Biochem. J. (1986) 236, 889–893 (Printed in Great Britain)

Active transport of *myo*-inositol in rat pancreatic islets

Trevor J. BIDEN and Claes B. WOLLHEIM
Institut de Biochimie Clinique, Université de Genève, Centre Médical Universitaire, 1211 Genève 4, Switzerland

myo-Inositol transport by isolated pancreatic islets was measured with a dual isotope technique. Uptake was saturable with a half-maximal response at approx. 75 μM. With 50 μM-inositol, uptake was linear for at least 2 h during which time the free intracellular concentration rose to double that of the incubation medium. Inositol transport is therefore active and probably energized by electrogenic co-transport of Na^+ down its concentration gradient as uptake was inhibited by ouabain, Na^+ removal or depolarizing K^+ concentrations. Inositol transport was abolished by cytochalasin B which binds to hexose carriers, but not by carbamoylcholine or Li^+ which respectively stimulate or inhibit phosphoinositide turnover. Uptake of inositol was not affected by 3-*O*-methylglucose or L-glucose (both 100 mM) nor by physiological concentrations of D-glucose. The results suggest that most intracellular inositol in pancreatic islets would be derived from the extracellular medium. Since the transport mechanism is distinct from that of glucose, inositol uptake would not be inhibited during periods of hyperglycaemia.

INTRODUCTION

Although not itself a stimulator of insulin release [1,2] the presence of inositol in tissue culture medium is essential for cultured islets to maintain their secretory function [3]. This probably represents a requirement for exogenous inositol in the synthesis of the phosphoinositides, a group of membrane phospholipids with an inositol head group. It has been known for some time that enhanced turnover of this class of lipid is associated with the activation of islet cells by a variety of secretory stimuli [1,2,4]. More recently, attention has focused on PtdInsP_2, a quantitatively minor constituent of this lipid pool which is thought, in many cell types, to be the site of action of Ca^{2+}-mobilizing agonists [5]. Receptor activation leads to a phosphodiesterase-mediated cleavage of this lipid, producing two molecules: InsP_3, a water-soluble second messenger which mobilizes Ca^{2+} from the endoplasmic reticulum [5], and diacylglycerol, the lipophilic activator of protein kinase C [6]. In insulin secretory tissue, secretagogues such as glucose and the muscarinic agonist carbamoylcholine, promote hydrolysis of PtdInsP_2 [7–10]. Moreover there is strong evidence to suggest that, at least for carbamoylcholine, the generation of InsP_3 plays a key role in the activation of the secretory response [11,12].

In most tissues the intracellular concentration of inositol is several-fold higher than the circulating levels [13,14]. These high concentrations are maintained both by uptake mechanisms [15–18], and by direct conversion of glucose to inositol, a reaction sequence for which the enzyme inositol 1-phosphate synthase (EC 5.5.1.4) is rate limiting [19]. Since the activity of this enzyme is generally very low [19,20], the metabolic pathway is likely to be of only minor quantitative importance in those tissues which actively transport inositol. Indeed it is thought that elevated plasma glucose levels actually lower free inositol concentrations in these tissues [14,21] by inhibiting inositol uptake [15–18].

A full characterization of inositol uptake in the pancreatic islet is therefore essential for complete understanding of the effects of glucose on phosphoinositide turnover in this tissue. In the following study we show that inositol is actively transported by islet cells, but via a mechanism which is not inhibited by physiological glucose concentrations. These results suggest that most of the intracellular inositol found in pancreatic islets is likely to be derived from the extracellular milieu, but that the intracellular concentration could be maintained even during periods of prolonged hyperglycaemia.

EXPERIMENTAL

Materials

Dowex 1 X8 resin (200–400 mesh), ammonium formate, formic acid, choline chloride, lithium chloride, phloridzin and L-glucose were obtained from Fluka AG, Buchs, Switzerland. Cytochalasin B, choline bicarbonate, 3-*O*-methylglucose and carbamoylcholine were from Sigma Chemie GmbH, Munich, Germany; and dibutylphthalate, dinonylphthalate, urea, *myo*-inositol and ouabain were from E. Merck, Darmstadt, Germany. Radiochemicals were purchased from NEN, Du Pont de Nemours International SA, Zurich, Switzerland. Hydroluma scintillant was obtained from Fakola AG, Basel, Switzerland. All other reagents were of analytical grade or better.

Islet isolation and incubation

Pancreatic islets were isolated from male Wistar rats (180–220 g) by collagenase digestion of the excised pancreas. The basic incubation medium was a modified Krebs–Ringer solution buffered with 10 mM-Hepes and containing 5 mM-$NaHCO_3$, 1 mM-$CaCl_2$, 2.8 mM-glucose and 0.5% bovine serum albumin [22]. In some experiments the total Na^+ content of this medium was replaced with choline.

Measurement of inositol uptake

Inositol uptake by islets was measured by a method similar to that previously described for ^{45}Ca uptake [22],

Abbreviations used: inositol, *myo*-inositol; InsP_3, inositol 1,4,5-trisphosphate; PtdInsP_2, phosphatidylinositol 4,5-bisphosphate.

in which islets are rapidly separated from the incubation medium by centrifugation through an oil layer, and total islet-associated radioactivity corrected for extracellular contamination by a dual isotope technique. Groups of 10 islets in 50 μl of incubation medium were transferred to 0.4 ml polyethylene tubes containing 0.2 ml of a mixture of dibutyl- and dinonylphthalate (10:3, v/v) layered on top of 20 μl of 6 M-urea. The incubation was started by the addition of 50 μl of prewarmed incubation medium containing 2.0–3.0 μCi of *myo*-[2-^{3}H]inositol (or ^3H_2O), 0.35 μCi of L-[1-^{14}C]glucose and doubly concentrated experimental additions as described. After incubation at 37 °C, islets were separated from the medium by centrifugation (8000 *g* for 15 s). The tip containing the solubilized islets was cut from the rest of the tube, shaken vigorously with 5 ml of Hydroluma and the radioactivity was quantified by liquid-scintillation spectrometry. Uptake of inositol was calculated from the total ^{3}H radioactivity by subtracting the counts in the extracellular space (determined from the L-[^{14}C]glucose distribution space), and correcting for channel crossover and counting efficiency. In some experiments, the intracellular islet volume was calculated by measuring the total ^3H_2O distribution space and correcting for extracellular contamination.

Measurement of free intracellular inositol

For quantification of the intracellular inositol concentration it was necessary to separate free inositol from phosphorylated or lipid-bound derivatives. These experiments were performed similarly to those described above, except that the islets were spun through the oil into 5% (w/v) sucrose rather than 6 M-urea. The contents of the tube tip were then extracted for 1 h with 10% (w/v) trichloroacetic acid (1 ml). The extracts were then washed three times with a 5-fold excess of diethyl ether, neutralized and made up to a final volume with 5 ml of water. These samples were analysed by anion exchange chromatography using 1 ml Dowex 1 X8 columns prepared in the formate form. [^{3}H]Inositol was collected in the void volume and in the eluate of a 5 ml wash with 5 mM unlabelled inositol. The radioactivity in these samples was determined by liquid-scintillation spectrometry in the presence of 10 ml of Hydroluma. The recovery of a [^{3}H]inositol standard carried through the entire extraction and elution procedure was 90±2% (n = 12). Phosphorylated inositol derivatives were next washed from the columns by the addition of 5 ml of 1.0 M-ammonium formate/0.1 M-formic acid. Intracellular inositol concentrations were calculated from the amount of free inositol present (correcting for extracellular contamination, channel crossover, counting efficiency and recovery) divided by the islet intracellular volume, which was measured in each experiment in parallel.

Statistics

All results are expressed as the mean±S.E.M. Statistical analysis was performed by using Student's t-test for unpaired data.

RESULTS

Initial experiments to determine the time dependence of inositol uptake in pancreatic islets were performed using 50 μM-inositol, which approximates to the normal circulating concentration [13,14]. At this extracellular concentration, uptake of inositol was linearly dependent on time for at least 2 h (Fig. 1). The rate of uptake could be calculated as approx. 1.3 pmol of inositol/h per 10 islets.

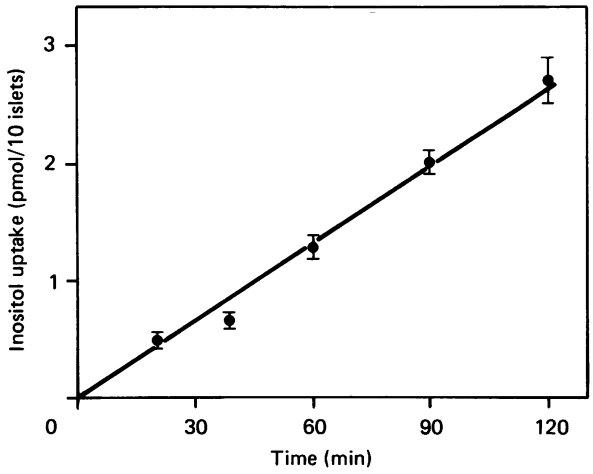

Fig. 1. Time dependence of net inositol uptake by isolated pancreatic islets

Isolated islets were incubated in the presence of 2.8 mM-glucose, 50 μM-inositol, 2 μCi of [2-^{3}H]inositol and 0.35 μCi of L-[1-^{14}C]glucose. Uptake was terminated by separating the islets from the incubation medium by centrifugation through an oil layer. Islet-associated radioactivity was quantified by liquid-scintillation spectrometry, and corrected for extracellular contamination. For further details see the Experimental section. Each point represents the mean±S.E.M. for 15–24 observations.

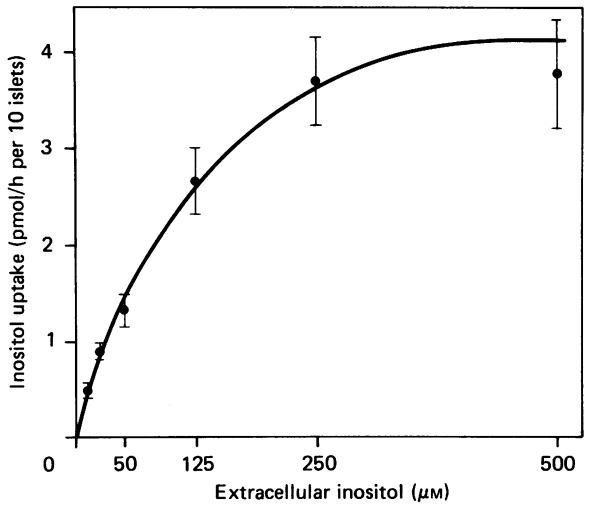

Fig. 2. Concentration-dependence of inositol uptake

Islets were incubated with 2.8 mM-glucose and various extracellular inositol concentrations. The experimental procedure was similar to that described in the legend to Fig. 1. Each point represents the mean±S.E.M. for 8–20 observations.

Further experiments were undertaken to determine whether inositol uptake was a saturable process in this tissue (Fig. 2). This proved to be the case. A maximal uptake of around 4.0 pmol/h per 10 islets was seen when

Table 1. Effects on inositol uptake of agents which alter phosphoinositide turnover

Islets were incubated in the presence of 2.8 mM-glucose, 50 μM-inositol and other additions as described for 30 min. For further details see the Experimental section. All values are the means ± S.E.M. for eight individual observations; *$P < 0.01$ compared with the 2.8 mM-glucose control.

Added agent (mM)	Inositol uptake (pmol/30 min per 10 islets)
None	0.91 ± 0.11
Glucose (14)	0.88 ± 0.05
Carbamoylcholine (0.5)	0.85 ± 0.09
K^+ (24)	0.51 ± 0.06*
Li^+ (10)	1.03 ± 0.11

Table 2. Effects of other hexoses on inositol transport

Islets were incubated for 30 min in the presence of 50 μM-inositol and other additions as described. For further details see the Experimental section. All values are the means ± S.E.M. for 14 individual observations; *$P < 0.01$ compared with the control.

Added hexose (mM)	Inositol uptake (pmol/30 min per 10 islets)
None	0.74 ± 0.10
D-Glucose (2.5)	0.77 ± 0.13
D-Glucose (50)	0.62 ± 0.08
D-Glucose (100)	0.39 ± 0.07*
L-Glucose (100)	0.51 ± 0.07
3-*O*-Methylglucose (100)	0.58 ± 0.10

the extracellular inositol concentration was raised to 0.5 mM. The half-maximal concentration of approx. 75 μM is well within the physiological range.

Secretory agonists such as glucose, carbamoylcholine and depolarizing K^+ concentrations stimulate phosphoinositide breakdown in pancreatic islets [7–10; T. J. Biden, B.-G. Peter, W. Schlegel & C. B. Wollheim, unpublished work] whereas Li^+ inhibits inositol phosphate metabolism [10]. Therefore, we tested the effects of these agents on inositol uptake, to test indirectly whether inositol transport is likely to be rate limiting for phosphoinositide turnover (Table 1). This is not the case because, of the compounds tested, only raised K^+ had any effect on inositol uptake. This is most likely due to a direct inhibition of inositol transport (see below).

In many tissues glucose and other sugars are competitive inhibitors of inositol transport. It is therefore possible that the uptake measured in the above experiments represents an underestimate, due to inhibitory effects of the 2.8 mM-glucose which was used to maintain islet metabolic function during the incubations. Accordingly, the rate of uptake of 50 μM-inositol was examined over the concentration range of glucose from 0 to 100 mM (Table 2). Uptake was not affected by a 1000-fold excess concentration of D-glucose, but was halved in the presence of 100 mM-D-glucose. Similar concentrations of L-glucose or 3-*O*-methylglucose had no significant effect.

Table 3. Inhibition of inositol uptake by cytochalasin B, phloridzin or alteration of the Na^+ gradient

Islets were incubated for 30 min in the presence of 2.8 mM-glucose and 50 μM-inositol with other additions as described, or in Na^+-depleted medium. For further details see the Experimental section. All values are the means ± S.E.M. for 12 individual observations; differences from control are denoted *$P < 0.005$ and **$P < 0.001$.

Experimental modification	Inositol uptake (pmol/30 min per 10 islets)
None	0.64 ± 0.08
Na^+ removal	0.10 ± 0.03**
Phloridzin (0.2 mM)	0.30 ± 0.05*
Cytochalasin B (0.01 mM)	0.32 ± 0.03*
Ouabain (1 mM)	0.12 ± 0.03**

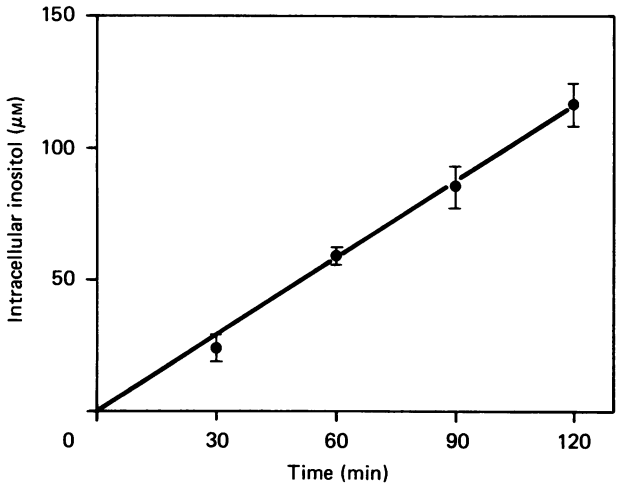

Fig. 3. Time dependence of intracellular free inositol accumulation

Islets were incubated with 2.8 mM-glucose and 50 μM-inositol under conditions identical to those described in the legend to Fig. 1. Islet associated radioactivity was extracted with 10% (w/v) trichloroacetic acid. The water-soluble extracts were washed with diethyl ether, and free inositol was separated from inositol phosphates by anion-exchange chromatography. By measuring in parallel the intracellular islet space with ^3H_2O, it was possible to calculate the actual concentration of intracellular free inositol at each experimental point. For further details see the Experimental section. The mean intracellular islet space in these experiments was 1.0 ± 0.1 nl/islet ($n = 30$). Each point represents the mean ± S.E.M. for 15–35 observations.

The results of experiments examining the sensitivity of inositol uptake to known inhibitors of sugar transport are presented in Table 3. Inositol transport showed a strict requirement for an inward Na^+ gradient because of the marked inhibitory effects of Na^+ depletion, or of blockade of the Na/K^+ pump with ouabain. In addition, inositol uptake was significantly decreased by concentrations of cytochalasin B and phloridzin which have been previously shown to inhibit carrier-mediated and active sugar transport respectively in other tissues [24–26].

To determine whether inositol is actively transported in pancreatic islets it is necessary to compare its

intracellular and extracellular concentrations at various time intervals. Calculations based upon net inositol uptake (Fig. 1) are misleading in this respect since much of the total intracellular radioactivity represents not free, but phosphorylated or lipid-bound inositol. We have therefore made aqueous extracts of incubated islets to recover non-bound material, and then further separated free inositol from its phosphorylated derivatives by anion-exchange chromatography. In a series of parallel experiments net [^{3}H]inositol uptake was measured as 2.59 ± 0.25 pmol/2 h per 10 islets whereas only 0.84 ± 0.07 pmol was recovered from the aqueous extract as free [^{3}H]inositol ($n = 39$). The radioactivity in the inositol phosphate fraction was less than 2% of that eluting with free inositol (result not shown) ($n = 39$). Hence over a 2 h period 68% of the inositol accumulated intracellularly is metabolically incorporated into trichloroacetic acid-precipitable material. This almost undoubtedly represents incorporation into phospholipids since, using a virtually identical protocol, it has been previously shown that 73% of total islet cell [^{3}H]inositol was associated with the phosphoinositides [27]. The accumulation of free inositol, calculated as an intracellular concentration, is shown in Fig. 3. With 50 μM extracellular inositol nearly 1 h was necessary before a similar concentration was reached inside the islet cells. The accumulation increased linearly with time, until after 2 h the intracellular concentration was almost double that of the incubation medium.

DISCUSSION

Hexose transport can be characterized as either facilitated diffusion or active transport depending on whether the sugar is actually concentrated intracellularly, and by its Na^+-dependence and sensitivity to inhibition by phloridzin [15–18,24,25]. Recent evidence suggests that two completely different carrier molecules are used for each process [28]. Active transport, in terms of either glucose or inositol, is best characterized in preparations of intestine [15,29] and renal brush border [16,30]. On the other hand, non-active uptake of both inositol and glucose occurs in the liver [26,31], exocrine pancreas [32,33] and skeletal muscle [26,31]. Finally, lens and nerve preparations display active inositol transport [17,18,35], but apparently take up glucose by facilitated diffusion [36,37].

In the present study we have shown that inositol transport in the islet of Langerhans is also an active process because free inositol was concentrated intracellularly with respect to the extracellular medium. The energy for this process was most probably derived from the electrogenic co-transport of Na^+ down its concentration gradient, since inositol uptake was blocked by extracellular Na^+ removal, by raising the intracellular Na^+ concentration with ouabain, or by membrane depolarization with 24 mM-K^+. On the other hand, glucose is not concentrated in islet cells [38] nor is the uptake process dependent on an inward Na^+ gradient [39]. Therefore the pancreatic islet is comparable with lens or nerve tissue. Indeed, similarities between the islet and lens have previously been commented upon in terms of the rather low affinities that these tissues show for glucose transport [37]. It was suggested that this might indicate the existence of a second uptake pathway operating at high glucose concentrations [37]. However, in the pancreatic islet, as opposed to nerve [17,18], active inositol transport was not inhibited by physiological glucose concentrations, suggesting a lower affinity of the active transporter for glucose in the secretory cells. Moreover, in the present study inositol transport was not inhibited by 3-*O*-methylglucose, routinely used as a marker for glucose transport. These results are one indication that the pancreatic islet would be able to maintain its intracellular inositol concentration independently of fluctuations of the plasma glucose.

A second indication of this independence is derived from the very fact that the transport of inositol is active, and displays sufficient affinity to be operative at normal circulating inositol concentrations. This suggests that the islet cell is unlikely to be dependent upon the synthetic pathway from glucose for the maintenance of intracellular inositol. This is not to say that such a pathway does not exist in the islet, nor that it could not play some role in the stimulation of insulin release by glucose. However, the present results indicate that unless inositol 1-phosphate synthase is much more active in islets than it is in most tissues, then newly synthesized inositol is unlikely to significantly dilute the extracellularly derived pool when there is a rise in plasma glucose. This in turn suggests that the incorporation of glucose-derived carbon atoms into the head groups of the phosphoinositides would be relatively slow, and that therefore synthesis *de novo* of InsP_3 from glucose, although theoretically possible, is not a likely explanation for the stimulatory effects of glucose on insulin release. Such a conclusion is consistent with the earlier observations that the concentrations of Li^+ which maximally inhibit the function of inositol 1-phosphate synthase [10] do not inhibit glucose-induced insulin release [40].

Inositol transport in pancreatic islets was not affected by short-term incubation with agents which either stimulate or inhibit phosphoinositide turnover. This is not surprising in a tissue which actively transports inositol and has therefore already stockpiled sufficient reserves to meet the demands of increased turnover. This probably also explains why inositol does not act as a stimulator [1,2] of insulin release in short-term studies, but is necessary to maintain functionally responsive islets during tissue culture [3].

In conclusion, it is apparent that the pancreatic islet, like lens and nerve, transports glucose and inositol by separate carriers. However, in contrast with other tissues, the active transport of inositol was not inhibited by physiological glucose concentrations. The strict segregation of inositol from glucose transport in pancreatic islets may thus represent a functional adaptation whereby inositol concentrations are maintained independently of fluctuations in the levels of blood glucose. Since increased phosphosinositide turnover appears important for activation of insulin release, this adaptation may play a role in the maintenance of secretory capacity during long-term hyperglycaemia

We gratefuly acknowledge the expert technical assistance of Mrs. Danielle Nappey. This work was supported by grant no. 3.246-0.82 of the Swiss National Science Foundation.

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Received 23 December 1985/31 January 1986; accepted 19 February 1986