 48-h hyperglycaemic period results in a high response of pancreatic beta cells to low glucose. The response is immediately maximal and cannot be increased with higher glucose concentrations. This situation could explain the apparent lack of effect of high concentrations on in vitro insulin secretion in previously hyperglycaemic rats and may provide insights into the sequence of events leading to the impairment of beta-cell function in Type 2 diabetes.

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