Glucose-stimulated efflux of indo-1 from pancreatic β-cells is reduced by probenecid

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Indo-1 loaded pancreatic β-cells, isolated from obese hyperglycaemic mice, were studied with respect to cytoplasmic free Ca^{2+} concentration ($[Ca^{2+}]_i$), efflux of indicator and insulin release. In the absence of glucose there was a continuous efflux of indo-1 which increased upon stimulation with 20 mM of the sugar. The anion exchange inhibitor probenecid reduced both basal efflux of indo-1 and prevented that promoted by glucose. Measurements of $[Ca^{2+}]_i$ and insulin release revealed similar results as previously reported with quin-2 and fura-2. Furthermore, probenecid did not influence the $[Ca^{2+}]_i$ responses. It is thus possible to reduce efflux of indo-1 probenecid and thereby improve the measurements of $[Ca^{2+}]_i$ in pancreatic β-cells.

Pancreatic β-cell; Cytoplasmic free Ca^{2+}; Indo-1; Probenecid

1. INTRODUCTION

The fluorescent indicators quin-2 and fura-2 are now used routinely to investigate the intracellular Ca^{2+} metabolism in different cell systems [1–3]. However, due to leakage of indicator, the information obtained from such measurements may be of limited value [4,5]. Since most measurements are performed at extracellular Ca^{2+} concentrations which will readily saturate the indicator, leakage will increase background fluorescence and thereby reduce the signal-to-noise ratio, resulting in shortening of the effective experimental time. This is especially critical when measuring the free cytoplasmic Ca^{2+} concentration ($[Ca^{2+}]_i$) in β-cell suspensions in a finite volume such as a cuvette. Another problem of interest is the non-linear leakage of fura-2 observed in pancreatic β-cells upon glucose stimulation [6,7]. Such a phenomenon makes it even harder to differ between true changes in $[Ca^{2+}]_i$ and variation in indicator leakage.

When searching for ways of minimizing leakage, an interesting finding was that the anion exchange inhibitor probenecid reduced fura-2 leakage from J-744 macrophages without affecting the physiological responses of these cells [8]. Similarly, 1 mM probenecid lowered the basal, and abolished the glucose-stimulated, fura-2 leakage in pancreatic β-cells without influencing changes in $[Ca^{2+}]_i$ or stimulation of insulin secretion [7]. Another representative of the second generation of fluorescent Ca^{2+} indicators is indo-1 which is closely related to fura-2 [2]. With the drawbacks related to fura-2 leakage in mind, this study was performed in order to evaluate possible advantages of using indo-1 instead of fura-2 in measurements of $[Ca^{2+}]_i$ in pancreatic β-cells.

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2. MATERIALS AND METHODS

2.1. *Media*

The basal medium used for preparation of cells as well as in all experiments was a Hepes buffer, pH 7.4, with chloride as the sole anion [9] containing 1.28 mM Ca^{2+} and 1 mg/ml bovine serum albumin.

2.2. *Animals and preparation of cells*

Adult obese hyperglycaemic (*ob/ob*) mice of both sexes were taken from a local non-inbred colony [10] and starved overnight. The islets were isolated with a collagenase technique and a cell suspension was prepared essentially as described in [11]. The cell suspension was cultured overnight as previously described [12].

2.3. *Measurement of indicator efflux and insulin release*

Cell suspensions were incubated with 1 μM indo-1/acethoxymethylester (AM) (Calbiochem, La Jolla, CA, USA) for 45 min. The dynamics of indicator efflux and insulin release were subsequently studied by perifusing about 1×10^6 loaded cells mixed with Bio-Gel P-4 polyacrylamide beads (Bio-Rad Laboratories, Richmond, CA, USA) in a 0.5 ml column at 37°C [13]. The flow rate was 0.3 ml/min and 1 min fractions were collected. The indicator content in each fraction was measured fluorometrically and the results corrected for background fluorescence. The insulin content in each fraction was measured radioimmunologically, using crystalline rat insulin as the standard.

2.4. *Measurements of $[Ca^{2+}]_i$*

Cell suspensions were incubated with 1 μM indo-1/AM for 45 min. After washing and resuspension in 1.5 ml of the above described medium the suspension was put in a polystyrene cuvette in an

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Aminco-Bowman spectrofluorometer, slightly modified to allow constant stirring. Changes in indo-1 fluorescence were measured at 37°C with excitation and emission wavelengths of 335 and 400 nm [2,14], respectively. Samples of the suspension were taken at the beginning and at the end of the experiments, to determine the extracellular fluorescence. Due to unlinear leakage of indo-1 in the controls, calibration of the experiments in terms of absolute values of $[Ca^{2+}]_i$ turned out unreliable. Thus, all recordings are presented as values of relative fluorescence only.

3. RESULTS AND DISCUSSION

When perifusing indo-1 loaded pancreatic β-cell aggregates in a column system, there was a continuous efflux of indicator, as evident from measurements of indo-1 specific fluorescence in the perifusate fractions (Fig. 1). Stimulation with 20 mM glucose (upper curve) evoked an increase in the efflux of indo-1 from the cells. The latter efflux had similar kinetics and magnitude as that previously demonstrated for fura-2 [7]. However, in cells run in parallel, the continuous presence of 1 mM probenecid (lower curve) reduced basal, and prevented glucose-induced, efflux of indo-1. This suggests that basal leakage of indo-1 is both unspecific, originating from damaged as well as intact cells, and specific, being inhibitable by probenecid.

Fig. 2 demonstrates measurements of $[Ca^{2+}]_i$ using indo-1 loaded cell suspensions. The cells were stimulated with 20 mM glucose in the absence (A) or presence (B) of 1 mM probenecid. Addition of the sugar induced a small decrease in fluorescence followed by a pronounced increase in both situations. As can be observed in A, subsequent addition of 50 μM D-600, a blocker of voltage-activated Ca^{2+} channels, did not bring fluorescence back to the prestimulatory level.

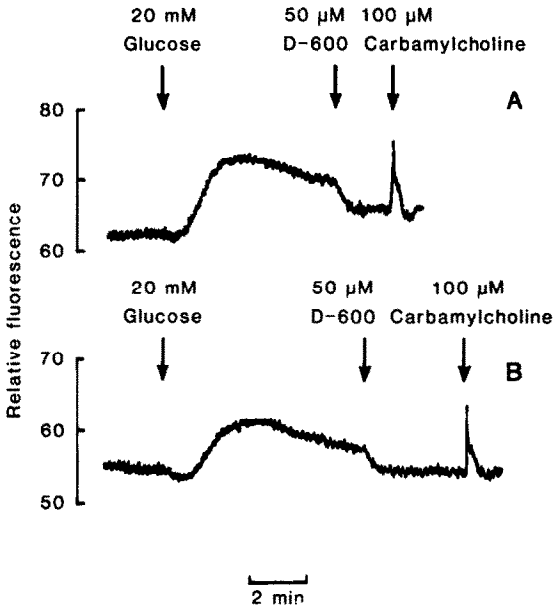

Fig. 2 Effects of glucose, D-600 and carbamylcholine on $[Ca^{2+}]_i$ in indo-1 loaded pancreatic β-cells. (A) control conditions; (B) 1 mM probenecid present throughout the experiment. The traces shown are representative of experiments performed on at least 3 different cell preparations.

This suggests that apart from basal leakage from damaged and intact cells, glucose-stimulated efflux of dye accumulates extracellularly and enhances background fluorescence. The seemingly larger increase in $[Ca^{2+}]_i$ in A is likely to reflect both variations among different cell preparations and extracellular indo-1 fluorescence. Further stimulation with 100 μM of the muscarinic receptor agonist carbamylcholine elicited a

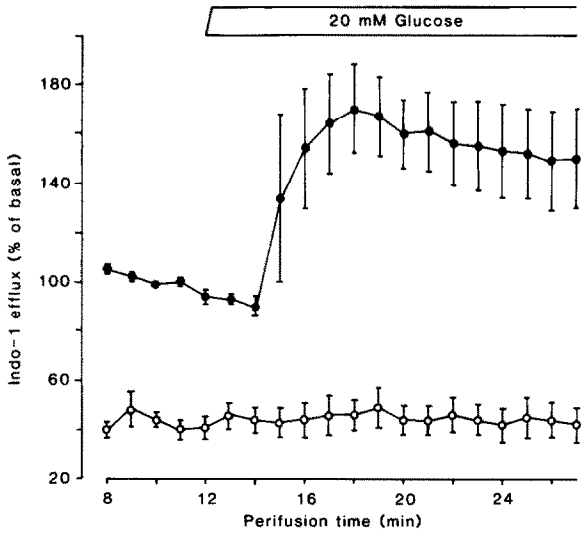

Fig. 1. Efflux of indicator from indo-1 loaded pancreatic β-cells. Cells were perifused in the absence (●—●) or presence (○—○) of 1 mM probenedid and 0 mM glucose. Glucose was added as indicated. The efflux is expressed as percent of the basal efflux in the absence of probenecid during the 5 min preceding the introduction of glucose. Values are given as mean ± SE ($n = 4$).

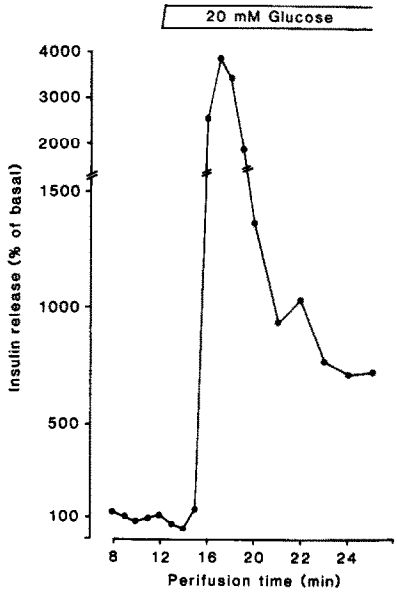

Fig. 3. Insulin release from indo-1 loaded pancreatic β-cells. The experiment shown is representative of experiments performed on 3 different cell preparations. Hormone release is expressed as percent of the basal release during the 5 min preceding the introduction of glucose.

transient peak in $[Ca^{2+}]_i$. In the presence of 1 mM probenecid (B) the prestimulatory level of $[Ca^{2+}]_i$ was reestablished after the addition of D-600. The subsequent stimulation with 100 μM carbamylcholine was not affected by the presence of probenecid (Fig. 2A). In 5 paired experiments, like those shown in Fig. 2, the accumulation of extracellular dye was followed during 12 min. Expressed in arbitrary units of indo-1 specific fluorescence, this accumulation was 12.2 ± 1.1, under the conditions in Fig. 2A, and 6.2 ± 1.1 (mean values $\pm$ SE) under the conditions in Fig. 2B. This further emphasizes the effectiveness of probenecid in reducing indo-1 efflux.

Fig. 3 shows glucose-stimulated insulin release from indo-1 loaded β-cells. As can be observed, the kinetics and magnitude were comparable to what has previously been reported for β-cell preparations, loaded or not with quin-2 or fura-2 [6]. Probenecid, used at a concentration of 1 mM, has previously been shown not to interfere with the increase in $[Ca^{2+}]_i$ [7] or insulin release [7,15] upon stimulation with 20 mM glucose [7]. Therefore, the effect of probenecid, with respect to insulin release, was not tested in this study.

Since indo-1 and fura-2 are structurally similar compounds [2], it is tempting to speculate that their leakage properties should be similar. Indeed, the fact that probenecid inhibited efflux of both fura-2 and indo-1 suggests that these indicators are extruded by a similar mechanism, the more precise nature of which is still to be investigated. As is the case for fura-2, indo-1 may be used for measurements of $[Ca^{2+}]_i$ in pancreatic β-cell suspensions, provided leakage is prevented with probenecid.

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