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In vitro kinetics of insulin release by microencapsulated rat islets: effect of the size of the microcapsules

D. Chicheportiche and G. Reach

Service de Diabétologie, Hôtel-Dieu, Paris, France

Summary. Microencapsulation has been proposed to protect islets of Langerhans against immune rejection in xenogenic transplantation. However, to achieve glucose homeostasis in human diabetic patients, insulin release by microencapsulated islets must increase in response to a glucose load. We microencapsulated isolated rat islets using the alginate-polylysine procedure. Capsule size was found to range from 300 to 800 μm, and microencapsulated islets were separated according to their size. Groups of 10 microencapsulated islets, either small (350 μm) or large (650 μm) were placed in plastic microwells, in minimal Eagle's culture medium containing either 5.5 mol/l glucose (basal) or 16.5 mol/l glucose and 5.5 mol/l theophylline (stimulatory medium). The increase in insulin concentration in the surrounding medium was then serially determined over 30 min: (1) With the small capsules, insulin concentration rose from 199 ± 20 to 297 ± 58 μU/ml in basal medium, and from 236 ± 23 to 510 ± 121 μU/ml in stimulatory medium ($n = 10$ preparations), the difference between the data obtained with the basal or the stimulatory medium being significant ($p < 0.01$) from the 5th min onwards. (2) With large capsules, insulin concentration increased from 182 ± 9 to 266 ± 44 μU/ml, and from 216 ± 19 to 297 ± 34 μU/ml in basal and stimulatory medium, respectively, with no apparent significant difference. The magnitude of insulin secretion in response to glucose by unencapsulated islets was, under similar conditions, seven-fold greater. We conclude therefore that the size of the microcapsules is an essential parameter which has to be considered for the optimisation of the microencapsulation procedure.

Key words: Microencapsulation, bioartificial pancreas, insulin kinetics, islets of Langerhans, Type 1 (insulin-dependent) diabetes mellitus, closed-loop insulin therapy.

Microencapsulation of islets of Langerhans has been proposed to protect the cells against immune rejection, allowing the use of xenogenic pancreatic tissue for the treatment of human diabetes mellitus [1]. Islets microencapsulated in alginate-polylysine capsules were found to remain functional for several months under culture conditions [2], and the intraperitoneal implantation of microencapsulated rat islets of Langerhans into diabetic rats [3, 4] or mice [5, 6] was followed by a sustained normalisation of weight, diuresis and blood glucose concentration. Moreover, it has been demonstrated that the capsule membrane provides immunoprotection of the pancreatic cells against the cytotoxic effect of the serum of diabetic patients [7].

Kinetic modelling of glucose homeostasis in man indicated that the lagtime of the increase in insulin delivery in response to a glucose load by an artificial pancreas must be shorter than 15 min in order to avoid the overexcursion of blood glucose postprandial levels [8]. The aim of this work was therefore to determine in vitro the kinetics of insulin release by microencapsulated islets of Langerhans in response to glucose. Furthermore, when microencapsulating the islets, we observed that the size of the capsules was heterogeneous, ranging from 300 to 800 μm. Since the volume of the islet chamber can obviously be involved in the response time of the system, the effect of the capsule size was also investigated.

Materials and methods

Rat islet isolation and microencapsulation

Islets of Langerhans were isolated from the pancreata of fed male Wistar rats (250–350 g body weight) by the collagenase method [9] and microencapsulated by the alginate-polylysine method, according to O'Shea et al. [5], with a polylysine molecular weight of 47,000. Microencapsulated islets were sorted according to their size by gentle pipetting through a Pasteur pipette under a stereomicroscope. Figure 1 depicts microencapsulated islets before and after sorting,

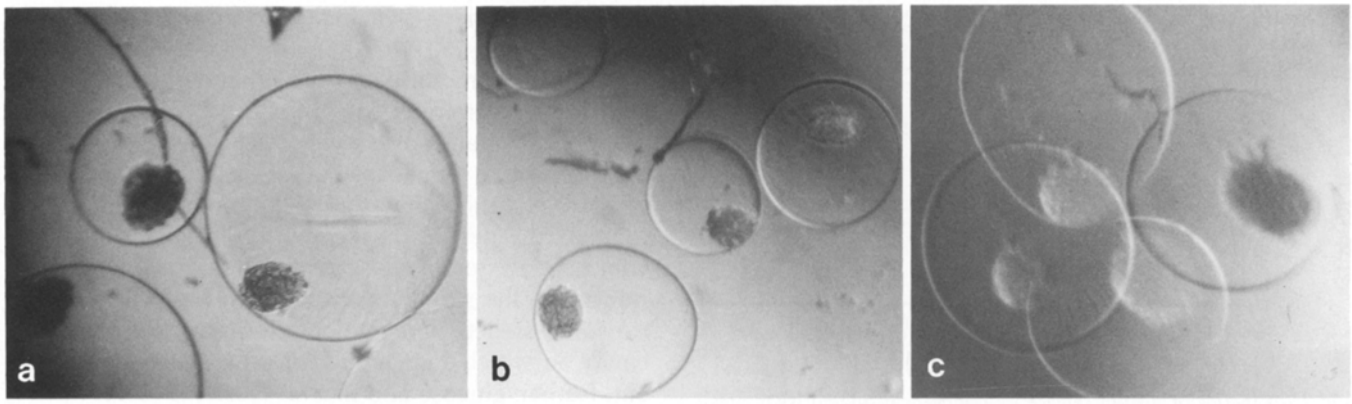

Fig. 1. Microencapsulated islets of Langerhans before (**a**) and after sorting into small (**b**) and large (**c**) capsules (same magnification: ×40)

demonstrating the heterogeneity of the sizes of the capsule preparation, and the final sizes of the two populations of capsules, which were approximately 350 and 650 μm, and are referred to below as "small" and "large" microcapsules respectively. As can be seen the size of the islets inside the two capsule populations was homogeneous.

From each initial batch, two groups of 10 microencapsulated islets of each size were incubated overnight in a CO2 incubator (6% CO2, 37 °C), inside 250 μl wells of microtest plates, in Roswell Park Memorial Institute (RPMI 1640) culture medium (Gibco Laboratories, Grand Island, NY, USA), supplemented with heat-inactivated 10% fetal calf serum, 2 mol/l L-glutamine, 100 U/ml penicillin and 100 μg/ml streptomycin.

Evaluation of the kinetics of insulin release by microencapsulated islets in response to glucose

After overnight culture, the four groups of encapsulated islets were washed four times with 200 μl of basal medium (Minimal Eagle's Medium (MEM), Gibco, Paisley, Scotland, containing 5.5 mol/l glucose). During each wash, encapsulated islets were left in the medium and maintained at room temperature, for 10 min, to remove insulin which might have built up during the previous culture period. After completion of the washing, the kinetics of insulin release in response to glucose by the islets present either in small or in large microcapsules, were determined by adding 200 μl of prewarmed (37 °C) MEM medium, containing either 5.5 mol/l glucose (basal medium) or 16.5 mol/l glucose supplemented with 5.5 mol/l theophylline (stimulatory medium). Immediately after adding the solutions (time zero), a 10 μl sample was collected in each of the four wells, and the plate was placed inside the incubator (37 °C). Ten μl samples were again collected at 5, 10, 15, and 30 min ($n = 10$ different experiments). The total sampling time, during which the plates were out of the incubator was minimised as far as possible and was usually 30 to 45 s. This is of importance since insulin secretion in response to glucose is reduced at room temperature [10].

One experiment using unencapsulated islets was performed under identical stimulatory conditions, except that from one islet preparation, 12 groups of 5 islets were incubated overnight in RPMI culture medium and then stimulated in basal MEM (6 wells), or in stimulatory medium (6 wells) as described above.

Insulin assay and presentation of data

Immediately after sampling the aliquots were diluted with 90 μl of Krebs bicarbonate buffer and stored at −20 °C until insulin assay. The insulin concentration was assayed by the radioimmunological method using rat insulin as standard and double antibody separation [11]. Insulin secretions over 30 min were calculated taking into account the final 160 μl incubation volume and the insulin removed in the 4 successive 10-μl samples, according to the formula:

Insulin secretion over 30 min (expressed in μU/10 microencapsulated islets)

$$= I_{30} \times 0.160 + (I_0 + I_5 + I_{10} + I_{15})/100,$$

were I_i is the insulin concentration, expressed in μU/ml at time i.

Statistical analysis

All data in text and figures are presented as mean ± SEM, and their statistical significance was assessed by the non parametric Wilcoxon test [12].

Results

Evaluation of the kinetics of insulin release by microencapsulated and unencapsulated islets in response to glucose

Figure 2 represents the results of 10 successive microcapsule preparations. If we first consider the small microcapsules, the insulin concentration increased significantly more between 0 and 30 min when the microencapsulated islets were incubated in stimulatory medium (from 236 ± 23 to 510 ± 121 μU/ml) than when they were incubated in basal medium (199 ± 20 to 297 ± 58 μU/ml), the difference being significant from 5 min onwards ($p < 0.01$). Insulin secretion over 30 min was significantly more important when the microencapsulated islets were incubated in stimulatory medium than when they were incubated in basal medium (95 ± 21 vs 57 ± 10 μU/10 microencapsulated islets, $p < 0.01$).

In the case of large microcapsules, the insulin concentration of samples increased between 0 and 30 min of incubation from 182 ± 9 to 266 ± 44 μU/ml when microencapsulated islets were incubated in basal medium, and from 216 ± 19 to 297 ± 34 μU/ml when they were incubated in stimulatory medium. Here, insulin secretion over 30 min did not significantly differ between basal and stimulatory medium (51 ± 8 vs 57 ± 6 μU/10 microencapsulated islets, NS). Finally, insulin secretion in the stimulatory medium was greater with the small microcapsules than with the large elements ($p < 0.05$). Such a difference was not observed in basal medium.

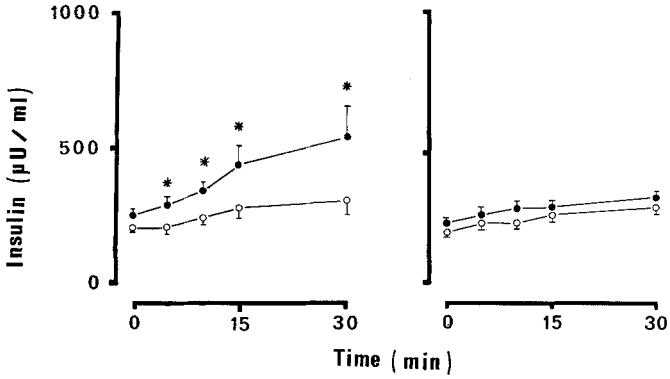

Fig. 2. Kinetics of insulin release by microencapsulated islets present in small (left panel) or large (right panel) microcapsules, incubated in basal medium (open circles, ○) or in stimulatory medium (closed circles, ●). Mean and SEM, $n=10$, * $p<0.01$

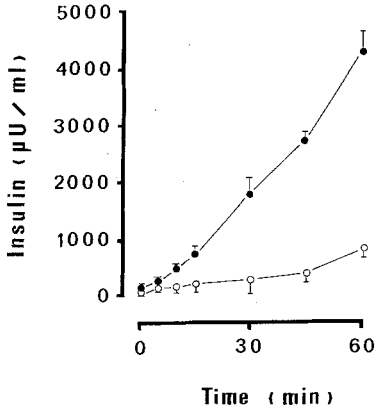

Fig. 3. Kinetics of insulin release by unencapsulated islets incubated in basal medium (open circles, ○) or in stimulatory medium (closed circles, ●). Mean and SEM of six wells

Figure 3 gives the data of one experiment in which groups of 5 unencapsulated islets were incubated under similar conditions in basal or in stimulatory medium. The islets responded immediately to the stimulation, and the insulin concentration reached at 30 min a level much higher than that observed with 10 microencapsulated islets stimulated under similar conditions.

Discussion

This study documents for the first time the kinetics of insulin release by microencapsulated islets in response to glucose under incubation conditions, and demonstrates a major effect of the size of the microcapsules. It should be stressed that the well-established ability of microencapsulated islets to correct experimental diabetes in rodents does not demonstrate whether they are able to deliver more insulin in response to a glucose load, since the same clinical results (namely disappearance of glycosuria, normalisation of glycaemic profile, prevention of cataract) can be achieved with insulin polymere matrices, which release insulin without any regulation [13-15]. On the other hand, in their first publication, Lim and Sun found that the increase in insulin release in response to glucose from perifused microencapsulated islets was delayed by 5 min, in comparison to unencapsulated islets [1]. However, it could be argued that these experimental conditions (namely the perifusion technique), in which insulin is continuously removed by the perifusion flow, did not reproduce the situation of in vivo use of the microencapsulated islets, i.e. implantation into the peritoneal cavity, in which such a solvent drag effect is not present. Rather, glucose and insulin cross the membrane of the microcapsule by diffusion only, which is a slow process [16].

Our data, obtained by using an experimental design of static incubation, clearly demonstrate that small capsules (mean diameter, 350 µm) containing isolated islets of Langerhans can and do respond rapidly (within 15 min) to stimulation by increasing insulin secretion, and that this is not the case for large microcapsules (650 µm) from the same batch, although the size of the islets within the two kinds of capsule was similar. These data are only in appearance inconsistent with our previous data on microencapsulated islets, which demonstrated a 6-fold increase in insulin release, within 30 min, by a population of unsorted encapsulated islets [7]. Indeed, the protocol was quite different and was based on a sequential design in which the same microencapsulated islets were successively incubated in basal and stimulatory medium. Obviously the new experimental design, presented in this paper, in which two groups of microcapsules, after washing with basal medium, are incubated simultaneously either in basal, or in stimulatory medium, is more suitable for investigating the kinetics of insulin release by microencapsulated islets.

Two hypotheses can explain the absence of significant response observed with large microcapsules. Firstly, possibilities for exchange across the microcapsule membrane decrease as the capsule diameter increases, since they are related to the surface over volume ratio. Thus, the absence of response of islets enclosed in large capsules might be due either to an inadequate influx of a factor essential to islet survival or function, or to the accumulation inside the capsule of an inhibiting substance (for instance, somatostatin). Secondly, it can be assumed that islets contained in small and large capsules secrete the same amount of insulin in response to glucose. Thus, intracapsular insulin concentration will be much smaller in large microcapsules, since the secreted insulin will be diluted in a volume approximately 8-fold larger (the increase in the volume of a sphere is proportional to the cube of the radius). Since the driving force for insulin diffusion is the difference in insulin concentration across the membrane, this can explain a slower insulin release from the large microcapsules.

Consistent with this hypothesis, it can be observed that under the same experimental conditions, unencapsulated islets of comparable size secreted 7-fold more insulin in response to glucose than did those islets contained in the small microcapsules. This would suggest that "small" microcapsules are in fact still too large, their volume being approximately 8-fold larger than that of an islet placed in a virtual volume. This might explain why 4000 encapsulated islets were required to correct experimental diabetes in rats [3], while data with free islets indicate a need for 2-3 islets/g body weight [17].

In conclusion, the volume of the microcapsule is an essential parameter in the kinetics of insulin secretion in response to glucose by microencapsulated islets. Since the achievement of glucose homeostasis in diabetic man requires a fast increase in insulin delivery during a glucose challenge [8], the volume of microcapsules should therefore be considered for the optimisation of the microencapsulation procedure, as well as the use of devices designed to control this parameter [18].

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Dr. Gérard Reach
Service de Diabétologie
Hôtel-Dieu
1 Place du Parvis Notre Dame
F-75004 Paris
France