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Originals

Different effects of glucose and glyburide on insulin secretion in rat pancreatic islets pre-exposed to interleukin-1β. Possible involvement of K^+ and Ca^{2+} channels

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Summary. In vitro islet exposure to interleukin 1β inhibits the beta-cell response to glucose. We have studied whether a similar inhibition also occurs in response to the sulphonylurea glyburide. Rat pancreatic islets were cultured for 24 h in the presence or absence of 50 U/ml interleukin 1β and then stimulated with either glucose or glyburide for 1 h at 37 °C. In control islets basal insulin secretion was 117 ± 32 pg · islet^{-1} · h^{-1} (mean ± SEM, $n = 7$) and greatly increased in response to 16.7 mmol/l glucose (2140 ± 293) or 10 μmol/l glyburide (1464 ± 234). When islets were pre-exposed to interleukin 1β, insulin release was significantly reduced in response to glucose (323 ± 80, $p < 0.001$) but not in response to glyburide (1316 ± 185). Since both glucose and glyburide influence beta-cell K^+ and Ca^{2+} efflux, to further investigate this different response in islets exposed to interleukin 1β we measured both Rb^+ efflux (as index of the ATP-sensitive K^+ channel activity) and Ca^{2+} uptake. In control islets, the increased insulin secretion in response to 16.7 mmol/l glucose or 10 μmol/l glyburide was associated with a reduction of ^{86}Rb efflux (decrement of −50 ± 1.2 % and −49 ± 2.3 %, respectively, mean ± SEM, $n = 5$). In contrast, in interleukin 1β pre-exposed islets both glucose and glyburide stimulation only slightly modified ^{86}Rb efflux (decrement of −19 ± 1.9 % and −5.3 ± 3.1 %, respectively, $n = 5$, $p < 0.001$). $^{45}Ca^{2+}$ uptake in control islets was 2.6 ± 0.4 pmol · islet^{-1} · 20 min^{-1} under basal conditions (at 2.8 mmol/l glucose), and increased to 16.8 ± 3.2 and 10.7 ± 2.1 pmol · islet^{-1} · 20 min^{-1} in islets stimulated with 16.7 mmol/l glucose or 10 μmol/l glyburide, respectively (mean ± SEM, $n = 6$). $^{45}Ca^{2+}$ uptake in interleukin 1β treated islets was higher than in control islets under basal conditions (4.6 ± 0.6 pmol · islet^{-1} · 20 min^{-1} at 2.8 mmol/l glucose, $p < 0.05$), but was significantly reduced in response to glucose 16.7 mmol/l (7.1 ± 1.1, $p < 0.01$ with respect to control islets). In contrast to glucose, 10 μmol/l glyburide was able to stimulate calcium uptake in interleukin 1β treated islets in a similar way to control islets (12.8 ± 2.5). The present data demonstrate that rat pancreatic islets treated with interleukin 1β for 24 h lose their responsivity to glucose, but not to glyburide. The difference between the two secretagogues is associated with the persistent ability of glyburide to influence Ca^{2+} uptake even in islets with impaired K^+-channel function.

Key words: Interleukin-1β, islets, insulin secretion, ion channels, glyburide.

Type 1 (insulin-dependent) diabetes mellitus, is characterized by a chronic lymphocytic infiltration associated with a selective dysfunction and ultimately destruction of insulin-producing beta cells [1–3]. Although the mediators of the immune-induced damage to the beta cells during the course of Type 1 diabetes are still unknown, it has been proposed that interleukin-1β (IL-1β), a cytokine primarily produced by activated macrophages and released locally during the preclinical stage of Type 1 diabetes may contribute to the process leading to beta-cell dysfunction and death [4, 5]. In vitro studies have shown that a chronic exposure of pancreatic beta cells to IL-1β induces suppression of glucose-induced insulin release and, eventually, beta-cell destruction [6–11]. The IL-1β-induced functional impairment is reversible, since islets previously exposed to IL-1β resume normal function when cultured in IL-1β-free medium [9, 10, 12]. Moreover, the inhibitory effect of IL-1β on insulin release is mainly evident when beta cells are stimulated with glucose and is less evident or absent when secretagogues other than glucose (such as amino acids or agents that increase intracellular cAMP levels) are used [13].

In the present study we compared the effect of either glucose or the sulphonylurea glyburide in eliciting insulin secretion in rat pancreatic islets pre-exposed to IL-1β. We found that glucose-stimulated but not glyburide-stimulated insulin release was significantly impaired by pretreatment of islets with IL-1β. To better understand the

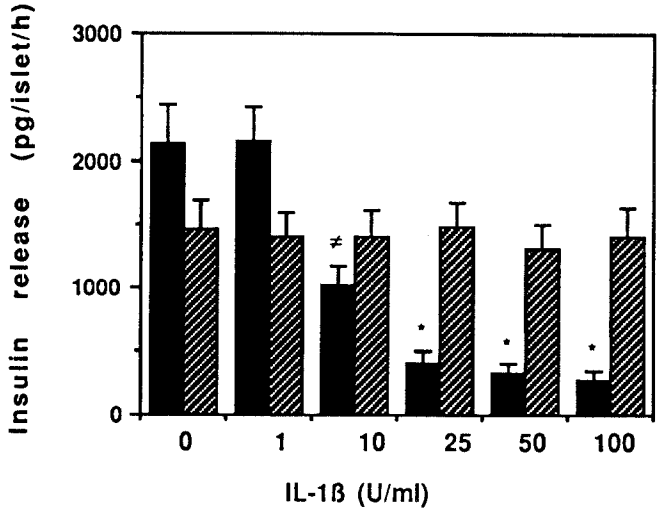

Fig. 1. Glucose (■) and glyburide (▨)-stimulated insulin release in rat pancreatic islets pre-incubated for 24 h with or without increasing concentrations of interleukin-1β (IL-1β). At the end of the 24 h pre-incubation period, islets were washed three times and incubated for 1 h at 37°C in KRHB containing either 16.7 mmol/l glucose or 10 μmol/l glyburide. Results (mean ± SEM of three separate experiments) are expressed as insulin released in the medium ($pg \cdot islet^{-1} \cdot h^{-1}$)
$^{*}p < 0.001$, $^{\neq}p < 0.05$ vs islets pre-incubated without IL-1 β

mechanism(s) of this different response, the function of both K^+ and Ca^{2+} channels was investigated since these are two important steps in recruitment and extrusion of insulin granules. We found that in IL-1β-exposed islets Rb^+ efflux (a marker of the ATP-sensitive K^+ channel activity) was always altered while Ca^{2+} uptake was impaired after glucose but not glyburide stimulation. Thus, the two secretagogues elicit different secretory responses.

Materials and methods

Materials

Crude collagenase was obtained from Boehringer Mannheim (Mannheim, FRG). Culture medium CMRL-1066, heat inactivated fetal calf serum (FCS), glutamine and gentamicin were obtained from Gibco (Glasgow, UK). Human recombinant IL-1β (specific activity 5×10^8 U/mg), rubidium (^{86}RbCl) (0.33 Ci mmol, 12.2 GBq/mmol) and $^{45}CaCl_2$ (25 mCi/mg) were from Amersham (Amersham, Bucks., UK). Glyburide was purchased from Sigma (London, UK). Silicone oil (density 1.040) was from Merck (Darmstadt, FRG). All other chemicals were of analytical grade.

Islet preparation and culture conditions

Pancreatic islets were isolated by the collagenase method from 200 to 250 g fed male Wistar rats injected i.p. with 0.2 ml of a 0.2% pilocarpine solution 2 h before death by decapitation. With this technique, 300–400 islets were isolated from each pancreas [14]. The whole procedure was completed within 120 min. Purified islets were cultured in CMRL-1066 medium (5.5 mmol/l glucose) containing 10% FCS, 2 mmol/l L-glutamine and gentamicin at 37°C in a 95% O_2/5% CO_2 atmosphere with or without IL-1β for 24 h. After this period, insulin secretion was studied both during static incubation and perifusion. K^+ channel activity was studied by measuring $^{86}Rb^+$ efflux and calcium influx by measuring $^{45}Ca^{2+}$ uptake.

Insulin secretion, calcium and $^{86}Rb^+$ uptake in static experiments

To study insulin secretion, groups of five purified islets were incubated in Krebs' Ringer Hepes buffer (KRHB, containing 115 mmol/l NaCl, 5.4 mmol/l KCl, 2.38 mmol/l $CaCl_2$, 0.8 mmol/l $MgSO_4$, 1 mmol/l Na_2HPO_4, 10 mmol/l Hepes, 0.5% bovine serum albumin, pH 7.35) containing either glucose (2.8, 8.25 or 16.7 mmol/l), or glyburide (dose-range 0.01–10 μmol/l). Glucose at 2.8 mmol/l was present in all islet groups stimulated with glyburide. Insulin in the medium and in the acid-alcohol extract of islets was measured by radioimmunoassay after 1 h incubation at 37°C. Results are expressed as insulin released in the medium ($pg \cdot islet^{-1} \cdot h^{-1}$).

$^{45}Ca^{2+}$ uptake was measured according to the method described by Henquin et al. [15]. After a 24-h pre-incubation with or without 50 U/ml IL-1β, groups of 15 islets were transferred into 50 μl Krebs'-bicarbonate buffer modified by replacing phosphate and sulphate with equimolar amounts of chloride [16] and layered on silicone oil. The uptake period was started by adding 50 μl of medium containing $^{45}Ca^{2+}$ (2.5 mmol/l) and either glucose (final concentration 2.8 or 16.7 mmol/l), or glyburide (final concentration 10 μmol/l). The reaction was stopped by centrifuging the islets for 2 min in a microfuge (Beckman Instruments, Palo Alto, Calif., USA) through the layer of silicone oil. The bottoms of the 400 μl tubes (Beckman) were then cut and the radioactivity of the pellet counted. Tubes without islets were run as blanks. The uptake of [U-^{14}C]-sucrose was measured to correct for label in the extracellular space.

Similar experiments were performed to study $^{86}Rb^+$ uptake, a marker of K^+ permeability [17]: groups of 15 islets, incubated for 24 h with or without 50 U/ml IL-1β, were transferred into 50 μl of KRHB containing 25 mmol/l Hepes and 5.5 mmol/l glucose. The uptake period was started by adding 50 μl of medium (glucose 5.5 mmol/l) containing $^{86}Rb^+$ with or without ouabain (final concentration 1.0 mmol/l). After 10 min at 37°C, the islets were separated from the incubation medium by centrifugation for 2 min through the oil layer. The islet pellet was then examined for its radioactive content by liquid scintillation.

Insulin secretion and $^{86}Rb^+$ efflux in perifusion experiments

The $^{86}Rb^+$ efflux and the insulin release kinetics were studied in a perifusion system [18]. After a 24-h pre-incubation in the presence or in the absence of IL-1β (50 U/ml), groups of 150 islets were incubated for 2 h at 37°C in CMRL-1066 medium containing 0.2 mmol/l $^{86}Rb^+$, then washed three times with fresh Rb-free medium, placed in an Endotronics chamber (Endotronics, Coon Rapids, Mn., USA) in a Biogel-P2 matrix (Biorad, Richmond, Calif., USA) and perifused at a flow rate of 1 ml/min at 37°C in KRHB. After a 10-min perifusion with the buffer containing 2.8 mmol/l glucose to equilibrate the system, either 16.7 mmol/l glucose or 10 μmol/l glyburide was added. Effluent fractions were collected at 1-min intervals and aliquots were analysed for both $^{86}Rb^+$ radioactivity and insulin content. Insulin secretion was expressed as insulin released in the medium ($pg \cdot islet^{-1} \cdot min^{-1}$) and $^{86}Rb^+$ efflux was expressed as fractional efflux of Rb^+ ($^{86}Rb^+$ released per min divided by $^{86}Rb^+$ retained in islets) [19].

Statistical analysis

The statistical analysis of results and the significance of the differences was assessed by the Student's unpaired *t*-test.

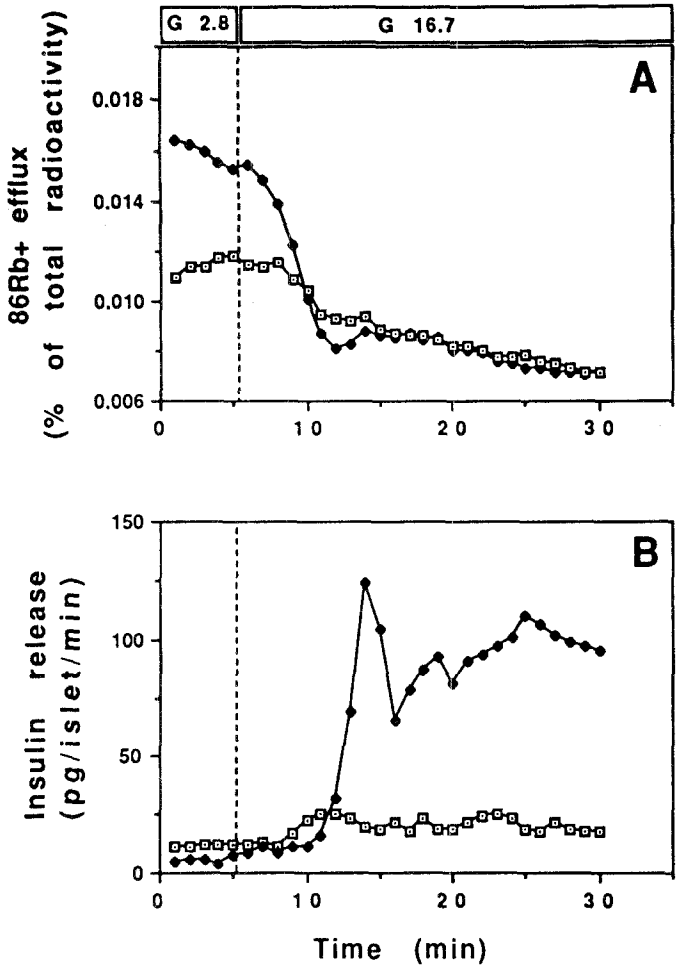

Fig. 2 A, B. $^{86}Rb^+$ efflux and glucose-induced insulin release in rat pancreatic islets pre-incubated for 24 h with (⊡) or without (◆) 50 U/ml interleukin-1β. At the end of the 24-h pre-incubation period, groups of 150 islets were incubated for 2 h in CMRL-1066 medium with 0.2 mmol/l $^{86}Rb^+$ at 37 °C. After extensive washing, islets were perifused at a flow rate of 1 ml/min at 37 °C in a $^{86}Rb^+$-free medium. After a 10-min perifusion with buffer containing 2.8 mmol/l glucose (G) to equilibrate the system, the islets were stimulated with glucose 16.7 mmol/l. Perifusate samples were collected at 1-min intervals, and 400 µl aliquots were analysed for $^{86}Rb^+$ radioactivity (**A**, upper panel) and insulin release (**B**, lower panel). A representative of five separate experiments is shown

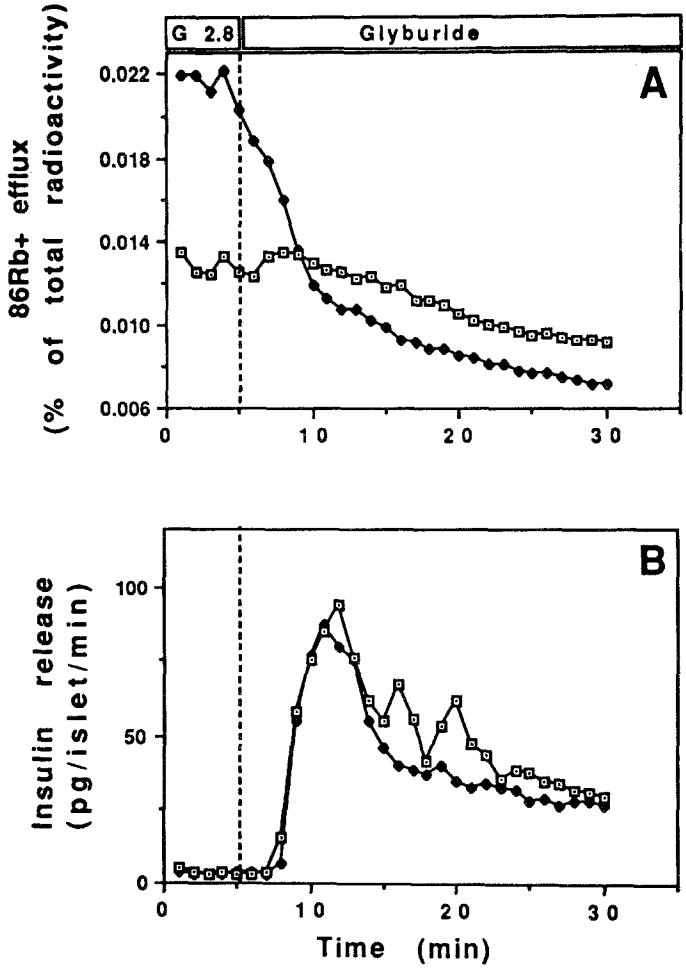

Fig. 3 A, B. $^{86}Rb^+$ efflux and glyburide-induced insulin release in rat pancreatic islets pre-incubated for 24 h with (⊡) or without (◆) 50 U/ml interleukin-1β. At the end of the 24-h pre-incubation period, groups of 150 islets were incubated for 2 h in CMRL-1066 medium with 0.2 mmol/l $^{86}Rb^+$ at 37 °C. After extensive washing, islets were perifused at a flow rate of 1 ml/min at 37 °C in a $^{86}Rb^+$-free medium. After a 10-min perifusion with buffer containing 2.8 mmol/l glucose (G) to equilibrate the system, the islets were stimulated with 10 µmol/l glyburide. Perifusate samples were collected at 1-min intervals, and 400 µl aliquots were analysed for $^{86}Rb^+$ radioactivity (**A**, upper panel) and insulin release (**B**, lower panel). A representative of five separate experiments is shown

Results

Effect of IL-1β on insulin release (static experiments)

When control pancreatic islets were cultured for 24 h and then incubated for 1 h at 37 °C in KRHB, insulin release was 117 ± 32 pg·islet^{-1}·min^{-1} in the presence of 2.8 mmol/l glucose (basal insulin release, $n = 7$), and progressively increased at 8.25 (802 ± 96, $n = 3$) and 16.7 mmol/l glucose (2140 ± 293, $n = 7$). Under the same conditions, glyburide also stimulated insulin secretion in a dose-dependent manner. Insulin release was 345 ± 96 pg·islet^{-1}·min^{-1} at 0.1 µmol/l glyburide ($n = 3$), and progressively increased at 1 (960 ± 163, $n = 3$) and 10 µmol/l (1464 ± 234, $n = 7$).

When islets were pre-exposed for 24 h to increasing IL-1β concentrations (dose-range 1–100 U/ml), basal insulin release was not significantly affected, while glucose-stimulated insulin release was progressively inhibited in a dose-dependent manner (Fig. 1). Maximal effect was reached at 50 U/ml IL-1β (323 ± 80, $p < 0.001$ in respect to control islets, Fig. 1) and therefore this cytokine concentration was used in all subsequent studies. Under these experimental conditions (pre-exposure to 50 U/ml IL-1β) islets were unresponsive to glucose concentration up to 25 mmol/l (2099 ± 312 and 358 ± 76 in control and IL-1β-exposed islets, respectively, $n = 3$, $p < 0.05$). In contrast, insulin release in response to 10 µmol/l glyburide was not affected by islet pre-exposure to IL-1β (Fig. 1).

Since both glucose and glyburide influence beta cell K^+ and Ca^{2+} efflux, two important steps in the cascade of in-

tracellular events that lead to insulin secretion, we measured both Rb^+ efflux (an index of the ATP-sensitive K^+ channel activity) and Ca^{2+} uptake in control and IL-1β-exposed islets after stimulation with either 16.7 mmol/l glucose or 10 μmol/l glyburide.

K^+ channel studies

$^{86}Rb^+$ efflux and insulin release in response to glucose

In control islets the rate of $^{86}Rb^+$ efflux declined slowly during the initial period of perifusion with low (2.8 mmol/l) glucose. A sharp and marked decrease was observed when the glucose concentration was raised to 16.7 mmol/l (Fig. 2). The average fractional $^{86}Rb^+$ efflux declined from 0.0165 ± 0.002 % (when glucose concentration was increased) to 0.0081 ± 0.001 % (at the nadir value; $n = 5$, decrement of –50 ± 1.2 %). In the same perifusion fractions insulin release increased from 6 ± 2 pg · islet^{-1} · min^{-1} (basal condition), to 124 ± 22 at the first phase peak, and 110 ± 29 at the maximum level of second phase insulin release (Fig. 2). In islets pre-exposed to 50 U/ml IL-1β the initial rate of $^{86}Rb^+$ efflux was significantly lower than in control islets (0.0115 % vs 0.0165 %, $p < 0.05$), and only a small and delayed decrease of $^{86}Rb^+$ efflux was observed when the glucose concentration was increased to 16.7 mmol/l (Fig. 2). The average fractional $^{86}Rb^+$ efflux declined from 0.0115 ± 0.001 % to 0.0092 ± 0.001 % at the time when the nadir value was observed in control islets ($n = 5$; decrement of –19 ± 1.9 %, $p < 0.001$ vs control islets). In the same perifusion fractions both first- and second-phase glucose-stimulated insulin release were markedly blunted in IL-1β-treated islets (23 ± 8 and 25 ± 11 pg · islet^{-1} · min^{-1}, respectively; $n = 5$, $p < 0.001$ vs control islets) (Fig. 2).

$^{86}Rb^+$ efflux and insulin release in response to glyburide

In control islets the addition of 10 μmol/l glyburide resulted in a rapid decrease of the $^{86}Rb^+$ efflux rate (Fig. 3). The average fractional $^{86}Rb^+$ efflux declined from 0.0210 ± 0.002 % (prior to the addition of glyburide) to 0.0108 ± 0.001 % at 12 min (the time when the peak of insulin release was reached; $n = 5$; decrement of –49 ± 2.1 %). In the same perifusion fractions insulin release increased from 4 ± 1 pg · islet^{-1} · min^{-1} under basal conditions, to 87 ± 12 at the peak of glyburide stimulation (Fig. 3). In IL-1β-treated islets the initial rate of $^{86}Rb^+$ efflux was lower than in control islets (0.013 % vs 0.021 %, $p < 0.01$) and no significant change of $^{86}Rb^+$ efflux was observed after stimulation with 10 μmol/l glyburide (0.012 ± 0.001 %, $n = 5$, decrement of –5 ± 3.1 %, $p < 0.001$ vs control islets) (Fig. 3). In contrast to what we had observed with glucose stimulation, however, in the same perifusion fractions from IL-1β-exposed islets, glyburide stimulated insulin release similar to that observed in control islets (94 ± 16 pg · islet^{-1} · min^{-1}) (Fig. 3).

Other studies on ^{86}Rb flux

To clarify the mechanism causing a significantly lower basal $^{86}Rb^+$ efflux in IL-1β-exposed islets in comparison to control islets, we first examined the possibility that by repolarizing the beta-cell membrane, the effect of IL-1β on $^{86}Rb^+$ efflux could be reversed. For this purpose we used diazoxide, a well-known repolarizing agent [20]. Islets pre-exposed to IL-1β were perifused with 0.1 mmol/l diazoxide-containing medium, but the low basal $^{86}Rb^+$ efflux of IL-1β exposed islets was not affected by this procedure (data not shown). We then examined the possibility that the basal $^{86}Rb^+$ efflux alterations in IL-1β-exposed islets were due to a decrease in $^{86}Rb^+$ uptake. To address this issue we measured the $^{86}Rb^+$ uptake both after a 10-min incubation (when the net $^{86}Rb^+$ uptake mainly reflects the K^+ inflow rate into the islet cells) and after a 2-h loading period (when the steady state of intracellular K^+ content is reached).

While the $^{86}Rb^+$ content after the 2-h loading period was similar in control and IL-1β-exposed islets (257.0 ± 31 vs 240.3 ± 42 pg · islet^{-1} · 2 h^{-1}, respectively), a significant inhibition of the acute (10 min) $^{86}Rb^+$ uptake was observed in rat islets pre-exposed to 50 U/ml IL-1β (58.8 ± 4.8 pg · islet^{-1} · 10 min^{-1} vs 80.3 ± 5.8 in IL-1β-exposed and control islets, respectively; mean ± SEM, $n = 3$, $p < 0.05$), indicating that IL-1β blunted $^{86}Rb^+$ influx within the islet cells. These experiments were performed either in the absence or in the presence of ouabain, an inhibitor of the Na^+, K^+-ATPase [17]. Thus, it was the ouabain-sensitive K^+ inflow which was impaired in IL-1β-exposed islets in comparison to control islets (30.6 ± 2.8 and 53.8 ± 5.8 pmol/islet, respectively; $p < 0.05$) (Table 1).

Ca^{2+} channel studies

In control islets, $^{45}Ca^{2+}$ uptake was 2.6 ± 0.4 pg · islet^{-1} · 20 min^{-1} (mean ± SEM, $n = 6$) under basal conditions (i.e. in the presence of 2.8 mmol/l glucose) and increased to 16.8 ± 3.2 and 10.7 ± 2.1 in the presence of either 16.7 mmol/l glucose or 10 μmol/l glyburide, respectively (Table 2). In pancreatic islets pre-exposed to 50 U/ml IL-1β, calcium uptake under basal conditions was higher than in control islets ($p < 0.05$), but only slightly increased after 16.7 mmol/l glucose stimulation ($p < 0.005$ vs control islets). In contrast to glucose, 10 μmol/l glyburide was able to stimulate calcium uptake in IL-1β-treated islets similar to that observed in control islets (12.8 ± 2.5 pg · islet^{-1} · 20 min^{-1}) (Table 2).

Discussion

The present studies indicate that pre-incubation with IL-1β impairs the glucose-induced insulin release from isolated rat pancreatic islets. By contrast, under the same conditions, the glyburide-induced insulin release is not affected. After chronic (24 h) exposure to IL-1β, therefore, the hormone storage is intact and the beta-cell secretory

machinery is functionally capable, although unresponsive to glucose.

In the cascade of intracellular events leading to insulin granule recruitment and extrusion from the beta cell, the closure of the ATP-sensitive K^+ channels is a key step leading to membrane depolarisation and subsequent Ca^{2+} influx, the suggested direct trigger of insulin secretion [21–23]. Exposure to IL-1β affected the function of the K^+ channels in isolated rat pancreatic islets in two different ways. First, it impaired K^+ influx as indicated by kinetic experiments with $^{86}Rb^+$ showing that the short-term radioactivity accumulation was blunted after IL-1β treatment. This mechanism may account, at least in part, for the lower basal $^{86}Rb^+$ efflux in IL-1β-treated islets and was due to the impairment of the Na^+, K^+-ATPase related K^+ uptake, either a direct effect of the IL-1β or the consequence of the cytokine effect on the beta-cell glucose metabolism [24] and energy (ATP) production. Secondly, IL-1β exposure markedly impaired the K^+ channel closure in response to beta-cell secretagogues such as glucose and glyburide. Our studies cannot exclude that these two impairments induced by IL-1β are related (i.e. that the secretagogues are unable to further reduce $^{86}Rb^+$ efflux that is already markedly reduced before stimulation). In fact one possible explanation for the defective effect of beta-cell stimulation under these conditions is that the membrane is already depolarised and no further depolarisation can be obtained. However, our studies with diazoxide which demonstrated that this well-known repolarizing agent [20] did not restore a normal basal $^{86}Rb^+$ efflux , do not support this possibility.

As a consequence of the effect of IL-1β on the K^+ channel function, glucose stimulation was unable to produce the Ca^{2+} influx and the subsequent insulin release in IL-1β-exposed islets. However, this was not the case for glyburide. Despite the altered K^+ channel function, this agent was able to elicit a normal Ca^{2+} uptake and a normal insulin release in islets pre-exposed to IL-1β. These findings suggest that in our experimental model glyburide is able to act directly on the Ca^{2+} influx independent of its activity on K^+ channels. The possibility for glyburide to directly stimulate Ca^{2+} uptake in beta cells has already been suggested for the HIT insulinoma cultured cells [25]. However, there is also evidence that glucose may increase Ca^{2+} influx in mouse beta cells by directly modulating the activity of dihydropyridine-sensitive Ca^{2+} channels [26].

The in vitro observation that IL-1β has an inhibitory effect on insulin release in rat pancreatic islets raises the possibility that the impairment of insulin response to glucose observed in vivo in the early stages of Type 1 diabetes [27, 28], may reflect a defective response to glucose and not necessarily an irreversible damage of the beta-cell mass. A similar observation of selective unresponsiveness to glucose, while responsive to other secretagogues (including glyburide) has already been reported in Type 2 (non-insulin-dependent) diabetes [29, 30]. In subjects at risk for developing Type 1 diabetes, the response to an intravenous glucose tolerance test (IVGTT) has been proposed as a marker for assessing changes in the beta-cell mass over time [31]. Data supporting this view have been obtained in streptozotocin-treated baboons, which develop hyperglycaemia and diabetes with no evidence of infiltration by macrophages or autoimmune insulitis [32]. Nevertheless, in these animals the acute beta-cell response to glucose ($AIR_{glucose}$) is undetectable when 40–50 % of the pancreatic mass is still present, thus showing that beta cells have become unresponsive to glucose [32]. Our in vitro data raise the possibility that IL-1β, released by activated macrophages, may selectively impair the response of the beta cell to glucose. We suggest the use of other secretagogues, in combination with the IVGTT, to better differentiate the irreversible loss of beta-cell mass from reversible functional defects for the assessment and monitoring of beta-cell damage in Type 1 diabetes prediction studies.

Table 1. $^{86}Rb^+$ uptake in pancreatic islets

	$^{86}Rb^+$ Uptake (pmol · islet^{-1} · 10 min^{-1})	
	Control Islets	IL-1β (50 U/ml) Pre-exposed islets
No Ouabain	80.3 ± 6.9	58.8 ± 5.0[a]
Ouabain (1 mmol/l)	26.5 ± 1.0	28.2 ± 2.2
Na^+/K^+ ATPase related uptake	53.8 ± 5.8	30.6 ± 2.8[a]

[a] $p < 0.05$ vs control islets.
Rb uptake was measured in groups of 15 islets for 10 min at 37 °C. Islets were separated from the incubation medium by centrifugation for 2 min through the oil layer, and the islet pellet counted. Data represent mean ± SEM of three separate experiments

Table 2. Effect of glucose and glyburide on $^{45}Ca^{2+}$ uptake

Secretagogue (mmol/l)		Treatment during pre-incubation	
		None	IL-1β (50 U/ml)
Glucose	2.8	2.6 ± 0.4	4.6 ± 0.6[a]
Glucose	16.7	16.8 ± 3.2	7.1 ± 1.1[b]
Glyburide	0.01	10.7 ± 2.1	12.8 ± 2.5

[a] $p < 0.05$, [b] $p < 0.005$ vs control islets.
$^{45}Ca^{2+}$ uptake was measured in groups of 15 islets for 20 min in buffer containing either 2.8 or 16.7 mmol/l glucose or 0.01 mmol/l glyburide. Ca^{2+} uptake is expressed as pmol · islet^{-1} · 20 min^{-1}. Data represent mean ± SEM of separate experiments

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