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Regulation of energy metabolism in pancreatic islets by glucose and tolbutamide

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Summary. The kinetics of insulin secretion and oxygen uptake in response to D-glucose and tolbutamide were compared in mouse pancreatic islets. In addition, the role of decreased ATP as a driving force for secretagogue-induced oxygen consumption was examined. D-glucose (10-30 mmol/l) triggered a biphasic insulin release which always coincided with a monophasic increase in islet oxygen uptake. In the presence of D-glucose (5-30 mmol/l), tolbutamide (3-500 μmol/l) consistently elicited an initial peak of insulin secretion which was followed by a continued decline. Tolbutamide-induced secretory profiles were accompanied by similar respiratory profiles. Oxygen consumption per ng of insulin released during the test phase was higher after elevation of the glucose concentration than after addition of tolbutamide. In conjunction with 5 or 10 mmol/l D-glucose, but not with 15 or 30 mmol/l D-glucose, tolbutamide (30-100 μmol/l) lowered islet ATP content significantly ($p < 0.02$). Phosphocreatine was not found in isolated islets, although they contained substantial creatine kinase activity. It is concluded that the driving force for tolbutamide-induced oxygen uptake is a decrease in the phosphorylation potential caused by the work load imposed by stimulation of the secretion process. However, a major proportion of the respiratory response to glucose also results from enhancement of biosynthesis.

Key words: Islets of Langerhans, insulin secretion, O_2 uptake, ATP, creatine kinase, D-glucose, tolbutamide.

Glucose metabolism in the pancreatic B cell has been suggested to control both secretion and biosynthesis of insulin [1]. Evidence has emerged that β-cytotropic fuels and their analogues stimulate insulin release via a primary enhancement of hydrogen (reducing equivalents) supply to the respiratory chain of B cell mitochondria [2-4]. This implies a putative signal function of the proton motive force across the mitochondrial inner membrane and of the cytosolic phosphorylation potential (free enthalpy for ATP hydrolysis). Recently a K^+ channel which is blocked by ATP has been found in the B-cell plasma membrane and was proposed to mediate the metabolism-induced depolarization of the B cell [5-7]. However, glucose concentrations less than half maximally effective on insulin secretion are sufficient to maintain a maximal ATP content of pancreatic islets [8, 9]. Thus, attribution of a major signal function to ATP requires the proof that in B cells the total ATP content is not representative of the ATP concentration in the vicinity of the K^+ channel.

Sulfonylureas (e.g. tolbutamide) trigger insulin release by acting upon specific receptors in the B-cell plasma membrane [10], probably by direct inhibition of the same K^+ channel which is the target for ATP [11]. These drugs neither stimulate catabolic processes nor cause an increase in the content of reduced pyridine nucleotides [NAD(P)H] in pancreatic islets [12-14]. Hence, the stimulation of ATP consumption in the B cells due to the work load imposed by the secretory response to sulfonylureas is expected to lower the cytosolic ATP concentration, thereby antagonizing partly the β-cytotropic effects of nutrient secretagogues. The kinetic of sulfonylurea-induced insulin release which declines after an initial maximum perhaps reflects such an interaction [15, 16]. However, the dependence of this kinetic upon the concentrations of sulfonylureas and nutrients is not established in isolated islets. Furthermore, it is unclear whether the sulfonylurea-induced work load is strong enough to modulate insulin secretion. Firstly, tolbutamide and glibenclamide caused both stimulation and inhibition of islet oxygen (O_2) uptake in experiments requiring long incubation periods [17, 18]. Secondly, conflicting results have been published concerning the effects of sulfonylureas on islet content of ATP. No change, an increase or a decrease were reported and experimental conditions were used which may produce effects not related to insulin release [8, 12, 18-20]. Thus, it is not possible to decide whether the re-

ported decrease in ATP content of islets reflects the energy requirements of the secretion process [21].

We have therefore undertaken a detailed comparison of the effects of D-glucose and tolbutamide on insulin secretion and islet energy metabolism in order to elucidate further the role of ATP in the function of the pancreatic B cell.

Materials and methods

Chemicals and media

The following substances were used: 3,5-diaminobenzoic acid hydrochloride from Fluka, Buchs, Switzerland; crystalline rat insulin from Novo, Bagsvaerd, Denmark; ^{125}I-labelled porcine insulin from Behringwerke, Frankfurt, FRG; bovine serum albumin (fraction V) from Miles, Frankfurt, FRG; imidazole (fluorimetric grade), dithiothreitol, P-creatine, p^1, p^5-di(adenosine 5') pentaphosphate (Ap_5A, lithium salt), calf thymus DNA (type I), charcoal (hydrochloric acid washed) and glucose-6-phosphate dehydrogenase (EC 1.1.1.49; from Leuconostoc mesenteroides) from Sigma, St. Louis, Mo, USA; collagenase (type IV) from Worthington, Freehold, NJ, USA. All other enzymes, the nucleotides, D-luciferin, glutathion, Tris and triethanolamine were purchased from Boehringer, Mannheim, FRG. Tolbutamide was a gift from Hoechst AG, Frankfurt, FRG. D-Glucose and all other reagents were analytical grade from Merck, Darmstadt, FRG.

Basal medium for isolation, incubation and perifusion of pancreatic islets was prepared as previously described [22] except that for measurement of O_2 consumption it was equilibrated with $O_2/N_2/CO_2$ (12:7:1). As indicated in the Results section, in some experiments calcium was omitted from all media and 1 mmol/l ethylene glycol bis(β-aminoethyl ether)-N,N,N',N'-tetracetic acid (EGTA; NaOH to give pH 7.4) was added. Stock solutions (20 mmol/l) of tolbutamide were prepared daily by dissolving tolbutamide in NaOH (50 mmol/l) and adjusting the pH to 8.5 with HCl.

Isolation of pancreatic islets

Pancreatic islets were isolated from male albino mice (NMRI, 11–15 weeks old, fed ad libitum) by collagenase digestion [23] in basal medium supplemented with glucose (5 mmol/l).

Measurement of insulin secretion

Batches of 50 islets were perifused at 0.9 ml/min at 37 °C as detailed previously [24]. At zero time (after perifusion for 44 min with control medium containing 0, 5, 10, 15 or 30 mmol/l glucose) the distribution valve of the system was switched to control or test medium. No correction for the dead space between valve and islets (about 0.4 ml) was made. Insulin content of 1–4 min fractions was determined by radioimmunosassay with rat insulin as reference [25].

Measurement of O_2 consumption

O_2 consumption by batches of 90–150 islets was recorded at 37 °C as described previously [26], except that the response time of the measuring system was 15–30 s for 99% change. After incubation for 45 min with control medium (0, 5, 10, 15 or 30 mmol/l D-glucose) the same medium with or without test substance (to give the final glucose or tolbutamide concentrations in the incubation chamber) was injected into the chamber (zero time). O_2 consumption by the islets was calculated from the decrease in the pO_2 during 1–4 min intervals and from the amount of medium in the closed incubation chamber, with corrections for atmospheric pressure, water vapour and drift, and with Bunsen's solubility coefficient of 0.0227 ml/ml (at 37 °C). The DNA content of the islets was measured as described [26]. In control experiments insulin secretion was measured by removing 10 µl aliquots from the medium. In the presence of 10 or 15 mmol/l D-glucose, tolbutamide (100 µmol/l) enhanced insulin secretion with an initial overshoot coinciding with the O_2 uptake peaks in separate experiments (Fig. 5).

Measurement of ATP and P-creatine content

Batches of 30 islets were preincubated (45 min) and incubated (15 min) at 37 °C in 100 µl of medium as described previously [22]. After preincubation, 90 µl of medium were removed and replaced by 90 µl of control or test medium. Incubations were stopped by removing rapidly 90 µl of medium, pipetting 90 µl of ice-chilled NaOH (0.1 mol/l) supplemented with EDTA (2 mmol/l) into the incubation tube, sonicating the stoppered tube in an ice bath by pressing it for 1 min against the 12 mm tip of a sonifier (Branson type B15P, pulsed mode with 40% duty cycle) and heating the tube for 10 min at 60 °C. After neutralization with 45 µl of Hepes (0.4 mol/l) supplemented with EDTA (2 mmol/l) and Ap_5A (20 µmol/l), ATP and P-creatine were measured according to Lust et al. [27] with minor modifications. Forty µl-aliquots were mixed with 20 µl of reaction mixture (50 mmol/l imidazole/HCl, pH 7.0, 15 mmol/l $MgCl_2$, 0.2 mg/ml albumin, 0.2 mmol/l ADP, 24 U/ml creatine kinase) or of the same mixture without ADP and creatine kinase. After 30 min at room temperature, 25 µl-aliquots were mixed with 100 µl of luciferase reagent (56 mmol/l Tris/acetate, pH 7.7, 1.5 mmol/l EDTA, 6 mmol/l dithiothreitol, 20 mmol/l magnesium acetate, 0.8 mg/ml albumin, 70 µmol/l luciferin, 0.6 µg/ml-firefly luciferase) and the luminescence was monitored with a Biocounter M2010 (Lumac, Meise, Belgium). Blanks, ATP standards and P-creatine standards were prepared with medium and run through the entire procedure together with samples. The DNA content of the islets was measured in 40 µl-aliquots of the neutralized samples using DNA standards in adequate volumes of mixtures of the different media with the neutralized quenching solution [26].

Measurement of creatine kinase

Pancreatic islets, total pancreas or brain from albino mice were homogenized in 10 mmol/l Tris/HCl, pH 7.0 at 0 °C immediately after isolation. 10 µl of the homogenate (corresponding to 1–10 µg of tissue protein) were added to 1 ml of reaction mixture (100 mmol/l triethanolamine/HCl, pH 7.0, 10 mmol/l $MgCl_2$, 20 mmol/l D-glucose, 10 mmol/l glutathion, 1 mmol/l ADP, 10 mmol/l AMP, 0.5 mmol/l NAD^+, 0.5 U/ml glucose 6-phosphate dehydrogenase, 0.5 U/ml hexokinase). After 5 min at 37 °C in the photometer, the reaction was started with 100 µl of P-creatine (350 mmol/l) and extinction at 340 nm was recorded after the lag phase. Recordings without P-creatine were used as parallel blanks. The protein content of the homogenates was determined as described [28].

Statistical analysis

Results are presented as mean ± SEM for independent experiments. Significances were calculated by the two-tailed matched-pairs signed rank test of Wilcoxon or by the two-tailed U-test of Wilcoxon and of Mann and Whitney. $p < 0.05$ was considered significant. Increase in insulin release or O_2 uptake was calculated by subtracting the last value before zero time. For calculation of the ratio of the increase in O_2 uptake to the increase in insulin release, the increase in O_2 uptake of tests was corrected by subtracting the corresponding increase of controls.

Results

In mouse islets perifused for 44 min with 5, 10 or 15 mmol/l D-glucose, elevation of the medium glucose concentration to 10, 15 or 30 mmol/l, respectively,

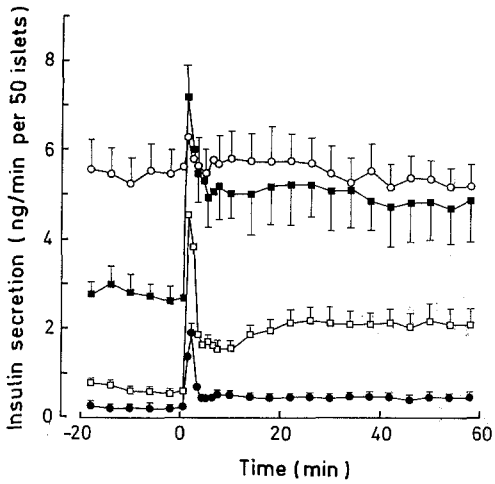

Fig. 1. Effects of glucose concentration on the kinetic of insulin secretion by mouse pancreatic islets. At zero time the D-glucose concentration of the perifusion medium was changed from 5 to 10 mmol/l (●), from 10 to 15 mmol/l (□), from 15 to 30 mmol/l (■) or from 30 to 40 mmol/l (○). Values in the curves are means ± SEM of results from 6 separate experiments and are drawn in the middle of the sampling intervals. For the sake of clarity SEM of some means are not shown. In each separate experiment of the series elevation of the glucose concentration to 10, 15 or 30 mmol/l enhanced insulin secretion from 1.5 min to 58 min as compared to the rate at −2 min

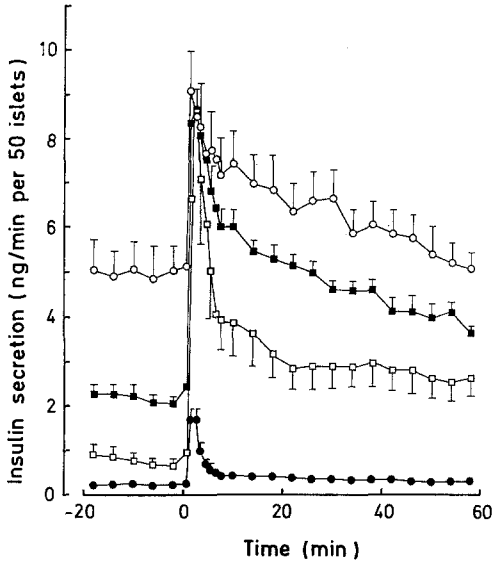

Fig. 3. Effects of glucose concentration on the kinetic of tolbutamide-induced insulin secretion by mouse pancreatic islets. Control and test medium contained 5 mmol/l (●), 10 mmol/l (□), 15 mmol/l (■) or 30 mmol/l (○) D-glucose. From zero time to 60 min all perifusion media contained 100 μmol/l tolbutamide. Values in the curves are means ± SEM of results from 6 separate experiments. In each separate experiment of the series tolbutamide enhanced insulin secretion in the presence of 5, 10, 15 or 30 mmol/l D-glucose from 1.5 min to 30, 58, 58 or 30 min, respectively, as compared to the rate at −2 min. Further details are the same as described in the legend to Figure 1

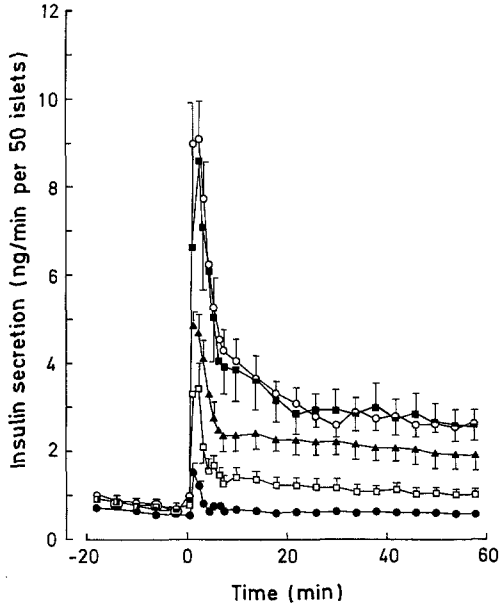

Fig. 2. Effects of tolbutamide concentration on the kinetic of insulin secretion by mouse pancreatic islets. All control and test media contained 10 mmol/l D-glucose. From zero time to 60 min the perifusion medium contained 3 μmol (●), 10 μmol/l (□), 30 μmol/l (▲), 100 μmol/l (■) or 500 μmol/l (○) tolbutamide. Values in the curves are means ± SEM of results from 6 separate experiments. In each separate experiment of the series 3 μmol/l or 10 to 500 μmol/l tolbutamide enhanced insulin secretion from 1.5 min to 6.5 or 58 min, respectively, as compared to the rate at −2 min. For further details see Figure 2

caused an increase in insulin release which was maximal at 1.5–2.5 min in each single experiment and reached a new steady state not later than at 24 min (Fig. 1). Transition from 30 to 40 mmol/l D-glucose did not change insulin secretion significantly, indicating that 30 mmol/l was already maximally effective (Fig. 1). Control studies demonstrated that during perifusion without change of glucose concentration at zero time, insulin secretion did not increase in the presence of 5, 10 or 15 mmol/l D-glucose from zero time up to 58 min (results not shown).

When islets were perifused with a slightly effective glucose concentration (10 mmol/l), tolbutamide (3, 10, 30, 100 or 500 μmol/l) stimulated a steep increase in insulin release (Fig. 2) culminating at 1.5–3.5 min and falling to less than 60% of the peak rate at 10 min in each single experiment. Subsequently insulin secretion decreased more slowly, but at all tolbutamide concentrations the secretory rate at 58 min was significantly ($p < 0.05$) lower than that at 10 min. The half maximally effective concentration of tolbutamide ranged between 10 μmol/l and 30 μmol/l (Fig. 2). In each single experiment testing the effect of glucose concentration (5, 10, 15 or 30 mmol/l) upon insulin release elicited by 100 μmol/l tolbutamide, rate of insulin secretion at 10 min was lower than that at 1.5–2.5 min and higher than that at 58 min (Fig. 3). In the absence of glucose,

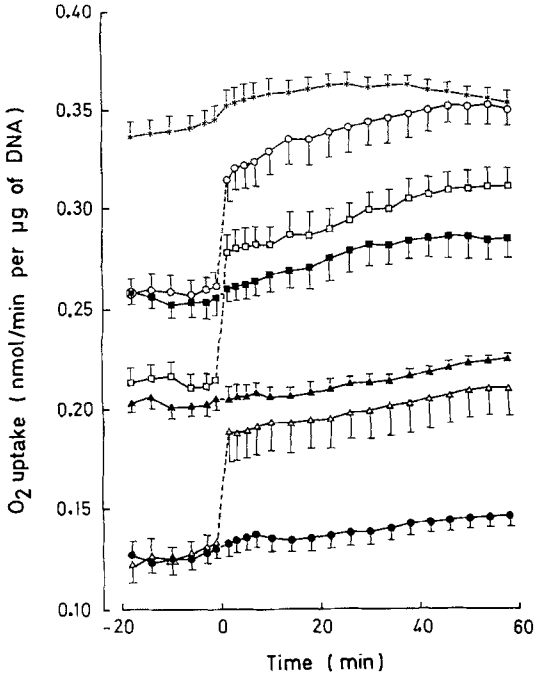

Fig. 4. Effects of glucose concentration on the kinetic of O_2 uptake by mouse pancreatic islets. At zero time the D-glucose concentration of the incubation medium was changed from 5 to 10 mmol/l (△), from 10 to 15 mmol/l (□) or from 15 to 30 mmol/l (○) or the glucose concentration of the incubation medium was kept constant by injection of control medium (5 mmol/l, ●; 10 mmol/l, ▲; 15 mmol/l, ■; 30 mmol/l, ✶). Values in the curves are means ± SEM of results from 6 to 9 separate experiments and are drawn in the middle of the sampling intervals. For the sake of clarity SEM of some means are not shown. The mean respiratory rate between 1.5 min and 58 min in profiles △, □ or ○ was significantly higher ($p < 0.02$, U-test) than this rate in the corresponding control profiles ●, ▲ or ■. The broken lines in the O_2 uptake profiles span the interval during which O_2 uptake could not be determined because injections caused pO_2 jumps

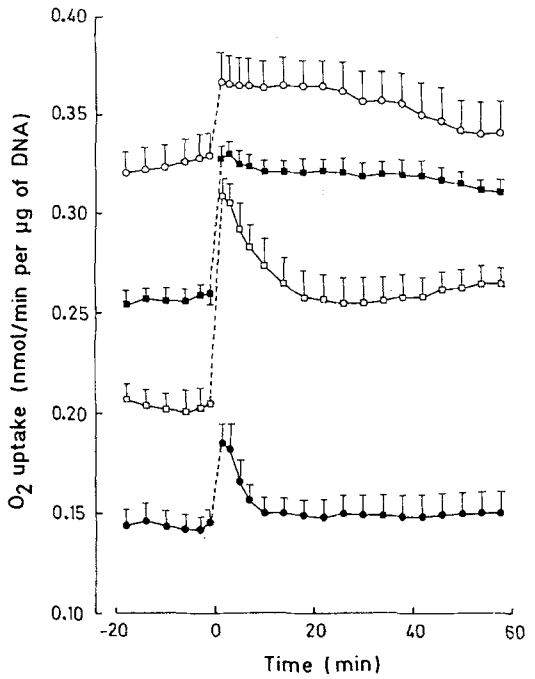

Fig. 5. Effects of glucose concentration on the kinetic of tolbutamide-induced O_2 uptake by mouse pancreatic islets. During control (-20 to 0 min) and test phase the D-glucose concentration of the incubation medium was 5 mmol/l (●), 10 mmol/l (□), 15 mmol/l (■) or 30 mmol/l (○). From zero time to 60 min all incubation media contained 100 µmol/l tolbutamide. Values in the curves are means ± SEM of results from 6 or 7 separate experiments. By subtracting the O_2 uptake rate at -2 min from the mean O_2 uptake rate between 1.5 and 58 min, the mean respiratory increase was calculated in each separate experiment. The mean respiratory increase of profiles □, ■ or ○ was significantly higher ($p < 0.05$, U-test) than the mean respiratory increase of the corresponding controls ▲, ■ or ✶ in Figure 4. Further details are the same as described in the legend to Figure 4

100 µmol/l tolbutamide was ineffective and 500 µmol/l tolbutamide caused merely an initial peak of insulin release (peak rate amounting to 0.90 ± 0.12 ng · min^{-1} per 50 islets, $n = 6$, secretory profile not shown). In the absence of extracellular Ca^{2+} ions, there was no secretory response to 100 µmol/l tolbutamide (in the presence of 10 mmol/l D-glucose, $n = 6$, results not shown).

Changing the medium glucose concentration from 5 to 10 mmol/l, from 10 to 15 mmol/l or from 15 to 30 mmol/l enhanced islet O_2 consumption by 42, 30 or 20%, respectively, within 2 min (in series △, □ and ○, $p < 0.05$ for comparison with the last value before zero time, Fig. 4). Then the respiratory rate increased gradually and an initial overshoot was never observed. In control experiments the rate of O_2 uptake rose slowly up to 58 min (except in profile ✶). In each single experiment of the series ●, △, ▲, □, ■ and ○ (Fig. 4), O_2 consumption at 10 min was lower than at 58 min. From 5 min up to 58 min the ratio of the increase in O_2 uptake to the increase in insulin release ranged above 170, 44 or 20 pmol of O_2/ng of insulin after transition from 5 to 10 mmol/l, from 10 to 15 mmol/l or from 15 to 30 mmol/l glucose respectively. The ratios were calcu-

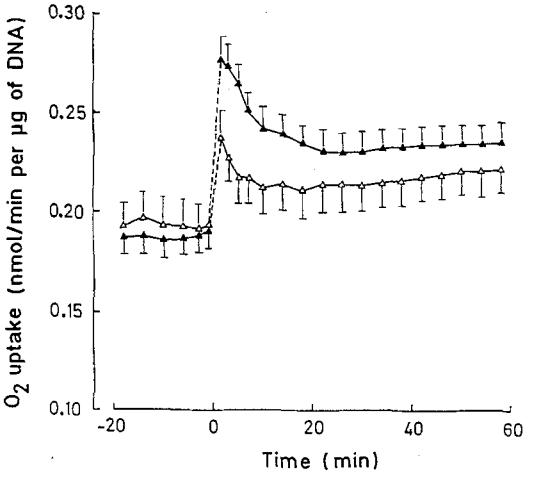

Fig. 6. Effects of tolbutamide concentration on the kinetic of O_2 uptake by mouse pancreatic islets. During control (-20 to 0 min) and test phase the D-glucose concentration of the incubation medium was 10 mmol/l. From zero time to 60 min the incubation medium contained 10 µmol/l (△) or 30 µmol/l (▲) tolbutamide. Values in the curves are means ± SEM of results from 5 or 6 separate experiments. The mean respiratory increase of both profiles was significantly higher ($p < 0.05$, U-test) than the mean respiratory increase of the corresponding control ▲ in Figure 4. Further details are the same as described in the legend to Figure 5

Table 1. Effects of glucose and tolbutamide on ATP content of pancreatic islets

	Glucose concentration (mmol/l)	Tolbutamide concentration (μmol/l)	*n*	ATP content (pmol/μg of DNA)	*p*
Control	5	0	13	220±21	
Test	5	100	13	193±20	<0.001
Control[a]	5	0	17	234±9	
Test[a]	5	100	17	227±8	NS
Control	10	0	12	239±10	
Test	10	10	12	234±17	NS
Control	10	0	12	250±16	
Test	10	30	12	217±13	<0.02
Control	10	0	19	240±10	
Test	10	100	19	218±8	<0.005
Control[a]	10	0	23	236±6	
Test[a]	10	100	23	244±7	NS
Control	15	0	11	245±12	
Test	15	100	11	241±10	NS
Control	30	0	11	237±20	
Test	30	100	11	235±16	NS

Preincubation and incubation (15 min) of the islets were performed in the presence of the indicated glucose concentrations. Each experimental series consisted of simultaneous incubations in the absence (control) or presence (test) of tolbutamide and in the presence of the same glucose concentrations. Values shown are means ± SEM for results from 11 to 23 separate experiments. Comparison of control and test was made by Wilcoxon's paired test. [a] During preincubation and incubation the media contained no calcium and were supplemented with 1 mmol/l EGTA

lated from the experiments of Figures 1 and 4 and from a DNA content of 20 ng/islet (average islet DNA content of the experimental series).

In the presence of 5, 10, 15 or 30 mmol/l D-glucose, a high concentration of tolbutamide (100 μmol/l) stimulated O_2 uptake in islets by 28, 51, 26 or 12%, respectively, within 2 min (in each series, $p < 0.05$ for comparison with the last value before zero time) and afterwards the increase in respiration fell rapidly at 5 or 10 mmol/l D-glucose and slowly at 15 or 30 mmol/l D-glucose (Fig. 5). Low (10 μmol/l) or intermediate (30 μmol/l) concentrations of tolbutamide, too, enhanced O_2 uptake with a maximum at 1.5 to 3 min in each single experiment (Fig. 6). In the presence of 10 or 15 mmol/l D-glucose, the tolbutamide-induced increase in O_2 uptake was still significant at 58 min ($p < 0.05$ for comparison of series □, ■ or ▲ in Figures 5 and 6 with the corresponding control series ▲ or ■ in Figure 4). In each single experiment of series ●, □, ■, △ or ▲ in Figure 5 and 6 the peak rate was higher than the rate at 10 min. Taking into account the gradual increase in O_2 consumption which was observed in control experiments (series ●, ▲, and ■ in Figure 4) and which may reflect augmentation of synthesizing reactions, the respiratory responses to tolbutamide decreased continuously up to 58 min in each series in Figures 5 and 6. The correlation between tolbutamide-induced secretory and respiratory responses is also documented by the ratios of the increase in O_2 uptake to the increase in insulin release which are calculated from the experiments of Figures 2-6 and from a DNA content of 20 ng/islet. This ratio ranged between 14 and 22 or between 10 and 14 pmol of O_2/ng of insulin from 1.5 to 58 min in the presence of tolbutamide (100 μmol/l) and 10 or 15 mmol/l D-glucose respectively. Except for two values, all other ratios ranged below 34 pmol of O_2/ng of insulin. In the absence of glucose, 500 μmol/l tolbutamide enhanced islet O_2 uptake for 12 min. The peak rate was higher by 18±3% than the last value before zero time ($n = 5$, respiratory profiles not shown). In contrast to experiments in the presence of extracellular Ca^{2+} ions [26], stirring caused some disintegration of the islets in Ca^{2+}-free media. In the absence of Ca^{2+} ions, there was no respiratory response to 100 μmol/l tolbutamide (in the presence of 10 mmol/l D-glucose, $n = 3$).

Since changes in the redox state of pyridine nucleotides probably were not the driving force for the O_2 consumption initiated by tolbutamide [12-14] and since previous results were equivocal [8, 12, 18-20], we examined the effects of tolbutamide on the ATP content of pancreatic islets. In conjunction with 5 or 10 mmol/l D-glucose, tolbutamide (30 or 100 μmol/l) lowered islet ATP content significantly (Table 1, $p < 0.02$). These effects were prevented in Ca^{2+}-free media. At higher glucose concentrations there was only a trend of lower ATP levels in the presence of tolbutamide (Table 1). P-creatine was not found in islets incubated at 10 mmol/l D-glucose ($n = 10$; the detection limit was 15 pmol/μg of DNA). However, substantial creatine kinase activity was measured in mouse islets (0.47±0.05 μmol·min^{-1}·mg^{-1} of protein, $n = 7$) ranging between the activities in total pancreas (0.17±0.03 μmol·min^{-1}·mg^{-1} of protein, $n = 3$) and brain (3.46±0.45 μmol·min^{-1}·mg^{-1} of protein, $n = 5$).

Discussion

Our study confirms that an increase in islet O_2 consumption starts within a few minutes after elevation of the medium glucose concentration [29, 30]. The initial respiratory responses in the present results were stronger than those observed in the experimental set-up of Hutton and Malaisse [30]. However, the equilibration kinetic in their system was rather slow [30], as indicated by a comparison with another perifusion system of these authors [31]. In the present study glucose-induced initial secretory maxima were never paralleled by O_2 uptake peaks. Similar dissociations between insulin release and O_2 consumption occurred in mouse islets exposed to endo-2-aminobicyclo [2.2.1]-heptane-2-carboxylic acid (BCH) [2]. These differences between the secretory and respiratory profiles presumably resulted from the additional work load imposed by fuel-induced synthesizing processes. This view is supported by the

finding that omission of calcium from the medium had only small effects on O_2 uptake in the presence of nutrient secretagogues [30]. In this situation the release but not the biosynthesis of insulin was inhibited [32]. Furthermore, O_2 consumption per ng of insulin released from min 5 to min 60 of the test phase was higher after elevation of the glucose concentration than after addition of tolbutamide, which did not stimulate insulin synthesis [10]. For instance, after transitions from 10 mmol/l D-glucose to 10 mmol/l D-glucose + tolbutamide (30 μmol/l) or to 15 mmol/l D-glucose, the ratios of the increase in O_2 uptake to the increase in insulin release were 20 or 48 pmol of O_2/ng of insulin, respectively, at min 30 (Figs. 1, 2, 4 and 6). This means that a major proportion of the respiratory response to glucose was not caused by stimulation of the secretory process.

The present finding that only a slight increase in islet O_2 uptake was initiated by tolbutamide in conjunction with 5 mmol/l glucose is in accordance with results obtained by the Cartesian diver technique [17]. However, we found increased respiration in response to tolbutamide in the presence of 15 mmol/l glucose, whereas Stork et al. [17] observed an inhibitory effect of tolbutamide in the presence of 16.7 mmol/l glucose. This discrepancy may be due to more efficient exchange of gas, ions, glucose and secretory products between medium and islets in the present experiments since smaller islets were used and since the medium was stirred [26, 33]. Moreover, in the Cartesian diver islet respiration was impaired by the absence of medium bicarbonate [30].

The correlation between tolbutamide-induced secretory and respiratory profiles in islets and the failure of sulfonylureas to enhance insulin synthesis [10] favour the view that tolbutamide evoked O_2 uptake by the work load imposed by stimulation of insulin secretion. Respiration is driven by the disequilibrium between the overall reduction potential span across the respiratory chain and the cytosolic phosphorylation potential [34]. Tolbutamide apparently does not enhance the availability of reducing equivalents in the B-cell mitochondria [12–14, 21]. Hence, a decrease in the cytosolic phosphorylation potential remains as the driving force for tolbutamide-induced O_2 consumption. This decrease was probably reflected in the diminished ATP content of islets incubated at 5 or 10 mmol/l glucose and at the same tolbutamide concentrations which enhanced islet O_2 uptake. This interpretation is supported by inhibition of the secretory, respiratory and ATP-lowering effects of tolbutamide in the absence of extracellular Ca^{2+} ions. In the presence of high glucose concentrations (15 or 30 mmol/l) the expected tolbutamide-induced decrease in islet content of ATP coinciding with the increase in O_2 consumption was not detected. This was not due to buffering by the P-creatine/creatine system, because P-creatine was not found in the mouse islets we used in the present study. It is therefore suggested that the total ATP content of islets does not reflect the ATP concentration in cytosolic microenvironments in which major changes in ATP utilization of B cells take place. Substantial microheterogeneity in ATP concentration probably occurs in the cytosol of liver cells [35].

It has been reported previously that sulfonylureas lowered islet content of ATP not only at low (3.3 or 5.6 mmol/l; [12, 19]) but also at high (16.7 mmol/l; [12]) glucose concentrations. The latter discrepancy to our findings may result from the high tolbutamide concentrations (0.74 mmol/l) used, which may have caused additional alterations in B-cell energy metabolism not related to insulin secretion. In the present study tolbutamide in conjunction with 10 mmol/l glucose was half maximally effective on insulin release at a concentration (around 20 μmol/l) in the range of therapeutic plasma concentrations of free tolbutamide [36, 37].

It has been suggested that the typical decline of the insulin secretory rate during stimulation with tolbutamide was due to slow inactivation of voltage-dependent calcium channels caused by depolarization [16]. However, this hypothesis does not explain why glucose-induced depolarization is accompanied by biphasic insulin release. It is therefore conceivable that the typical decline of the secretory response to tolbutamide reflects decrease in the ATP concentration in the vicinity of the ATP-regulated K^+ channel. Yet, it is premature to attribute the insulin releasing effects of tolbutamide and glucose solely to a blockade of this K^+ channel. The P-creatine content of islets in vivo [38] and the creatine kinase activity in islets indicate that islet cells may lose creatine during the isolation procedure and that the P-creatine/creatine system could buffer the concentration of cytosolic ATP in vivo. Furthermore, a secretory response to tolbutamide was even observed at a maximally effective glucose concentration which is believed to cause permanent depolarization of B cells [39]. This finding means either that not all B cells are permanently depolarized or that decreasing the membrane potential of B cells is not the sole mechanism by which sulfonylureas elicit insulin release.

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