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Transport of ascorbic acid and dehydroascorbic acid by pancreatic islet cells from neonatal rats

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Several amidated biologically active peptides such as pancreastatin, thyrotropin-releasing hormone, pancreatic polypeptide and amylin are produced in endocrine pancreatic tissue which contains the enzyme necessary for their final processing, i.e. peptidylglycine α-amidating mono-oxygenase (EC 1.14.17.3). The enzyme needs ascorbic acid for activity as well as copper and molecular oxygen. The present work shows that pancreatic islet cells prepared from overnight cultures of isolated islets from 5–7-day-old rats accumulate ^{14}C-labelled ascorbic acid by a Na^+-dependent active transport mechanism which involves a saturable process (estimated K_m 17.6 μM). Transport was inhibited by ouabain, phloridzin, cytochalasin B, amiloride and probenecid. Glucose inhibited or stimulated uptake, depending on the length of incubation time of the cells. The uptake of dehydroascorbic acid was linearly dependent on concentration. Dehydroascorbic acid was converted to ascorbic acid by an unknown mechanism after uptake. The uptake of both ascorbic acid and dehydroascorbic acid was inhibited by tri-iodothyronine, and uptake of ascorbic acid, but not of dehydroascorbic acid, was inhibited by glucocorticoids. Isolated secretory granules contained a fairly low concentration of iron but a high concentration of copper.

INTRODUCTION

Biologically active peptides are synthesized as large precursor molecules which undergo a series of processing steps before they achieve full biological activity. The last step, in about 50% of the peptides, involves a peptidylglycyl α-amidating mono-oxygenase (PAM, EC 1.14.17.3). This enzyme has been identified in the pituitary glands of rat, pig and ox (Eipper *et al.*, 1983*a*,*b*; Kizer *et al.*, 1984, 1986; Murthy *et al.*, 1986) and its activity has been found to be present in both (neuro)endocrine and exocrine tissues, e.g. in the hypothalamus (Emeson, 1984) and the parotid gland (von Zastrow *et al.*, 1986; see also Mains *et al.*, 1990). The enzyme has been cloned (Eipper *et al.*, 1987; Ohsuye *et al.*, 1988).

Several peptides in the endocrine pancreas, such as pancreastatin, thyrotropin-releasing hormone (TRH), pancreatic polypeptide (PP) and amylin (IAPP), are *C*-terminally amidated. Ouafik *et al.* (1986) reported the presence of PAM activity in a crude subcellular fraction containing secretory granules from whole pancreata of newborn rats. Later, the presence of PAM activity in secretory granules from Anglerfish islets (Mackin *et al.*, 1987) and fetal and neonatal rat islets (Scharfmann *et al.*, 1988), was reported and the characteristics of the enzyme and its dependence on certain factors (Zhou & Thorn, 1990) was established. The expression of PAM mRNA in pancreas tissue has also been demonstrated (Maltese *et al.*, 1989).

PAM needs ascorbic acid (AA) for activity, as well as copper and molecular oxygen. As highly developed mammals have lost their ability to synthesize AA or can only synthesize it in the liver, this factor must be taken up by cells that produce amidated peptides. Such cells usually have high contents of AA. Carrier-mediated AA/dehydroAA (DHA) uptake into different types of cells has been reported (e.g. Cullen *et al.*, 1986; review by Rose, 1988). Several papers have appeared concerning the possible relationship between amidation in the processing of peptide hormones and pancreas function (Paquette *et al.*, 1981; Ouafik *et al.*, 1986). Besides its role as essential factor for PAM, AA may have other functions in the endocrine pancreas, e.g. as an important factor in neutralizing free radicals, a function which may be disturbed in diabetes (Grankvist *et al.*, 1981). In the present work we have studied some basic aspects of the uptake of AA and DHA into isolated pancreatic islet cells. No previous reports on this topic could be found.

MATERIALS AND METHODS

Materials

L-[^{14}C]Ascorbic acid ([^{14}C]AA) (21 mCi/mmol), and 3-*O*-methyl-D-[^{14}C]glucose ([^{14}C]3-MeGlc) (59 mCi/mmol) were from Amersham. AA was from Merck. D-Isoascorbic acid (iso-AA) and DHA were from Aldrich. Ascorbate oxidase was from Boehringer Mannheim. Carbonyl cyanide *p*-trifluoromethoxyphenylhydrazone (FCCP), ouabain, phloridzin, probenecid, cytochalasin B, amiloride, corticosterone and BSA (fraction V) were from Sigma. Tri-iodothyronine (T_3) was from Glaxo, Greenford, Middx., U.K. Cortisol was from Upjohn, Puurs, Belgium. Hanks balanced salt solution (HBSS), newborn calf serum, RPMI 1640, L-glutamine, penicillin/streptomycin and trypsin were obtained from Gibco. Collagenase was from Boehringer, Mannheim, Germany and DNAase I was from BDH Chemicals.

Islet preparation, culture and cell isolation

Pancreatic islets from newborn Pan–Wistar rats (5–7 days old) were prepared by an enzymic digestion procedure (Brunstedt

Abbreviations used: AA, ascorbic acid; DHA, dehydroascorbic acid; FCCP, carbonyl cyanide *p*-trifluoromethoxyphenyl hydrazone; HBSS, Hanks' balanced salt solution; iso-AA, D-isoascorbic acid; PAM, Peptidylglycine α-amidating mono-oxygenase; PP, pancreatic polypeptide; TRH, thyrotropin-releasing hormone; 3-MeGlc, 3-*O*-methyl-D-glucose; IAPP, islet amyloid polypeptide (amylin); T_3, tri-iodothyronine.

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et al., 1984). The operating tools were sterilized by heating, and media were passed through a 0.22 μm-pore-size filter. After treatment by collagenase, islets were separated manually from exocrine tissue. In some early experiments, when cells from freshly prepared islets were used, the uptake increased almost linearly with increasing [^{14}C]AA concentration. There may have been damaged due to collagenase digestion and the effects of mechanical treatment on cell function (Brunstedt *et al.*, 1984; Zhou *et al.*, 1990). Thus for all experiments on AA or DHA uptake the islets were cultured overnight at 37 °C in RPMI 1640 culture medium (10.1 mM-glucose) supplemented with 2 mM-L-glutamine, 100 units of each of penicillin and streptomycin/ml, 10% (v/v) newborn calf serum, 20 mM-Hepes and 0.035% (w/v) $NaHCO_3$, pH 7.2 (adjusted at room temperature), in order to allow the islets to recover from the damage.

Dispersed cells were prepared from such islets by the procedure of Lernmark *et al.* (1984), which involved a trypsin/DNAase treatment step and a mechanical dispersal step in Ca^{2+}-free medium. Isolated cells were separated from preparation medium by centrifugation and were resuspended in experimental buffer. It was confirmed that over 75% of the cells prepared in this way were viable after a 2 h incubation in experimental buffer, as shown by the eosin exclusion test.

Isolation of secretory granules from islets

Secretory granules from pancreatic islets were prepared by placing islet homogenates on a sucrose gradient and collecting the granule-enriched fraction (Howell *et al.*, 1969).

Uptake experiments

Unless indicated otherwise, all uptake experiments were carried out in modified HBSS without Phenol Red, pH 7.4, containing (mM): $CaCl_2$ 1.26, KCl 5.37, KH_2PO_4 0.44, $MgCl_2$ 0.49, $MgSO_4$ 0.41, NaCl 136.89, $NaHCO_3$ 4.17, Na_2HPO_4 0.34, glucose 5.05 and Hepes 20.00. For the uptake of AA, the buffer also contained 0.5 mM-thiourea to prevent oxidation of AA. A [^{14}C]AA stock solution was made by dissolving [^{14}C]AA in redistilled water, and was stored at −20 °C. [^{14}C]DHA was prepared by incubating [^{14}C]AA with ascorbate oxidase at 37 °C for 20 min in medium without thiourea.

[^{14}C]AA or [^{14}C]DHA was added to the cell suspensions (100 μl incubation volume in routine experiments, containing approx. 1×10^5 cells, corresponding roughly to 9 μg of cellular protein) as indicated in the section. After incubation, cells were rapidly cooled on ice, pelleted in an Airfuge (Beckman) and washed twice with ice-cold medium without isotope. Before the counting of radioactivity, 0.5 ml of NCS tissue solubilizer was added to samples, and 10 min later 10 ml of Lumagel was added.

Analysis of AA content

AA concentrations were determined by using a fluorimetric method (Omaye *et al.*, 1979). Pancreatic tissue or dispersed islet cells were suspended in 5% metaphosphoric acid (10–20%, w/v). After three cycles of freezing–thawing, the samples were homogenized and centrifuged. Serum AA was extracted by mixing serum with 5% metaphosphoric acid (1:10, v/v), vortex-mixing for 30 s and centrifugation (800 *g*, 20 min). The supernatants were used for assay of AA. Before assay, samples were kept at −20 °C for not longer than 1 week (it was shown that AA content remained stable for at least 1 week under these conditions).

Determination of intracellular water space

Measurements were made by the procedure of Kletzien *et al.* (1975), using [^{14}C]3-MeGlc.

Determination of metal ions and protein

Cu^{2+}, Zn^{2+}, iron and Co^{2+} were measured as previously described (Thorn *et al.*, 1986). Protein content was determined by the method of Böhlen *et al.* (1973).

Analysis of AA and DHA by t.l.c.

Since the use of thiourea and similar substances in [^{14}C]AA preparations does not ensure that 100% of the molecules are present as AA (see Thorn *et al.*, 1986, and references therein), we tested this in the present procedure. The analysis was performed using the system employed in Amersham's radiochemical batch analysis [cellulose in ethyl acetate/water/acetic acid/formic acid (18:4:3:1, by vol.)]. By this method we demonstrated that the [^{14}C]AA preparation consisted of 93.5±1.8% ($n = 6$, mean±S.E.M.) of intact [^{14}C]AA before and 78.8% (average of two preparations) after a 2–3 h incubation with cells in a medium containing 0.5 mM-thiourea. The [^{14}C]DHA preparation consisted of 83.9±5.0% ($n = 5$) of [^{14}C]DHA before and 99.5% (assay of one preparation) after incubation (see Fig. 6).

RESULTS

Tissue and serum concentrations of AA during the neonatal period

AA concentrations in homogenates of whole pancreas tissue and in serum during the first 7 days after birth and on day 30 are shown in Fig. 1. The tissue concentration reached a plateau lasting from the third to the fifth day and then fell. The serum concentration showed a progressive fall throughout this period.

In a different set of experiments, the serum concentration of AA of 5–7-day-old rats was 66.8±3.7 μM (mean±S.E.M., $n = 5$; each sample represented the pool from two rats). In the pregnant mothers (on the day before delivery) this concentration was 47.5±6.5 μM ($n = 4$). For the studies of uptake characteristics, rats aged 5–7 days were used.

The cells from freshly prepared islets contained 0.74±0.02 nmol of AA/10^6 cells ($n = 4$, each sample represented the pool of islets from 10 animals). The cells from overnight-cultured islets contained 0.51 nmol of AA/10^6 cells (assay of a pool from 40 animals). Intracellular water space was determined to be 0.21 μl/10^6 cells for fresh islet cells and 0.20 μl/10^6 cells for cultured islet cells. Therefore the intracellular concentration of

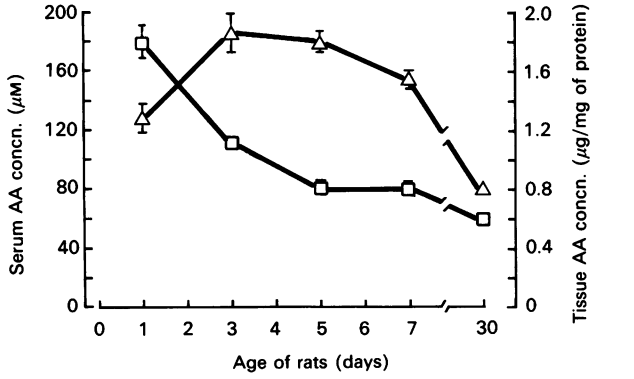

Fig. 1. Changes in pancreatic tissue (△) and serum (□) AA concentrations in the neonatal period

Tissue and blood samples were collected immediately after the decapitation of the rats. Tissue samples were suspended and homogenized in 5% metaphosphoric acid. Sera were diluted with 5% metaphosphoric acid. The suspensions were centrifuged and the supernatants were used for assay of AA. Each point represents the mean of six samples, with S.E.M.s indicated.

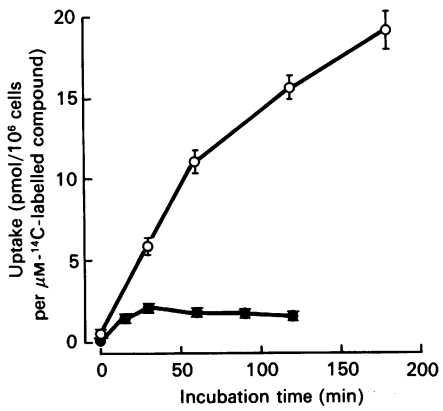

Fig. 2. Time course of uptake of [^{14}C]AA and [^{14}C]DHA into isolated neonatal rat pancreatic islet cells

Cells from overnight-cultured islets from 5–7-day-old rats were incubated with 32 μM-[^{14}C]AA (○) or 104 μM-[^{14}C]DHA (●) in modified HBSS. After different time intervals the cells were rapidly cooled, pelleted, washed and analysed for radioactivity. Means ± S.E.M. of triplicate determinations are shown.

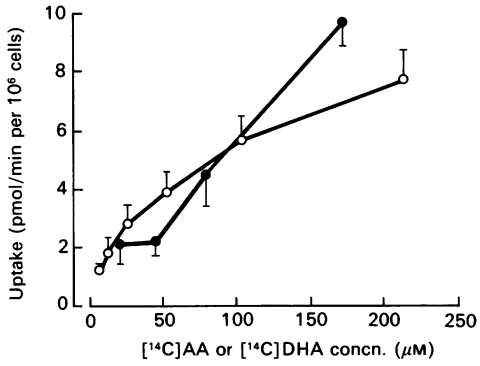

Fig. 3. Concentration-dependence of [^{14}C]AA and [^{14}C]DHA uptake into isolated islet cells

Cells were incubated with different concentrations of [^{14}C]AA (○) or [^{14}C]DHA (●) for 20 min. Means ± S.E.M. of three cultures ([^{14}C]AA uptake) or triplicate assays ([^{14}C]DHA uptake) are shown. Similar results were found in experiments using four other individual sets of cultures for [^{14}C]DHA uptake.

AA would be 3.53 ± 0.10 mM for fresh islet cells and 2.52 mM for overnight-cultured islet cells respectively if a free distribution of AA in intracellular water is assumed.

Characteristics of AA uptake into isolated pancreatic islet cells

When isolated cells were incubated with [^{14}C]AA they accumulated radioactivity in a time-dependent manner (Fig. 2). The uptake rate was linear for at least 30 min from the beginning of the incubation and then gradually decreased. The amount of radioactivity accumulated in the linear phase was proportional to the number of cells in the incubation (results not shown). The ratio of uptake after 30 min at temperatures of 37 °C, 22 °C and 10 °C to that at 0 °C was 12.4, 4.1 and 1.6 respectively (results not shown). The presence of 10 μM-FCCP inhibited uptake by 55% after a 30 min incubation with [^{14}C]AA.

When the concentration of [^{14}C]AA in the incubation medium was increased, the uptake into the cells increased. To ensure that the initial rate of uptake was studied, all of the concentration-dependence experiments involved a 20 min incubation. As shown in Fig. 3, the uptake of [^{14}C]AA seemed to be complex, involving two components: a saturable process, with an estimated K_m of 17.6 ± 0.3 μM and a $V_{max.}$ of 3.9 ± 1.7 pmol/min per 10^6 cells (means ± S.E.M.; results from three independent cultures); and a non-saturable process with an estimated permeability coefficient of 0.02 ± 0.0 μl/min per 10^6 cells. The presence of 310 μM non-radioactive AA or iso-AA in the incubation medium with 90 μM-[^{14}C]AA inhibited uptake by 56% and 25% respectively, whereas unlabelled DHA at the same concentration did not show any inhibitory effect.

AA uptake was increased about 4-fold on changing the pH from 5.0 to 7.0. Amiloride (1 mM) and probenecid (1 mM) decreased uptake by 35% and 22% respectively (results not shown).

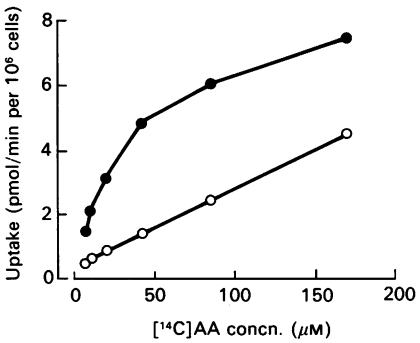

Fig. 4. Influence of extracellular Na^+ on [^{14}C]AA uptake

Cells were incubated with different concentrations of [^{14}C]AA for 20 min in the presences of 142 mM-Na^+ (●) or 0 mM-Na^+ (○) (replaced with 142 mM-Li^+). Other components of the buffer in this experiment were: 4.8 mM-KCl, 2.8 mM-$CaCl_2$, 0.5 mM-thiourea, 5.1 mM-glucose and 20 mM-Hepes (pH 7.4). The kinetic parameters of [^{14}C]AA uptake in this buffer system (when NaCl = 150 mM) were estimated to be: K_m, 9.2 μM; $V_{max.}$, 5.66 pmol/min per 10^6 cells; permeability coefficient, 0.02 μl/min per 10^6 cells (A. Zhou, unpublished work). Similar curves were obtained in a second experiment.

Dependence of AA uptake on Na^+

When Na^+ in the incubation medium was replaced by Li^+, AA uptake decreased and did not show any saturation with increased [^{14}C]AA concentration (Fig. 4). Decreasing the Na^+ concentration resulted in decreased uptake and an increase in the K_m value (see Fig. 4 legend). Uptake in the presence of 50 mM-K^+ and 100 mM-Li^+ in a Na^+-free medium was no different from uptake in medium containing 150 mM-Li^+ (results not shown). Presence of 100 μM-ouabain inhibited the uptake by about 50% after a 30 min incubation with [^{14}C]AA. In a Na^+-free medium there was still an accumulation of radioactivity. However, no inhibitory effect of ouabain was observed under these circumstances.

Effect of glucose on uptake

In preliminary experiments it was noticed that [^{14}C]AA uptake was higher in the presence of higher glucose concentrations. To study the influence of glucose on AA uptake, islet cells were incubated with [^{14}C]AA for 20 min or 2 h in the presence of different concentrations of glucose. As shown in Fig. 5, glucose inhibited AA uptake in a concentration-dependent manner (20 min incubation). Prolonging the incubation time or pre-incubating the cells with glucose all resulted in enhancement in the [^{14}C]AA uptake. When islets had been cultured in a medium with supplemented glucose (final concentration 21 mM) overnight, the uptake of [^{14}C]AA into isolated cells also increased. In contrast with the effect of glucose, 3-MeGlc inhibited [^{14}C]AA

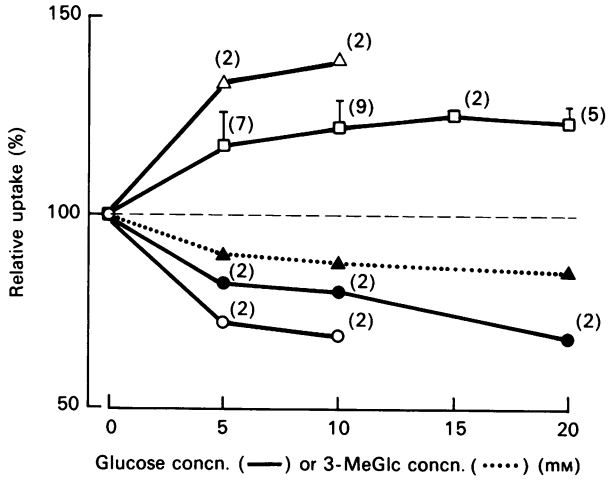

Fig. 5. Influence of extracellular glucose on [^{14}C]AA and [^{14}C]DHA uptake

Isolated cells were incubated with [^{14}C]AA for 20 min (○) or 2 h (□) in the presence of different concentrations of glucose (continuous line). In some experiments cells were preincubated with glucose for 1 h before the 2 h incubation with [^{14}C]AA (△). [^{14}C]DHA uptake (●) was carried out for 1 h after a 2 h preincubation with glucose. The effects of 3-MeGlc on [^{14}C]AA uptake in a 2 h incubation are also presented (broken line). The uptake in the absence of glucose was defined as 100%. For each point the number of experiments is indicated in parentheses.

uptake in a 2 h incubation. Both 2 mM-phloridzin and 50 μM-cytochalasin B (in the presence of glucose) inhibited uptake by 70% (results not shown).

Uptake of DHA

[^{14}C]DHA was taken up by isolated islet cells in a concentration-dependent manner (Fig. 3) without showing any clear saturation (up to 640 μM; results not shown for higher concentrations). Uptake was not influenced by the extracellular Na^+ concentration (results not shown), but was decreased by glucose (Fig. 5). Cytochalasin B (50 μM) inhibited DHA uptake by 80%. Uptake at pH 6.0 or 7.0 was approx. 30% higher than at pH 5.0 or 8.0. Amiloride or probenecid at 1 mM did not decrease uptake.

Subcellular distribution and properties of [^{14}C]AA and [^{14}C]DHA being taken up

To determine whether the accumulation of radioactivity in cell pellets was due to uptake by cells or just to an adsorption of radioactive material to the cell surface, two sets of experiments were carried out. First, the cells were loaded with [^{14}C]AA (2 h) or [^{14}C]DHA (1 h), pelleted, washed with buffer without isotope and subjected to three cycles of freezing and thawing. After this, the soluble cytosolic and membrane fractions were separated by centrifugation (90000 *g* for 10 min). For both [^{14}C]AA and [^{14}C]DHA the majority of the radioactivity being taken up was recovered from the supernatant (cytosolic fraction) (94.4±1.2% for [^{14}C]AA; 82.4±2.9% for [^{14}C]DHA; means±S.E.M. of three incubations). In the second type of experiment, after a 2 h loading with [^{14}C]AA cells were washed and resuspended in a medium containing no isotope. The residual radioactivity in these resuspended cells was determined at different time points from the beginning of resuspension. After suspension for 6 h in non-radioactive medium, approx. 50% of the initial activity in cell pellets was still present (results not shown).

To examine the subcellular distribution of newly taken up [^{14}C]AA, cells loaded with [^{14}C]AA were homogenized and

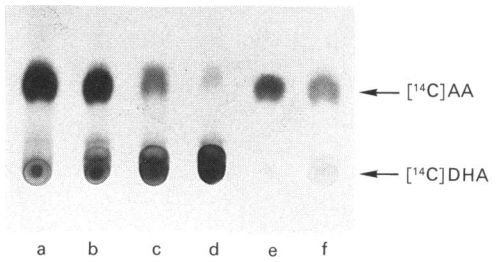

Fig. 6. T.l.c. analysis of [^{14}C]AA and [^{14}C]DHA

The samples were developed in cellulose in ethyl acetate/water/acetic acid/formic acid (18:4:3:1, by vol.). A typical experiment is shown. Shown are: standard [^{14}C]AA preparation before (lane a) and after (lane b) incubation with islet cells; standard [^{14}C]DHA before (lane c) and after (lane d) incubation with islet cells; and cytosolic fraction from cells loaded with 97.5 μM-[^{14}C]AA (lane e) or 72.6 μM-[^{14}C]DHA (lane f) for 1 h. For the analysis of the cytosolic fraction similar results were obtained in two other independent experiments.

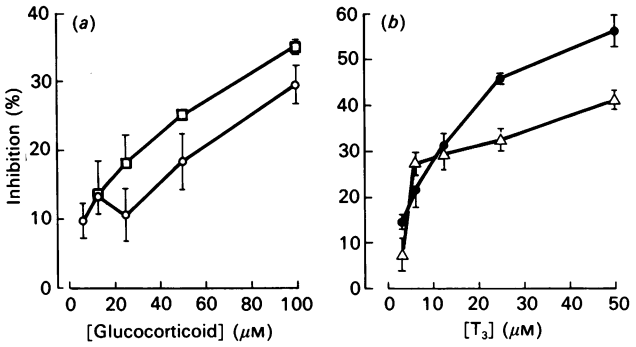

Fig. 7. Effects of glucocorticoids and T_3 on [^{14}C]AA and [^{14}C]DHA uptake

Cells were incubated with different concentrations of (*a*) corticosterone (○; $n = 5$) and cortisol (□; $n = 3$) or (*b*) T_3 (△; $n = 3$) for 2 h. Then [^{14}C]AA was added and uptake was measured over 1 h. The uptake at different hormone concentrations was compared with that with no hormone added. The influence of T_3 on [^{14}C]DHA uptake is also presented (●; $n = 3$). Means±S.E.M. are shown.

fractionated on a pre-generated discontinuous sucrose gradient. Radioactivity was recovered in several fractions, with the highest recovery of radioactivity and highest specific radioactivity (i.e. per mg of protein) being present in a fraction corresponding mainly to mitochondria (Howell *et al.*, 1969).

As shown in Fig. 6 (lanes e and f), [^{14}C]AA was the predominant form in the cytosolic fraction whether the cells had been incubated with [^{14}C]AA or [^{14}C]DHA.

Hormonal regulation of AA and DHA uptake

Glucocorticoids had concentration-dependent inhibitory effects on [^{14}C]AA uptake but not on the uptake of [^{14}C]DHA. T_3 inhibited both [^{14}C]AA and [^{14}C]DHA uptake (Fig. 7). Culturing islets with 1 μM-cortisol for 3 days resulted in a 26.6±0.3% (mean±S.E.M. of three cultures) decrease in uptake of [^{14}C]AA into isolated cells.

Contents of metal ions

Freshly prepared islets contained the following concentrations of metal ions (nmol/mg of protein; single set of analyses on a pool from 30 animals): Zn^{2+}, 3.86; iron, 1.23; Cu^{2+}, 0.027. In the secretory granule preparation (from 30 animals), the concentrations were: Zn^{2+}, 7.06; iron, 0.72; Cu^{2+}, 1.04. Co^{2+} was not detectable in the tissue or granule samples.

DISCUSSION

As the major part of the radioactivity taken up in the present experiments could be recovered from the soluble cytosolic fraction, the ability of islet cells to accumulate AA could be roughly estimated. For example, after a 2 h incubation with 32 μM-[^{14}C]AA, the cellular concentration of [^{14}C]AA calculated from the uptake and the intracellular water space was about 2.39 mM, or more than 70-fold higher than the external medium concentration. This capacity for AA accumulation was of the same order as that of primary culture cells of rat anterior and pars intermedia pituitaries (Cullen *et al.*, 1986), of cultured bovine adrenomedullary chromaffin cells (Diliberto *et al.*, 1983), and of mononuclear leukocytes (Bergsten *et al.*, 1990), when calculated as the uptake rate per unit number of cells. However, estimation of the concentration will be correct only when AA is freely distributed in the intracellular water phase, which has not been studied thoroughly in any of the systems. Binding of AA to intracellular components and compartmentation of newly taken up AA have been reported (Kabrt, 1982; Knoth *et al.*, 1987; see also present results).

The inhibitory effects of FCCP and of low temperatures and the presence of a saturable phase in the uptake of AA indicate that an energy-dependent carrier-mediated transport system is involved. A K_m of 17.6 μM in the saturable phase implies that there is a high-affinity transport system in rat islet cells.

Na^+-dependent AA uptake has been found in different tissues (for references, see Rose, 1988). Our results seem to be in agreement with the hypothesis that a Na^+/AA symport exists in rat islet cells. First, the uptake of AA was specifically dependent on Na^+. Neither Li^+ nor K^+ could replace Na^+, and ouabain showed an inhibitory effect only in the presence of Na^+. Secondly, different extracellular Na^+ concentrations resulted in different K_m values, indicating an interaction between Na^+ and the AA transporter. Furthermore, Na^+-dependent AA uptake seemed to be coupled to Na^+–H^+ exchange, as shown by the pH-dependence of AA uptake and the inhibitory effect of amiloride (an inhibitor of Na^+/H^+ antiports).

The fact that there was still uptake to a certain extent in Na^+-free medium might be related to the fact that there are two mechanisms involved: a carrier-mediated one, possibly a Na^+/AA symporter, and a diffusion process which is independent of Na^+. In an estimation of uptake by the unsaturable process (Fig. 3), the calculated result was very close to what was observed at '0 mM'-Na^+ (Fig. 4). Similar patterns of uptake (biphasic) have been reported in other types of cells (Moser, 1988). With regard to the mechanism of AA transport, an ion-pair seemed to be formed between AA (being negatively charged at physiological pH) and Na^+, as indicated by the effect of probenecid on AA uptake.

AA and glucose resemble each other structurally. Although in rat islet cells the characteristics of AA uptake are similar to those of glucose transport in some aspects (e.g. inhibited by cytochalasin B, a specific inhibitor of the glucose facilitated diffusion transporter), there is not enough evidence to confirm that AA and glucose share the same transporter. Mammalian cell glucose transporters have been divided into two classes: facilitative diffusion transporters and Na^+/glucose cotransporters. Since it has been stated that only the facilitative diffusion transporter of glucose is found in endocrine pancreas (Bell *et al.*, 1989), it is likely that AA is taken up by a specific transporting system, whereas DHA is transported via the glucose carrier in this tissue. The latter transport might be affected by changes in this glucose transport system in diabetes (Thorens *et al.*, 1990).

Our knowledge of the molecular properties of putative Na^+-dependent AA transporter(s) is poor. In bovine adrenal chromaffin cells (Diliberto, *et al.*, 1983) and pars intermedia cells (Zhou *et al.*, 1990), AA uptake was inhibited by phloridzin but not by glucose. On the other hand, in small intestine brush border (Siliprandi *et al.*, 1979) and kidney cortex brush border (Toggenburger *et al.*, 1981), AA uptake was not depressed by phloridzin, but was inhibited by glucose. In bovine pituitary neurosecretosomes, AA uptake was inhibited by both phloridzin and glucose. Whether this diversity indicates the existence of multiple forms of AA transporter in different tissues or is due to the differences in experimental conditions is not clear.

The effect of glucose on AA/DHA uptake in islet cells may be of interest in elucidating their function. AA uptake was inhibited by glucose during short-term incubations but was enhanced during prolonged incubation experiments, whereas 3-MeGlu, a non-metabolizable analogue of glucose, inhibited the uptake in long-term incubations, suggesting that the stimulatory effect of glucose is a metabolic one. We imagine that this may be important in the regulation of islet cell function, e.g. through regulating the production of peptide amides such as pancreastatin (Tatemoto *et al.*, 1986) and IAPP (Nishi *et al.*, 1990). If hyperglycaemia enhances AA uptake into islet cells, one may expect an overproduction of these amidated peptides in type I diabetes. On the other hand, since IAPP appears to be co-secreted with insulin, it may be speculated that the hyperactivity of the β-cells in type II diabetes may lead to insufficiency in AA availability (Som *et al.*, 1981; Rose, 1986) and defective amidation of IAPP, possibly resulting in precipitation as islet amyloid, one of the characteristics in type II diabetic patients.

Our results that AA was predominant in the cytosolic fraction whether cells had been incubated with [^{14}C]AA or [^{14}C]DHA, and that high specific radioactivity was located to the mitochondrial fraction after cells had been loaded with [^{14}C]AA, suggest that a DHA → AA reducing system exists and that compartmentation took place for newly taken up AA. It is noteworthy that the concentration of Cu^{2+} is enriched in the islet granule fraction where the peptide amidation reaction takes place. The concentration of iron (a component of cytochrome b_{561}) in islet granules is about 10 times lower than in secretory granules from ox neurohypophyses (Thorn *et al.*, 1986), where cytochrome b_{561} as well as PAM activity and AA uptake have been shown to be present.

We have little information on the mechanisms of the inhibitory effects of steroids or T_3 on AA/DHA uptake. Whether this is a direct effect or is secondary to inhibition of glucose uptake (A. Zhou & N. A. Thorn, unpublished work) is not yet clear. Similar effects have been observed in bovine neurohypophysis (Thorn *et al.*, 1991), where the effect of cortisol, but not that of T_3, was shown to be dependent on the presence of Na^+.

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