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The intracellular handling of insulin-related peptides in isolated pancreatic islets

Evidence for differential rates of degradation of insulin and C-peptide

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Islets of Langerhans isolated from adult rats were maintained in tissue culture for 3 days in the continued presence of [^{3}H]leucine. Labelled proinsulin, C-peptide and insulin were measured by quantitative h.p.l.c., a method which also allowed for resolution of C-peptide I and II, and of insulin I and II (the products of the two rat insulin genes). The results showed that: (1) at early times, proinsulin was the major radiolabelled product; with progressive time in culture, intra-islet levels of [^{3}H]proinsulin decreased, despite continuous labelling with [^{3}H]leucine, indicating that the combined rates of proinsulin conversion into insulin and of proinsulin release, exceeded the rate of synthesis; (2) insulin I levels were always greater than those of insulin II, both in the islets and for products released to the medium; (3) the molar ratio of [^{3}H]insulin I and II to their respective ^{3}H-labelled C-peptides increased with time for products retained within islets, reaching a value close to 3:1 by 3 days; by contrast, for products released to the medium during the culture period, the ratio was always close to unity; (4) when islets were incubated with [^{3}H]leucine for 2 days, and then left for a further 1 day without label (chase period), the intra-islet [^{3}H]insulin/[^{3}H]C-peptide ratios rose to values as high as 9:1. Again, for material released to the medium, the values were close to 1:1; (5) it is concluded that C-peptide is degraded more rapidly than insulin within islet cells, thereby accounting for the elevated insulin/C-peptide ratios. The difference between the ratios observed in the islets and those for material released to the medium is taken to indicate that degradation occurs in a discrete cellular compartment and not in the secretory granule itself.

INTRODUCTION

Insulin production by the pancreatic B-cell consists of a series of intracellular events encompassing biosynthesis, precursor processing and packaging into secretory granules, and exocytosis of granule contents [1,2]. On the basis of both morphological [3–5] and biochemical [6,7] studies, it is evident that insulin production is counterbalanced by its degradation within the B-cell itself. The intracellular degradation of insulin is thought to proceed primarily by crinophagy [8,9] (fusion of granules with primary lysosomes) [3–5,10], a pathway probably common to most secretory-cell types [11,12].

Morphological studies have shown the presence of insulin, but not of the other known granule constituents proinsulin and C-peptide, in multigranular bodies (secondary lysosomes representing the degradative compartment) in B-cells [5]. Since insulin is known to form crystals within the secretory granules of B-cells from most species, including the rat [13], we suggested [5] that the insulin crystal would be relatively stable even after its introduction into lysosomes, thereby protecting the insulin molecules from rapid degradation. Proinsulin and C-peptide, neither of which can form crystals, would, by contrast, be expected to be rapidly degraded. The partial resistance of the insulin crystal towards lysosomal enzymes has been further supported by studies *in vitro* [14], but the hypothesis of differential rates of degradation of insulin, C-peptide or proinsulin within the B-cell itself has yet to be confirmed directly. This is largely due to the lack of appropriate assay techniques. We have now combined long-term labelling of islet-cell proteins in culture [6,7] with an h.p.l.c. technique that allows for quantitative analysis of proinsulin, C-peptide and insulin [15,16]. The results of the present study provide dramatic evidence for insulin being degraded less rapidly than C-peptide within the pancreatic B-cell.

EXPERIMENTAL

Animals

Male Sprague–Dawley rats (180–200 g; Charles River Laboratories, Wilmington, MA, U.S.A.) were allowed free access to water and standard laboratory chow.

Materials

Unless otherwise stated, all biochemical reagents were purchased from Fisher Scientific, Lexington, MA,

Abbreviation used: BSA, bovine serum albumin.

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U.S.A., or Sigma Chemical Co., St. Louis, MO, U.S.A., and were of the highest grade or purity available. L-[4,5-^{3}H]Leucine (141–153 Ci/mmol) was from Amersham International, Amersham, Bucks., U.K., and Biocount scintillation cocktail was from Research Products International, Mount Prospect, IL, U.S.A. Tissue-culture media TC 199 and modified RPMI 1640, as well as newborn-calf serum, were from Irvine Scientific, Santa Ana, CA, U.S.A. Collagenase (Type IV, 165 units/mg) was obtained from Cooper Biomedical, Malvern, PA, U.S.A.

Rat pancreatic-islet isolation

Rat islets were isolated by collagenase digestion as previously described in detail [17]. In brief, the method involved distension *in situ* of the pancreas by injecting collagenase through the pancreatic duct, followed by dissection and incubation at 37 °C. After washing the pancreatic digest, islets were picked away from the digested exocrine material under a dissection microscope (not by a discontinuous Ficoll gradient as previously described [17]). Freshly isolated islets were then used for studies *in vitro* directly after isolation.

Maintenance and radiolabelling of islets *in vitro*

(*a*) Continuous labelling with [^{3}H]leucine for 3 days. Approx. 500–600 isolated rat islets were suspended in 4 ml of modified tissue culture medium [RPMI 1640, containing 10% (v/v) newborn-calf serum, glucose (11.2 mM), penicillin (100 units/ml), streptomycin (10 μg/ml) and 0.5 mCi of L-[4,5-^{3}H]leucine]. In order to obtain adequate incorporation of [^{3}H]leucine into islet-cell protein, the concentration of unlabelled leucine in the RPMI 1640 was decreased 20-fold from 0.38 to 0.02 mM.

The islets were maintained in tissue culture at 37 °C in a humidified atmosphere of air/CO_2(19:1) in an incubator (Queue Systems, Parkersburg, WV, U.S.A.) for up to 72 h. Samples of either 100 freshly isolated islets (zero time) or 100 islets removed from culture after 2, 6, 24, 48, or 72 h were taken in a 50 μl aliquot. These islets were then washed with phosphate-buffered saline (10 mM-phosphate/0.14 M-NaCl, pH 7.4) containing 0.1% (w/v) bovine serum albumin (BSA) by centrifugation in 1.5 ml Microfuge tubes (Bio-Rad, Richmond, CA, U.S.A.) at 1500 *g* for 1 min in a bench-top clinical centrifuge (International Equipment Co., Needham Heights, MA, U.S.A.). The supernatant was discarded and the islets resuspended in 100 μl of 0.1 M-HCl/0.1% (w/v) BSA. These islet samples were sonicated [25 W; 10 s; Branson (Danbury, CT, U.S.A.) Sonifier, model B15-P] and centrifuged [10000 *g*, 2 min; Beckman Instruments (Palo Alto, CA, U.S.A.) Microfuge]. The pellet was discarded and the supernatant stored at −20 °C pending analysis.

(*b*) 2-Day labelling and 1-day chase. Islets were maintained in culture in the presence of [^{3}H]leucine as described above, except that the glucose concentration was lowered to 8.3 mM and the labelling was terminated at 2 days. The islets were washed in RPMI 1640/2.8 mM-glucose/10% newborn-calf serum, containing 0.38 mM-unlabelled leucine. Some islets were set aside for analysis (washing and extraction as above). The remaining islets were maintained for a further 1 day in tissue culture, using the same medium as that used for washing. For this chase period, 200 islets were suspended in 0.6 ml of the medium in one well of a 24-well plastic culture plate. At the end of the chase period, all of the islets were harvested and then washed and extracted in HCl/BSA as described above. The chase-period culture medium was centrifuged (10000 *g*, 2 min) to remove cell debris before analysis.

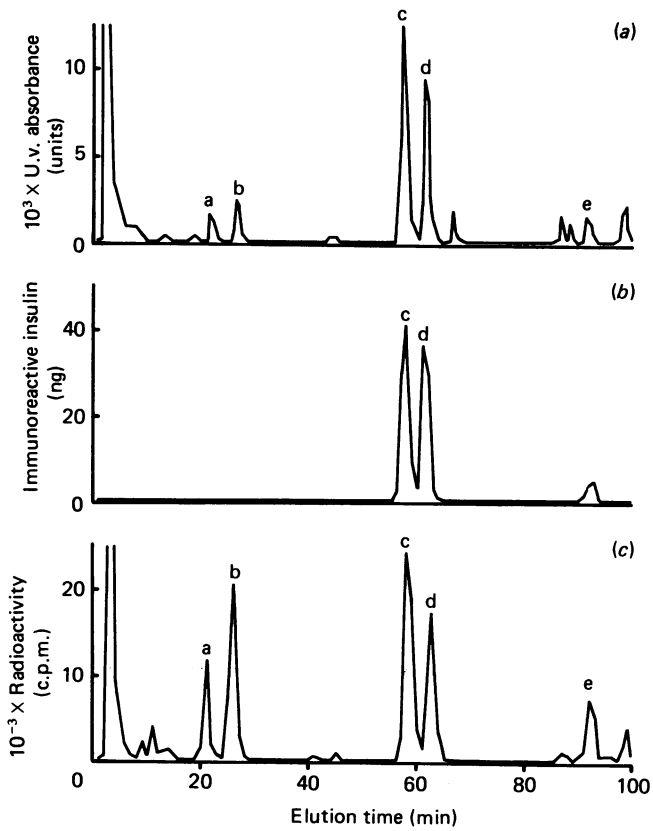

Fig. 1. Elution profile for the separation of rat proinsulins, insulin I and II and C-peptides I and II from isolated islets

The h.p.l.c. analysis of sonicated rat islets or medium is described in the Experimental section. Results in this Figure refer to a 10 μl portion of islets sonicated (100 islets/100 μl of HCl/BSA) after an incubation *in vitro* for 24 h with [^{3}H]leucine. The elution profile depicts u.v. absorbance at 213 nm in (*a*), insulin immunoreactivity in (*b*), and radiolabel incorporation in (*c*). a, Rat C-peptide II; b, C-peptide I; c, insulin I; d, insulin II; and e, proinsulins (which are eluted together).

Analysis of islet and medium samples

The total insulin immunoreactivity in portions of chase medium, sonicated islet samples and fractions collected from h.p.l.c. was measured by radioimmunoassay [18], an equal mixture of rat I and II insulin being used as the standard (generously provided by Eli Lilly and Co., Indianapolis, IN, U.S.A.). In order to quantify islet insulin content and secretion relative to islet cell number, islet DNA content was determined by a fluorescent DNA assay [19].

Separation of rat proinsulins, insulins I and II, and C-peptides I and II (Fig. 1) was achieved by a modification of our h.p.l.c. technique for proinsulin/insulin separation [15,16]. In brief, a Beckman model-332 h.p.l.c. system was used with an Altex Ultrasphere ODS 5 μm column (Beckman, Altex Division, San Ramon,

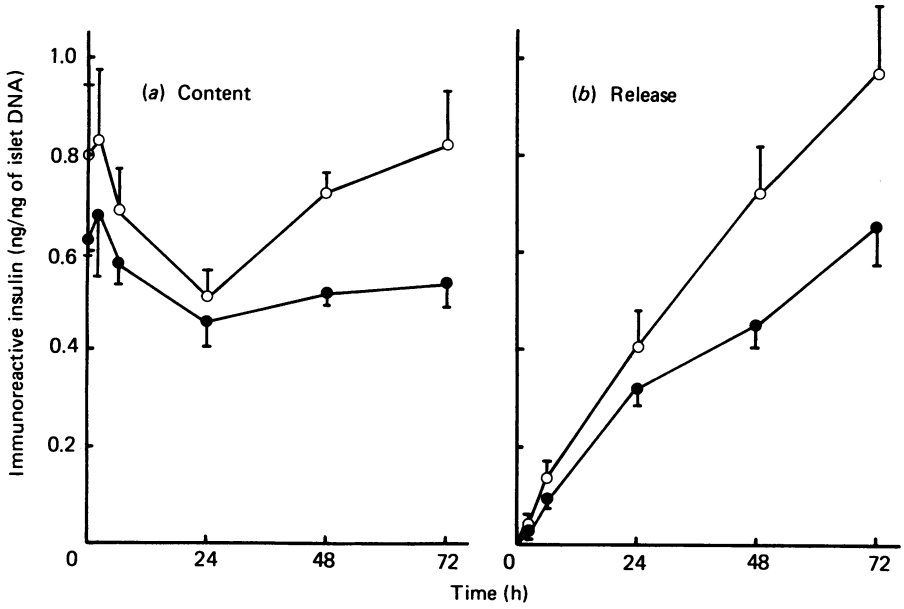

Fig. 2. Total immunoreactive insulin in islets and medium during the 3-day labelling period

Total immunoreactive rat insulins I and II were first separated by h.p.l.c. and then assayed by radioimmunoassay. Islet insulin content (*a*) and release (*a*) were related to islet DNA content (see the Experimental section). Mean values ± S.E.M. are shown for three individual observations for rat insulins I (○) and II (●).

CA, U.S.A.). U.v. absorption was monitored at 213 nm with a model LC-90 detector connected with a RC50 pen recorder (Perkin–Elmer, Norwalk, CT, U.S.A.). The two buffers used were as follows: (1) Buffer A (TEAP), 5 mM-H_3PO_4/20 mM-triethylamine/50 mM-$NaClO_4$, adjusted to pH 3.0 with 5 M-NaOH; (2) Buffer B (H_2O/ACN), acetonitrile/water (9:1, v/v). The flow rate was 1 ml/min, and 1 min fractions were collected. Radioactivity and insulin immunoreactivity in these fractions were determined as described previously [15,16]. The rat C-peptides were separated by isocratic elution at 31.2% buffer B over 30 min (C-peptide II being eluted before I). Buffer B was then increased to 35.0% over 5 min, and the two rat insulins were then separated isocratically over the next 40 min (insulin I being eluted before II). Buffer B was finally increased to 39.0% over 5 min, and the rat proinsulins were eluted together isocratically over 20 min. It has not proved possible in our hands to resolve rat proinsulins I and II with this reversed-phase system. The column was then washed as previously described [16]. A representative elution profile of an extract of rat islets incubated with [^{3}H]leucine for 24 h is shown in Fig. 1, with the peaks for rat C-peptides, insulins and proinsulins indicated.

Presentation of results

The results are means ± S.E.M. for the numbers of observations indicated. Statistical significance for differences between experimental groups was determined by Student's two-tailed unpaired *t* test.

RESULTS

Analysis of rat insulins and related products by h.p.l.c.

Quantitative h.p.l.c. analysis used in the present study was a further refinement of our previously described method [15,16] and by means of which the rat C-peptides have now been additionally separated. A representative h.p.l.c. elution profile is shown (Fig. 1) for a portion of sonicated islets after their incubation with [^{3}H]leucine for 24 h. As indicated in Fig. 1, C-peptide II was eluted between 19 and 24 min, C-peptide I between 25 and 30 min, insulin I between 57 and 60 min, insulin II between 61 and 65 min, and both rat proinsulins (which were eluted together in this h.p.l.c. system) between 90 and 95 min. Unlike the insulins and proinsulins, the rat C-peptides were not detected by insulin radioimmunoassay. The ratio between the insulins and proinsulins determined by radiolabel incorporation (at 72 h 16:1), compared with the same ratio determined by insulin immunoreactivity (at 72 h 32:1), indicates approx. 50% cross-reactivity of the anti-insulin serum for proinsulin in this radioimmunoassay system.

Total (immunoreactive) rat insulins I and II in islets and medium

Owing to the variation in insulin content during maintenance *in vitro*, the DNA content of the islets was used as an index for data presentation. The mean DNA content of the islets in this study was 34.0 ± 5.24 ($n = 3$) ng of DNA/islet (for freshly isolated islets) and 31.9 ± 2.45 ($n = 15$) ng of DNA/islet for islets maintained for 72 h in tissue culture. This confirms the previous findings that the DNA content of isolated islets remains remarkably constant under a wide variety of conditions [19].

Measurements of the total immunoreactive rat insulin I and II content of freshly isolated islets revealed 1.26 times more rat insulin I than II (zero-time value, Fig. 2). During the first 24 h of maintenance *in vitro* there was a small and parallel decrease in the islet insulin I and II content (Fig. 2), followed by an increase during the remaining 48 h in tissue culture.

The release of immunoreactive insulin I and II from

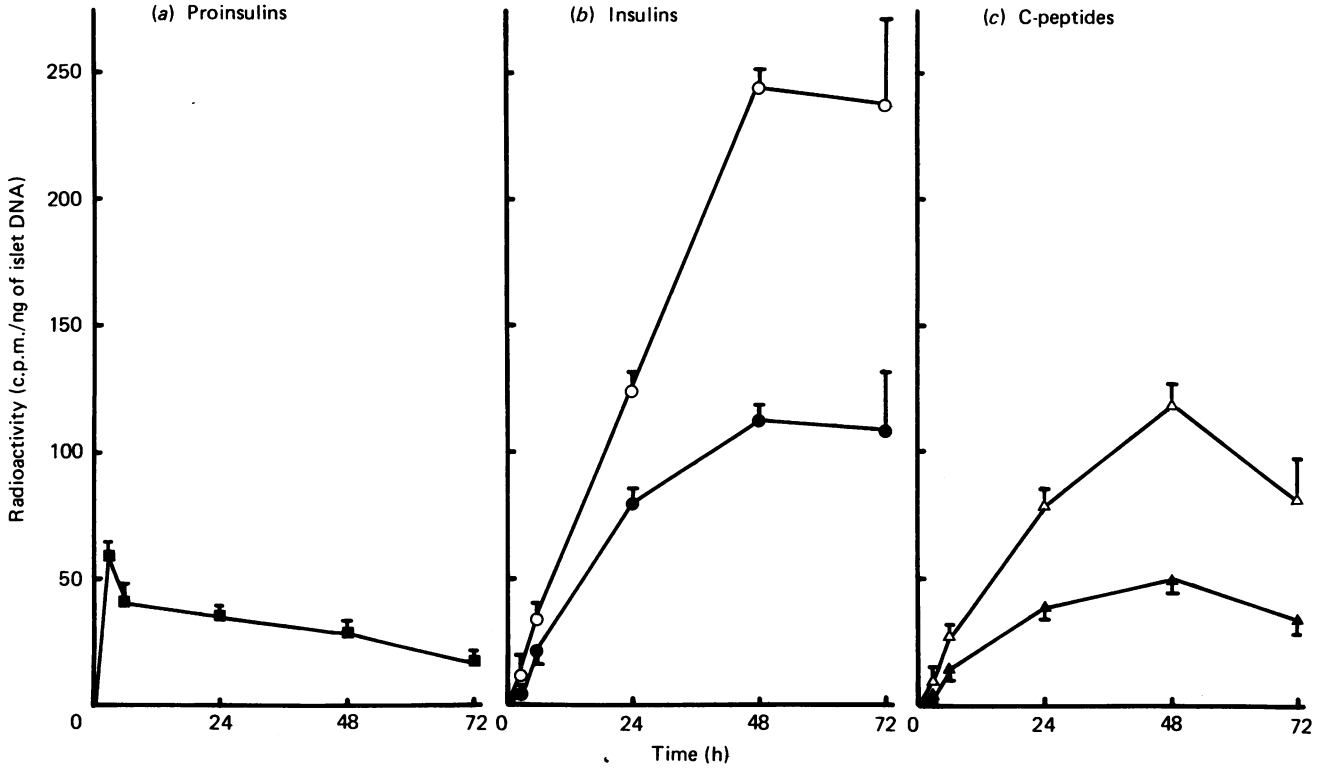

Fig. 3. Incorporation of [^{3}H]leucine into rat (*a*) proinsulins, (*b*) insulins and (*c*) C-peptides in isolated rat islets during 3-day labelling period

Rat islets were maintained *in vitro* for up to 72 h in the presence of [^{3}H]leucine. Incorporation into rat proinsulins (■), insulin I (○), insulin II (●), C-peptide I (△) and C-peptide II (▲) was determined by quantitative h.p.l.c. analysis (see the Experimental section). Mean values ± S.E.M. for three individual observations are shown.

Table 1. Molar ratio between equivalent rat insulins and C-peptides in islets and medium during continuous labelling for 3 days

Insulin/C-peptide ratios were determined by h.p.l.c. analysis of [^{3}H]leucine incorporation into rat insulins and C-peptides (see the Experimental section). To obtain a molar ratio, the incorporation into C-peptide has been adjusted by 6/5, as insulin I and II contain six leucine residues, and C-peptides I and II five leucine residues, in their sequences. Mean values ± S.E.M. are shown for three separate experiments. Levels of significance for difference between an islet value and its 'medium' value: $*P < 0.05$; $**P < 0.01$; $***P < 0.005$.

	Insulin/C-peptide molar ratio				
Time (h)...	2	6	24	48	72
Insulin I/C-peptide I					
Islets	1.09±0.07	1.02±0.04	1.30±0.06**	1.82±0.13*	3.18±0.20***
Medium	1.03±0.03	0.98±0.02	0.96±0.05	1.18±0.09	1.16±0.12
Insulin II/C-peptide II					
Islets	1.09±0.12	1.18±0.21	1.57±0.15**	1.91±0.20**	2.96±0.24***
Medium	1.02±0.11	1.02±0.08	0.95±0.04	0.97±0.06	1.00±0.06

the islets was essentially linear with time. The release of insulin I and II appeared to reflect the islet content of both insulins, with more insulin I than II being released at all times.

Continuous (3 days) radiolabelling of proinsulins, insulin I and II, and C-peptides I and II

In the islets that were labelled continuously for 3 days, incorporation of [^{3}H]leucine into rat proinsulins, insulins I and II, and C-peptides I and II, indicated that, at 2 h, there was markedly more incorporation into proinsulins (65.3%) than into insulins or C-peptides (Fig. 3). With further time in culture, the level of [^{3}H]proinsulin decreased. The [^{3}H]insulins I and II in the islets progressively increased with time, reaching a plateau between 48 and 72 h. At all times, there was more [^{3}H]insulin I than [^{3}H]insulin II, and the ratio between labelled insulin I and II tended to be higher than that for immunoreactive insulins I and II (compare Figs. 2 and 3).

Since rat insulins I and II both contain six, and C-peptide I and II five, leucine residues in their sequences [20,21], a 6/5 correction factor was applied to calculate the molar ratio of both [^{3}H]insulins compared with their equivalent [^{3}H]C-peptides at all times during maintenance *in vitro* (Table 1). Radiolabelling of C-peptides I and II increased up to 48 h, with more incorporation into C-peptide I than into II (Fig. 3). At 2 and 6 h, equimolar amounts of corresponding [^{3}H]insulins and [^{3}H]C-peptides were measured (Table 1). However, unlike the released labelled products (see below), increasingly more [^{3}H]insulin I and II relative to the respective [^{3}H]C-peptides was found in the islets from 24 h onwards, as reflected by the increasing [^{3}H]insulin-to-[^{3}H]C-peptide molar ratios (Table 1).

The release of [^{3}H]proinsulins, [^{3}H]insulin I and II and [^{3}H]C-peptides I and II from the islets during the 3 days labelling period is shown in Fig. 4. Even though there was release of [^{3}H]proinsulins from islets with continuing maintenance *in vitro*, the contribution of [^{3}H]proinsulins to the total released material decreased from 28.9% at 2 h to 7.7% between 48 and 72 h (Fig. 4). The rate of

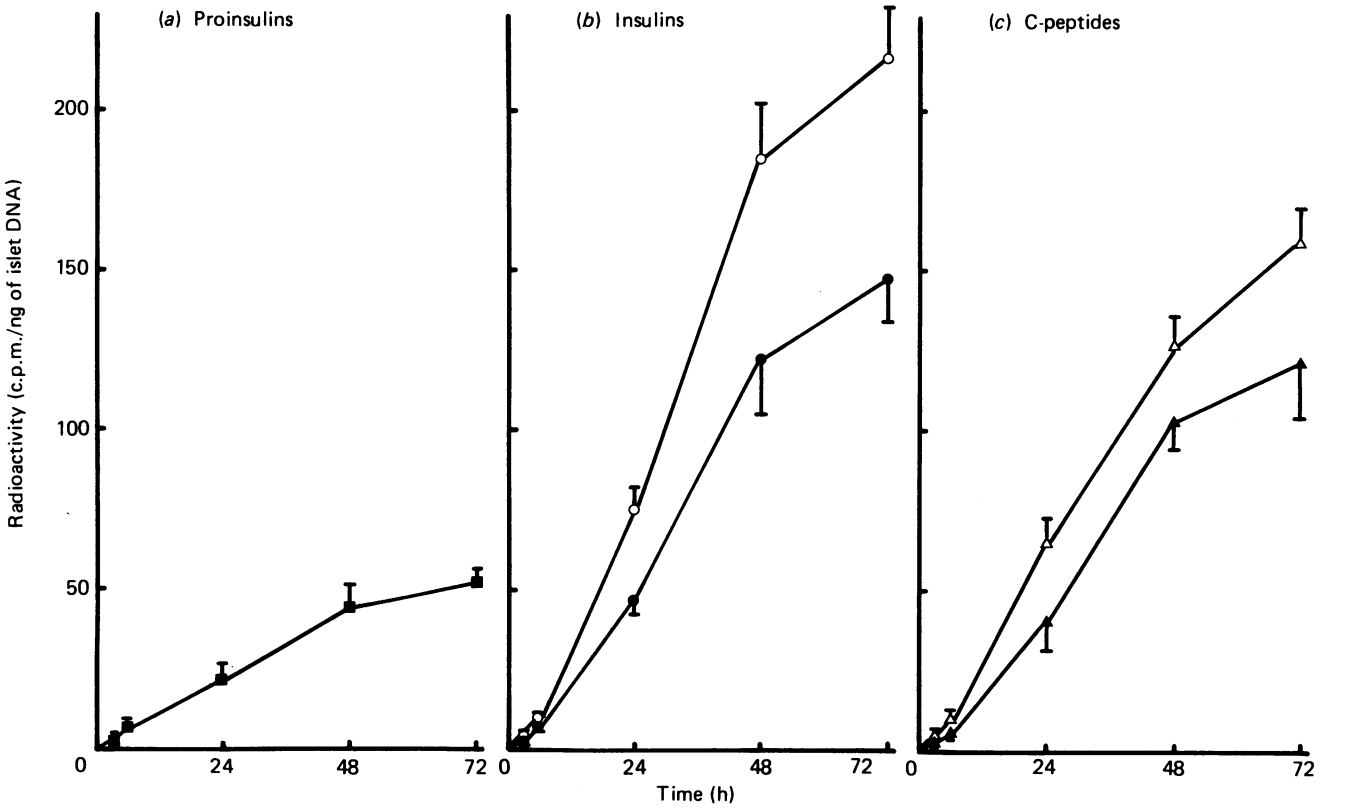

Fig. 4. Release of radiolabelled rat (*a*) proinsulins, (*b*) insulins and (*c*) C-peptides from rat islets during a 3-day labelling period

Rat islets were maintained *in vitro* for up to 72 h in the presence of [^{3}H]leucine. Release of radiolabelled rat proinsulins (■), insulin I (○), insulin II (●), C-peptide I (△) and C-peptide II (▲) was determined by quantitative h.p.l.c. analysis (see the Experimental section). Mean values ± S.E.M. for three individual observations are shown.

Table 2. Radioactivity associated with C-peptides I and II and insulins I and II in islets and medium after a 2-day labelling period with a 1-day chase period

Results are presented as in Table 1.

	Radioactivity (c.p.m./ng of islet DNA)				Insulin/C-peptide ratio	
	C-peptide		Insulin			
	I	II	I	II	Insulin I / C-peptide I	Insulin II / C-peptide II
2-Day labelled islets	66±2.7	25±0.7	222±6.7	105±13	2.84±0.14	3.53±0.49
1-Day chase						
Islets	13±1	4.9±0.3	119±18	55±3.2	7.57±0.55	9.23±0.70
Medium	24±3.9	8.9±1.1	35±6.3	11±2.0	1.18±0.02	1.06±0.06

release of [^{3}H]insulins and [^{3}H]C-peptides from islets was more rapid than that of the [^{3}H]proinsulins (Fig. 4). More [^{3}H]insulin I than [^{3}H]insulin II was released, and this was accompanied by an equivalent excess of released [^{3}H]C-peptide I compared with C-peptide II (Fig. 4). At all time points, the molar ratio of C-peptide to insulin in the medium was close to 1:1 (Table 1).

2-Day labelling of islets followed by 1-day chase

After 2 days of labelling with [^{3}H]leucine at 8.3 mM-glucose (compared with 11.2 mM for the 3-day labelling protocol), the islets again contained approximately twice as much labelled insulin I as insulin II (Table 2). The insulin/C-peptide ratios were elevated at 2 days and rose to even higher values in the islets by the end of the subsequent 1-day chase period (Table 2). By contrast, the ratios of insulin to C-peptide released to the medium during the chase period remained close to 1:1.

DISCUSSION

In the pancreatic B-cell, the initial product of translation of the insulin gene is preproinsulin, which is rapidly converted into proinsulin [22]. Proinsulin itself and the products of its proteolytic processing (conversion into insulin and C-peptide) are thus the molecules of primary interest when studying the differentiated function of pancreatic B-cells. Islets of Langerhans from rats have proved useful for such studies, principally because they are relatively easy to obtain (albeit in very limited amounts for biochemical work) and because they can be maintained in tissue culture for several days [23–26], which allows for long-term biosynthetic labelling of insulin and related peptides [6,7]. An additional feature of both interest and complication is that rats [27,28] and mice [29,30] have two insulins. These insulins are encoded by two non-allelic genes [31,32] and, in the rat, the preproinsulin molecules differ by a total of seven residues (three in the signal sequence, two in the C-peptide and two in the B-chain) [20,21]. For a complete understanding of rat B-cell function, it will be necessary to monitor the production and subsequent fate of all insulin-related peptides generated by both genes. To date this has not been possible, since assays for the independent measurement of the two rat proinsulins, insulins and C-peptides have not been available in any single laboratory. In the present study we have depended upon biosynthetic labelling of rat islets for two reasons. First, this has enabled us to study both newly synthesized and stored products, depending on the time points selected. Secondly, and more significantly, after the incorporation of a radioactively labelled amino acid, insulin and related peptides can be measured by quantitative h.p.l.c. [15,16]. In this way it has proved possible to measure the levels of C-peptide I and II, and of insulin I and II. The only limitation of the methodology at present is our inability to resolve proinsulin I from II.

During the early time points of the 3-day biolabelling period [^{3}H]proinsulin, as expected, was a major islet constituent. At later times, the amount of labelled precursor relative to [^{3}H]insulin or C-peptide decreased within the islets, as indeed did the absolute amount of [^{3}H]proinsulin. The balance between proinsulin synthesis on the one hand and its loss from B-cells on the other (by conversion and release or degradation) will govern the steady-state level of proinsulin in the B-cell. The gradual decline in islet [^{3}H]proinsulin content as from 2 h indicates that the rate of loss exceeds that of synthesis under the conditions of the present study. Proinsulin-to-insulin conversion is, indeed, a rapid process, occurring with a half-time of approx. 45 min [33], and it would appear that most of the proinsulin molecules in the islet cells in the present study were subject to conversion. It has furthermore been shown that islets incubated for long periods at high glucose concentrations (as here) convert proinsulin to insulin at an accelerated rate [34]. Only a limited amount of proinsulin was released from the islets. The amount of proinsulin degraded within B-cells is not yet clear. Certainly no proinsulin can be detected within multigranular bodies (secondary lysosomes) [5]. It must therefore be assumed that proinsulin is either degraded very rapidly once introduced into lysosomes [5] or, alternatively, that granules are not subject to crinophagy (granulolysis) before proinsulin is converted into insulin with the concomitant maturation of clathrin-coated to uncoated granules [2]. It has been shown, however, that if proinsulin-to-insulin conversion [35,36] and granule maturation [37] are blocked by incorporation of amino-acid-analogue islet proteins, the analogue-modified proinsulin is degraded rapidly within the B-cell [36].

Recent evidence would suggest that the synthesis and subsequent release of the two rat insulins occurs in tandem and with co-ordinate control [15,38,39]. It has been shown, however, that there is slightly more mRNA for insulin I than II in native islets [38], and this was reflected in the relative amounts of immunoreactive insulin I and II in the islets. Intriguingly, the ratio of [^{3}H]-insulin I to II was higher than that for immunoreactive insulin I to II (compare Figs. 2 and 3), even at late time points when the labelled and total (immunoreactive) insulin should be representative of a common pool. Although we have no explanation for this apparent discrepancy at present, it will be interesting to discover by further experiments whether there may indeed be some difference in the intracellular handling of insulin I and II which has yet to be exposed.

The conversion of proinsulin is known to produce equimolar amounts of insulin and C-peptide [33], and conversion occurs within the secretory granule itself [1,2]. In turn, the release of insulin and related products from the B-cell is thought to arise exclusively by exocytosis of granule contents [1,2]. The ratio of insulin to C-peptide in secretory granules and, by extension, in material released by exocytosis from B-cells, should therefore be equimolar. This was indeed found to be the case under all conditions in the present study, and we infer from this that insulin and C-peptide are not extensively modified or degraded within secretory granules or, if they are, that such a process occurs at the same rate for both insulin and C-peptide (a possible, but unlikely, scenario). Strikingly, the ratio of insulin to C-peptide within the islet cells increased from a value close to 1:1 at early times of labelling to much higher values by 3 days. If, as we have suggested [5,14], insulin, on account of its crystal state, is degraded much less rapidly than non-crystalline C-peptide after its introduction into B-cell lysosomes, a modest yet progressive rise in the insulin/C-peptide ratio would be predicted, reflecting the channelling of some granules towards the degradative compartment. The molar ratio of labelled insulin to C-

peptide observed at the end of the 3-day labelling period was close to 3:1. This value reflects both the granules contained in these islets, with their equimolar ratio of insulin to C-peptide, and multigranular bodies, with their elevated insulin/C-peptide ratio due to rapid C-peptide degradation in the face of only slow insulin degradation. The absolute value of 3:1 would suggest that there was twice as much labelled insulin in lysosomes (multigranular bodies) than in granules. On the basis of previous findings [5], this value is unusually high and would suggest that labelled insulin was not being synthesized at a constant rate throughout the 3-day labelling period. Any reduction in the rate of labelled insulin synthesis with time would result in a reduction in the amount of labelled products in the granule compartment with a consequent increase in the labelled insulin/C-peptide ratio in the islets. Such a reduction in the rate of labelled insulin synthesis could be due to either isotopic dilution of the [^{3}H]leucine during the 3-day labelling period or an absolute decrease in the total available leucine. If newly synthesized labelled products were no longer being generated at all, as was the case during the 1-day chase after a 2-day labelling period, the [^{3}H]insulin/[^{3}H]C-peptide ratio would be expected to be very much higher, and this was indeed found. It is also interesting to note that, after 2 days of labelling at 11.2 mM-glucose (the conditions used for labelling islets continuously for 3 days), the labelled insulin/C-peptide ratio was lower than that in islets labelled for 2 days at 8.3 mM (islets used for the 2-day-labelling and 1-day-chase protocol). This would suggest that there was less crinophagy occurring at 11.2 mM-glucose. This is exactly what we would predict on the basis of previous studies [6,7,40,41] showing an inverse relationship between rates of insulin release and degradation. Indeed, the slightly lower glucose concentration of 8.3 mM was selected for the label–chase experiments in order to maximize degradation [6,7] during the chase period (during which the glucose was further decreased to 2.8 mM).

The results of the present study show that the differential rates of insulin and C-peptide degradation within islet cells lead to higher levels of insulin than of C-peptide in B-cells. The difference between the levels of these two related products thus provides an index of intracellular degradation. The higher the insulin/C-peptide ratio, the more active the degradative compartment. Since radioimmunoassays for both human and rat insulin [18] and C-peptide [42,43] are available, it will now be possible to estimate intra-islet-cell insulin degradation by means of the insulin/C-peptide ratio without the need for complicated manipulation of islets *in vitro*. In this way, intra-B-cell insulin degradation can be examined even in pancreatic extracts obtained from animal or human autopsy, thereby opening the way to determining whether such degradation is unusual in disease states.

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