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An immunoradiometric assay for endopeptidase-24.11 shows it to be a widely distributed enzyme in pig tissues

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An immunoradiometric assay for endopeptidase-24.11, which depended on the absorption by tissues of a monoclonal antibody, GK7C2, was established. The optimum conditions for the assay were defined and its correlation with an enzymic assay determined. The immunoassay was used to survey the endopeptidase in crude homogenates of various tissues of the pig. Detergent treatment decreased the sensitivity of the assay but did not invalidate it. Although the endopeptidase was found in many tissues, it was neither uniformly nor ubiquitously distributed. Kidney cortex was confirmed as the major location of the endopeptidase, containing 5000ng/mg of protein. Lymph nodes were also very active (1370ng/mg), followed by chondrocytes from articular cartilage (650ng/mg). In the gut, the endopeptidase was concentrated mainly in the jejunum (130ng/mg). Various glands (salivary, adrenal, anterior pituitary and pancreas) also contained the antigen in the range 20–55ng/mg of protein. Lung contained only 5ng/mg of protein and, in other tissues examined, little or none was detectable. In particular, other lymphoid tissues (spleen, thymus, tonsillar tissues) were relatively poor sources, and none was detectable in peripheral-blood leucocytes or in peritoneal macrophages.

Endopeptidase-24.11 is an ectoenzyme that is most abundant in the brush border of renal proximal tubules (George & Kenny, 1973; Booth & Kenny, 1974; Gee *et al.*, 1983). It is capable of hydrolysing a wide range of peptides, including hormones (such as the chains of insulin and glucagon; Kerr & Kenny, 1974) and many neuropeptides (Matsas *et al.*, 1983, 1984*a*,*b*). The endopeptidase is also present in intestinal brush-border membrane (Danielsen *et al.*, 1980; Fulcher *et al.*, 1983; Gee *et al.*, 1983), and lower activities have been demonstrated in pituitary glands (Orlowski & Wilk, 1981) and brain (Relton *et al.*, 1983). We have suggested that the enzyme has a general role in the metabolism of regulatory and dietary peptides at cell surfaces, and that the physiological function in different sites is determined by the precise location and the susceptibility to attack of the peptides present at that site (Matsas *et al.*, 1983).

Little is known about the distribution of endopeptidase-24.11 in other tissues. By using a specific inhibitor of the enzyme, phosphoramidon (Kenny, 1977), a preliminary survey of pig tissues revealed phosphoramidon-sensitive activity in crude membrane fractions prepared from spleen, aorta, lung and myocardium (Kenny & Fulcher, 1983). In that study, ^{125}I-insulin B-chain was the substrate and the interpretation of the data rested on the assumption that endopeptidase-24.11 was the only phosphoramidon-sensitive peptidase in the tissues examined. The production of a monoclonal antibody, GK7C2, to endopeptidase-24.11 (Gee *et al.*, 1983) has provided the opportunity to set up an i.r.m.a. based on the depletion of the antibody by antigen present in unfractionated homogenates of pig tissues. In the present paper we describe the design of the assay, its optimization, its correlation with enzymic activity and its application in a survey of pig tissues. Among the tissues not previously examined, the most striking findings have been the abundance of the enzyme in peripheral lymph nodes and chondrocytes.

Abbreviation used: i.r.m.a., immunoradiometric assay.

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Experimental

Materials

Tissues were obtained from pigs kindly provided by ASDA Farm Stores, Lofthousegate, W. York-

shire, U.K., or from 2–5-week-old piglets obtained from the University of Leeds Field Station. Endopeptidase-24.11 was purified as described by Gee *et al.* (1983).

Methods

Preparation of homogenates. Tissues were homogenized at 4°C in 9 vol. of 50mM-Tris/HCl/500mM-NaCl, pH8.0, with an Ultra-Turrax homogenizer (Janke und Kunkel KG, Staufen, Germany).

Preparation of microvillar fractions. These were prepared by the method of Booth & Kenny (1974).

Preparation of monoclonal antibody, GK7C2. Pristane-primed Balb/c mice were inoculated by intraperitoneal injection of the hybridoma line and the resulting ascites fluid was harvested as described by Gee *et al.* (1983). It was stored at 4°C with 0.1% NaN_3. Control ascites fluid was produced by injecting the myeloma cell line (SP2/0-Ag-14).

Absorption of monoclonal antibody by tissue extracts. Ascites fluid (containing GK7C2 monoclonal antibody) diluted appropriately in homogenizing buffer containing 0.1% bovine serum albumin was dispensed (100μl) into 1.5ml polyethylene tubes and incubated for 1h at 4°C with 100μl of tissue homogenates, serially diluted in the same buffer. After centrifugation at 18000*g* for 5min, the supernatant fraction (50μl samples in triplicate) was subjected to i.r.m.a.

Immunoradiometric assay. The solid-phase i.r.m.a. of the monoclonal antibody employing a ^{125}I-labelled rabbit anti-(mouse IgG) antibody has been previously described (Gee *et al.*, 1980).

Other methods. The enzyme assay for the endopeptidase using ^{125}I-insulin B-chain and fluorimetric assays for exopeptidases have been described (Fulcher & Kenny, 1983). For the detergent treatment of tissue homogenates, Triton X-100 was added to 2% (w/v) immediately before the antibody-absorption step. Chondrocytes were isolated from pig metacarpal articular cartilage and cultured as described by Trechsel *et al.* (1982). Macrophages were obtained by peritoneal lavage (Gee *et al.*, 1983).

Results and discussion

Titration curve of GK7C2 in the immunoradiometric assay

The precision and sensitivity of the i.r.m.a. for endopeptidase-24.11 depended on selecting a suitable dilution of the monoclonal antibody. The titration curve of GK7C2 in ascites fluid in the i.r.m.a. is shown in Fig. 1. The uncorrected curve did not appear to saturate the antigen at high concentrations of antibody, thus making it impossible to define precisely the region in which GK7C2 was limiting. This phenomenon was attributable to non-specific binding at very high concentrations of the ascites fluid, as demonstrated by the curve for SP2/0 ascites fluid. The 'true' titration curve was obtained by subtraction of the control values, and this curve showed a maximum in bound radioactivity when GK7C2 was diluted approx. 10^3-fold. At high concentration of antibody, univalent binding predominates and the observed maximum probably corresponds to the antibody concentration at which bivalent binding is favoured. From the corrected titration curve, the dilution of GK7C2 selected for the antibody-depletion assay was usually in the range 10^4–10^5-fold dilution.

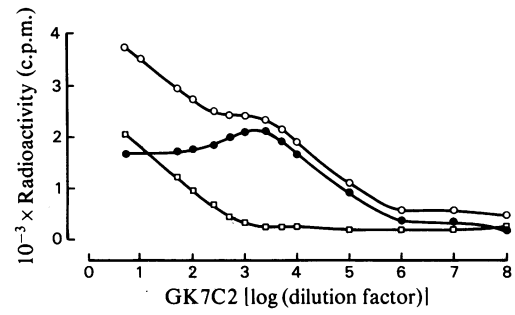

Fig. 1. *I.r.m.a. of GK7C2*

See the Experimental section for details. Two ascites fluids were titrated in this experiment: ○, that produced by GK7C2 hybridoma; □, that produced by the myeloma line SP2/0. The latter revealed non-specific binding at low dilutions, and the corrected curve for GK7C2 (●) was obtained by subtraction of these values.

Antibody-depletion assay: absorption curves

Fig. 2 shows the curves when GK7C2 was preabsorbed with various dilutions of some tissue homogenates. Kidney-cortex homogenate had the highest activity in the assay and provided a convenient reference standard. A unit of antigenic activity in the i.r.m.a. may be defined as the quantity of antigen present in the dilution that decreases the bound radioactivity to half-maximum value. The other curves in Fig. 2 show good parallelism for those tissues (jejunum and ileum) that contained sufficient antigen to absorb all the GK7C2. One tissue, stomach, contained no demonstrable antigenic activity. Spleen homogenates only partially absorbed GK7C2 at low dilutions under these conditions, but this tissue could be brought within the sensitivity range of the assay by selecting a higher dilution of GK7C2 (see below).

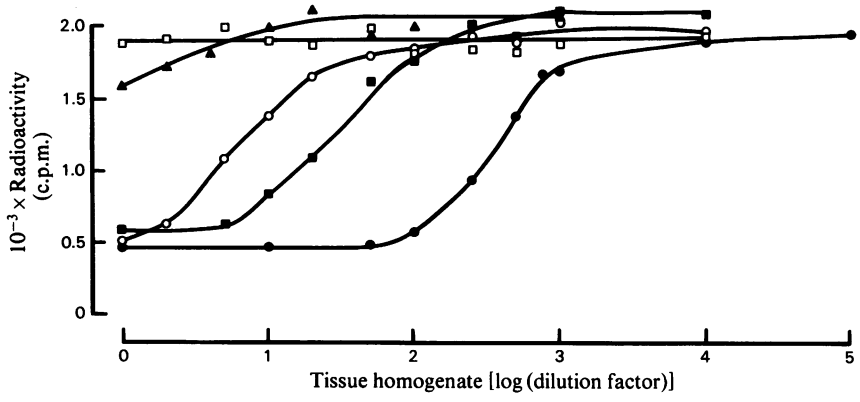

Fig. 2. *Antibody-depletion assay of pig tissues*
See the Experimental section for details. Unfractionated homogenates were diluted as shown: ●, kidney (cortex); ■, jejunum; ○, ileum; ▲, spleen □, stomach. GK7C2 was used at 10^4-fold dilution.

Optimum conditions for the assay of endopeptidase in tissues

Choice of dilution of GK7C2 ascites fluid. In the antibody-depletion step it is obvious that a decrease in the concentration of GK7C2 will raise the proportion of antibody bound by low quantities of antigen and hence increase the sensitivity of the assay. This phenomenon is illustrated in Fig. 3(*a*) for three different dilutions of GK7C2 ascites fluid used to assay a kidney homogenate. At the highest antibody dilution, half-maximum binding was obtained at about 10^4-fold dilution of antigen. At lower dilutions of antibody the curves were shifted to the left, giving half maximum binding between 10^3- and 10^2-fold dilutions of kidney homogenate. Two limitations are also apparent: at high dilutions of GK7C2 the range of values of bound radioactivity was compressed and the precision of the assay thereby impaired. At lower dilutions the background binding became greater.

Labelled second antibody. The effect of different amounts of the ^{125}I-labelled rabbit anti-(mouse IgG) antibody is shown in Fig. 3(*b*). The maximum bound radioactivity rose to higher levels with increased amounts of the second antibody. However, the calculated i.r.m.a units for the kidney homogenate were not significantly influenced by this experiment, since the dilution giving half maximum binding was essentially unchanged.

Two other variables were examined. No significant improvement was observed by increasing the amount of membrane protein in the wells, nor by extending the absorption period to 2 h.

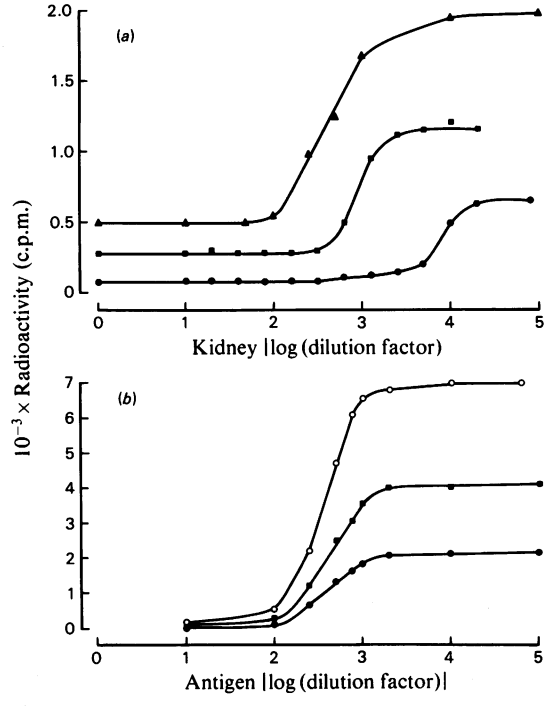

Fig. 3. *Effects of two variables on the immunoradiometric assay of kidney homogenates*
See the Experimental section for details. (*a*) Three dilutions of GK7C2 ascites fluid were tested: ●, 1:200000 ■, 1:100000; ▲, 1:20000. (*b*) Three different dilutions of the ^{125}I-labelled second antibody, rabbit anti-(mouse IgG), were tested: ●, 5×10^4 c.p.m./well; ■, 1×10^5 c.p.m./well; ○, 1.8×10^5 c.p.m./well.

Distribution of endopeptidase-24.11 in homogenates of pig tissues

Table 1 shows the i.r.m.a results expressed as specific activities on 22 different tissues or cell types. The antigen exhibited a strikingly non-uniform distribution. Along the length of the gastro-intestinal tract the antigen was undetectable in the stomach, increasing to a maximum in the jejunum. However, stomach was not devoid of

Table 1. *Immunoradiometric assay of endopeptidase-24.11 in pig tissues*

See the experimental section for details. Each tissue sample was homogenized in 9 vol. of buffer and, after suitable dilutions had been prepared, was assayed concurrently with a 'standard' kidney-cortex homogenate. The results are expressed in arbitrary units relative to the standard. In the last column, the values have been expressed in absolute terms (ng of endopeptidase-24.11/mg of protein, using values for the specific activities (enzymic assays) for kidney homogenates and for purified kidney endopeptidase-24.11 (Fulcher & Kenny, 1983). The pituitaries were obtained from an adult Landrace × White pig. Samples of lung, adrenals, spleen, cardiac and skeletal muscle, thyroid and aorta were from a mature Yucatan pig. The remaining tissues were from Yucatan piglets 2–5 weeks old. The values for the intestine are for mucosal scrapings. Abbreviations used: nd, not detectable; d, detectable, but too little to calculate activity. When three or more samples were assayed, the results are given as means ± S.D.

		Endopeptidase-24.11 content	
Homogenates examined	No. of samples	Arbitrary units relative to kidney-cortex homogenate (units/mg of protein)	Absolute terms (ng/mg of protein)
Tissues			
Kidney cortex (standard)	1	100	5000
Stomach	3	nd	nd
Stomach (microsomal fraction)	1	1.1	55
Duodenum	2	1.3	65
Jejunum	2	8.0	400
Ileum	2	2.6	130
Salivary glands	3	1.1 ± 0.3	55 ± 15
Pancreas	1	0.5	25
Lymph nodes			
Piglets	20	15.0 ± 8.6	748 ± 428
Adults	14	27.4 ± 13.9	1370 ± 695
Lingual lymphatic tissue	2	0.32	16.25
Thymus	1	0.07	3.5
Spleen	1	0.03	1.5
Adrenal glands	1	0.4	20
Pituitary-gland			
Adenohypophysis	1	0.5	25
Neurohypophysis	1	d	d
Liver	3	nd	nd
Skin	1	0.05	2.5
Lung	1	0.1	5
Skeletal muscle	1	d	d
Cardiac muscle	1	d	d
Thyroid	1	nd	nd
Aorta	1	nd	nd
Cells			
Chondrocytes			
Before culture	2	13.0	650
Cultured 3 days	1	1.9	80
Cultured 7 days	1	nd	nd
Cultured 10 days	1	nd	nd
Peripheral-blood leucocytes	1	nd	nd
Peritoneal macrophages	1	nd	nd

endopeptidase-24.11, but the antigen was detected only when a microsomal fraction was assayed. Salivary glands and pancreas also contained the antigen, but liver was devoid of activity. Lymph nodes were the richest source after kidney, but also showed much greater variability. Nodes from piglets contained 15.0 units/mg of protein (range 7.0–35 units), whereas those from adult pigs yielded higher values (mean 27.0, range 9.3–48). Other lymphoid organs, the tongue, thymus and spleen, contained relatively little antigen, and none was detectable in peripheral leucocytes or peritoneal macrophages. However, one cell type was found to be a rich source of antigen. Freshly prepared chondrocytes from articular cartilage contained 13.0 units/mg of protein, but this value

decreased during culture and the antigen was not detectable at 7 and 10 days. Phosphoramidon sensitive endopeptidase activity fell in parallel, although total endopeptidase activity showed little change.

Immunoradiometric assay of detergent-solubilized tissues

Membrane preparations were more effective in absorbing antibody than were the corresponding detergent-solubilized preparations. This phenomenon is illustrated in Fig. 4, in which homogenates of kidney and jejunum are compared. Two points are apparent: first, the effect of detergent was quantitatively similar for both tissues: the ratio untreated/detergent-treated membranes (units/mg of protein) was 5.4:1 for kidney and 6:1 for jejunum. Secondly, provided like was compared with like, the assay was equally valid for solubilized tissues, though at the cost of lowered sensitivity. Thus the abundance in the untreated jejunum (relative to kidney) was 5.6% and for the solubilized forms it was 5.0%. This effect of detergents has been noted by others (e.g. Mason & Williams, 1985) and it relates to the stability of the antibody–antigen complex, which is lower in the presence of detergents.

Detergents may also have an effect on the microvillar membranes immobilized in the polyvinyl wells in that some of the antigen may be solubilized during this phase of the assay. A low concentration of detergent (0.1%) increased the binding of GK7C2, perhaps by increasing accessibility of the antigen. However, 2% detergent reduced binding and decreased the maximum bound radioactivity, suggesting that some of the membrane antigen had been extracted. This phenomenon would explain the lower maximum bound radioactivity (seen in Fig. 5) for the 'detergent-treated' curves.

Since the antigen assayed in these tissues was available as a pure protein, it was desirable to convert the arbitrary i.r.m.a. units into absolute units in order to describe the abundance of the protein in different tissues. The experiment in Fig. 5 illustrates the problem and its solution. The detergent-solubilized microvillar membrane preparation was compared with the detergent form of endopeptidase-24.11. In arbitrary units the values were 138 and 3520 units/mg of protein, an enrichment of 25.5-fold. This agrees well with estimates, derived from enzyme assays, that the enzyme constitutes about 4% of the microvillar membrane protein (Booth & Kenny, 1976; Fulcher & Kenny, 1983). The corresponding specific activities for the untreated homogenate and microvillar membranes were 167 and 854 units/mg of protein, an enrichment of 5.1. Thus the overall enrichment (homogenate/enzyme) was 130-fold. Enzyme assays on the same fractions indicated comparable enrichment of 169-fold. Having established the validity of these relationships in the i.r.m.a., it became possible to express the arbitrary units in Table 1 as absolute values (as shown in the last column of this Table). From previous results (Fulcher & Kenny, 1983), the pure endopeptidase contained 177munits/mg of protein, hence the homogenate in this experiment (enzyme assay, 0.9munits/mg of protein, corrected for aminopeptidase N amplification) contained 5μg of endopeptidase-24.11/mg of protein.

Correlation of antigenic and enzymic activities of endopeptidase-24.11

There is no specific substrate for endopeptidase-24.11 and, for crude tissue preparations that are

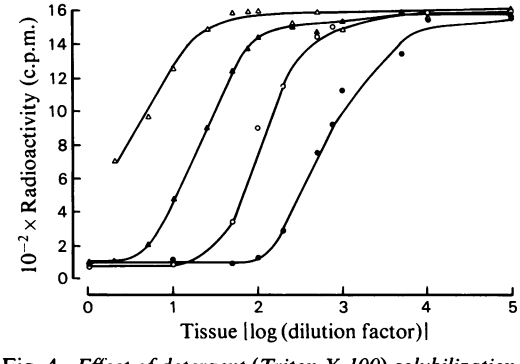

Fig. 4. *Effect of detergent (Triton X-100) solubilization on the i.r.m.a. of kidney and jejunum*

See the Experimental section for details. Kidney homogenates: ●, untreated; ○, detergent-treated. Jejunum homogenates: ▲, untreated; △, detergent-treated.

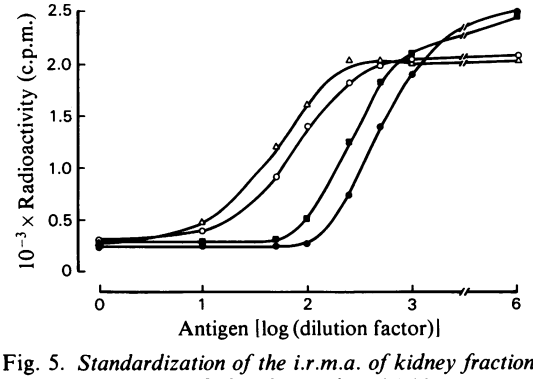

Fig. 5. *Standardization of the i.r.m.a. of kidney fractions with purified endopeptidase-24.11*

See the Experimental section for details. ●, Microvillus fraction; ■, homogenate; ○, detergent (Triton X-100)-solubilized microvillus fraction; △, purified kidney endopeptidase-24.11.

rich in other neutral-endopeptidase activities, the only means of identifying endopeptidase-24.11 is to assay the activity in the presence and absence of an appropriate inhibitor. Phosphoramidon is a potent inhibitor for this class of Zn^{2+}-containing metalloendopeptidase, including those of microbial origin (Suda *et al.*, 1973; Kenny, 1977). So far endopeptidase-24.11 is the only known mammalian representative, but it must be emphasized that the assumption that all phosphoramidon-sensitive endopeptidase activity in mammalian tissues can be equated with it may be undermined by future work. Indeed, peptidyl dipeptidase A has been shown to possess endopeptidase activity (Yokosawa *et al.*, 1983), and it can be inhibited by phosphoramidon, albeit at high concentrations (Matsas *et al.*, 1984*a*). The results of a survey of activities obtained by this enzymic assay have been compared with the i.r.m.a. values on the same samples. These values, normalized with respect to kidney, are plotted in Fig. 6. For non-lymphoid tissues, shown in Fig. 6(*a*), the correlation (excluding chondrocytes) was good ($r = 0.97$), but the slope (b) deviated from unity, being skewed in favour of the enzymic assay ($b = 1.5$). Within this group, only pancreas deviated substantially from the line of best fit. The two chondrocyte assays were anomalous, in that the enzymic assays were relatively low, for reasons that remain to be explored. The correlation observed when the results for homogenates of lymph nodes were plotted (Fig. 6*b*) was poor ($r = 0.67$): the line of best fit did not intersect through the origin and was skewed in favour of the i.r.m.a. ($b = 0.42$). When membranes were prepared from the same set of homogenates and re-assayed (Fig. 6*c*), the correlation improved markedly ($r = 0.92$) and the line now intersected close to the origin, but the slope was unchanged ($b = 0.44$).

The anomalies in the correlation of the assays on this large group of lymph nodes posed several questions and prompted some experiments that might provide answers. In brief, no single factor emerged, and it appeared that the anomalies resulted from a combination of factors. Pig IgG at high concentrations did not affect the i.r.m.a.; lymph-node cytosol contained no antigen detectable by i.r.m.a. and no heat-stable components affecting either assay. The cytosol did, however, contain some enzymic activity, in the range 7–20% of that of the homogenate. Various exopeptidases were detected in the membrane fraction. The activities relative to the content of the same fraction prepared from kidney cortex (= 100%) were: aminopeptidase N (EC 3.4.11.2), 3%; aminopeptidase A (EC 3.4.11.7), 3%; dipeptidyl peptidase IV (EC 3.4.14.5), 1.5%; peptidyl dipeptidase A (EC 3.4.15.1), 35%; and γ-glutamyl transferase (EC 2.3.2.2), 11.5%. Thus, relative to kidney, the lymph-node microsomal fraction was very low in exopeptidase activity. The exopeptidases, particularly aminopeptidase N, are known to amplify the enzymic assay by a factor that in kidney has been estimated to be 1.22 (Fulcher & Kenny, 1983). Intestine contains a higher ratio of aminopeptidase N to endopeptidase-24.11 than kidney, and this will have contributed to the greater slope of the line in Fig. 6(*a*). In contrast, the lower slope

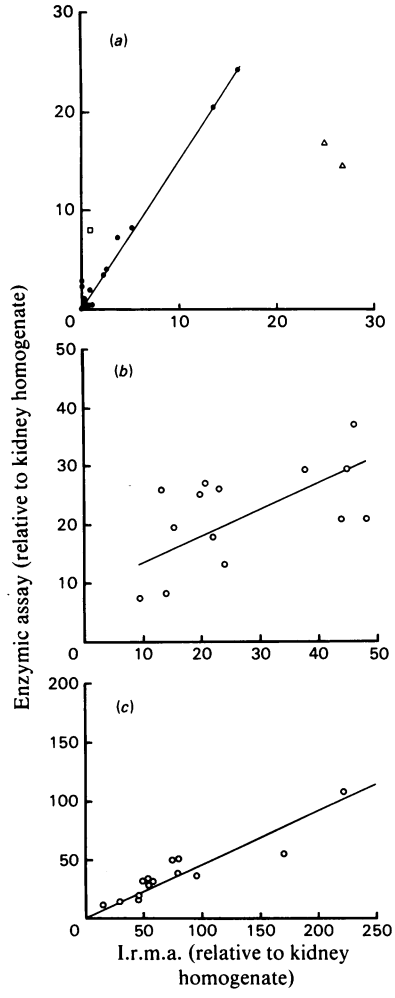

Fig. 6. *Correlation of i.r.m.a. values with endopeptidase activity*

See the Experimental section for details. The specific activities (units/mg of homogenate protein) have been normalized relative to kidney (= 100). (*a*) Homogenates of tissues other than lymph nodes; (*b*) homogenates of lymph nodes; (*c*) membrane fraction of lymph nodes (P_2 fraction obtained by centrifugation at 31000*g* for 60min after removal of the pellet, P_1 sedimenting at 8000*g* for 20min). Tissues: □, pancreas; △, chondrocytes; ○, lymph nodes; ●, unspecified.

in Fig. 6(*c*) is in part explained by using kidney as the standard. If the amplification factor for kidney is discounted, the slope would be slightly greater ($b = 0.51$).

The i.r.m.a. values for isolated chondrocytes were high relative to their enzymic activity, i.e. the deviation was similar to that observed with lymph nodes. The dissociation of chondrocytes from cartilage required treatment with collagenase, trypsin and hyaluronidase. Insofar as these enzymes may be sequestered on to the cell surface, this phenomenon clearly did not lead to erroneously high enzymic activities. Very strong immunofluorescence with GK7C2, consistent with the i.r.m.a. results, has been observed both in articular cartilage and isolated chondrocytes. During culture, the proportion of fluorescent cells diminished in parallel with the assays (R. M. Hembry, M. A. Bowes & A. J. Kenny, unpublished work). The antigen was confined to the chondrocyte membrane both before and after dissolution of the tissue. Any contribution of the hydrolytic enzymes to the i.r.m.a. seems highly unlikely.

General discussion

Advantages of i.r.m.a. over enzymic assay of endopeptidase-24.11. For the purified enzyme, enzyme assays using ^{125}I-insulin B-chain (Kenny, 1977), [D-Ala^2, Leu^5]enkephalin (Matsas *et al.*, 1983) or coupled enzymic assays with synthetic substrates (Orlowski & Wilk, 1981; Mumford *et al.*, 1981; Kenny & Fulcher, 1983) are adequate, simpler and more precise than i.r.m.a. They are less satisfactory for crude tissue extracts, even if the enzyme is designated as 'phosphoramidon-sensitive peptidase activity'. The labelled insulin B-chain assay is amplified by the presence of other peptidases, particularly aminopeptidase N (Kerr & Kenny, 1974; Fulcher & Kenny, 1983). Endogenous peptides in the extracts may obscure the h.p.l.c. analyses of the enkephalin products and may possibly compete for hydrolysis by the endopeptidase in all enzyme assays. The abnormally high enrichment factor reported by Mumford *et al.* (1981) in the purification of the kidney enzyme may have arisen from this latter phenomenon. For such extracts, the i.r.m.a. offers major advantages. The monoclonal antibody GK7C2 is specific for endopeptidase-24.11 in Western blotting of membranes from kidney (Gee *et al.*, 1983), and immunoaffinity chromatography has succeeded in purifying endopeptidase-24.11 uncontaminated by other proteins from crude membrane fractions of kidney (Gee *et al.*, 1983), brain (Relton *et al.*, 1983), intestine, lymph nodes, salivary glands (N. S. Gee, M. A. Bowes & A. J. Kenny, unpublished work). In each case the enzyme had the expected specific activity. Hence, in membrane fractions, no other antigen has so far been detected by GK7C2. However, the i.r.m.a. has the potential to detect inactive enzyme [e.g. that revealed by immunoblotting (Gee *et al.*, 1983)] and might detect smaller fragments bearing the epitope if they were generated in conditions where the enzyme was being degraded. The correlation of the two assays has been discussed in detail above. It seems likely that the poor correlation with unfractionated homogenates of lymph nodes was the result of several factors operating on the enzyme assay. We have so far found no factor which perturbed the i.r.m.a. and we favour the view that those assays were the more reliable.

In the conditions employed in these experiments, the i.r.m.a. had a similar sensitivity to our enzyme assays, though the latter was more precise at low levels of enzyme activity. The sensitivity of the i.r.m.a. is limited only by the detection of the GK7C2 in the solid-phase assay, and this might be improved by preparing a labelled second antibody of higher specific activity. In addition, the non-specific binding might be lowered by using $F(ab')_2$ fragments in place of IgG. Although we have preferred a radiometric assay, the method would be equally suitable for an enzyme-linked detection system. The principal weakness of the i.r.m.a. compared with enzyme assays was the lowered sensitivity when detergent-solubilized rather than membrane-bound antigen was determined.

The non-uniform distribution of endopeptidase-24.11. The physiological role of this enzyme in its different locations remains to be clarified. Endopeptidase-24.11 is known to hydrolyse a wide range of biological peptides, but is not known to attack native proteins (Kerr & Kenny, 1974; Matsas *et al.*, 1984*a*,*b*). An understanding of the distribution of the enzyme in organs and tissues is a first step to formulating the appropriate questions concerning its function. A subsequent step is a precise description of its cellular localization. This information then needs to be correlated with that on the identity of the peptides located at these sites. At present, this is mainly an area of speculation. In the jejunum it is probable that the enzyme plays a role in the final stage of hydrolysis of peptides generated by pancreatic and gastric proteinases. In the kidney, it may initiate the hydrolysis of some peptide hormones filtered at the glomerulus, though why the complete degradation of peptides already exteriorized should be mandatory remains unclear. Although the concentration of the enzyme in even the richest regions of the central nervous system is extremely low, it is likely that it plays an important role in the hydrolysis of some neuropeptides liberated at the synapse (Matsas *et al.*, 1983, 1984*a*,*b*). However, the relative abundance of the

enzyme in lymph nodes and chondrocytes raises intriguing questions concerning the likely peptide substrates in these locations. Catabolin is a protein (M_r 21000) that is synthesized by leucocytes and which promotes resorption of the matrix of cartilage and which appears to be identical with interleukin-1 (Saklatvala *et al.*, 1983). There is also a wealth of recent information on lymphokines, including the interleukins and interferons, as well as on the complex interrelationships between the neuroendocrinological and immunological systems (for review, see Blalock, 1984). There seems, therefore, to be no shortage of potential substrates for endopeptidase-24.11 in lymph nodes.

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