Development and hormonal modulation of postnatal expression of intestinal alkaline phosphatase mRNA species and their encoded isoenzymes

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In the rat, intestinal alkaline phosphatase (IAP) activity in the duodenum, but not jejunum, increases on day 22–24 after birth and exhibits higher activity hydrolysing phenyl phosphate (PhP) than β-glycerophosphate (βGP) [Moog and Yeh (1973) Comp. Biochem. Physiol. **44B**, 657–666]. The mechanism underlying these developmental changes remains unknown. To define possible mechanisms, we have measured IAP activity and mRNA levels, and analysed IAP mRNA species and isoenzymes on postnatal days 12, 18, 24 and 32. Duodenal IAP activity and mRNA content were identical on postnatal days 12 and 18, but were 7-fold and 3-fold higher on day 24, respectively than on day 18. The increased IAP activity exhibited a high PhP/βGP ratio and was accompanied by initial appearance of the 3.0 kb mRNA and 90 kDa isoenzyme. On day 32, duodenal IAP activity did not increase over the levels on day 24, whereas mRNA levels doubled. The lack of enzyme increase might be related in part to increased apical release, as luminal IAP activity increased from 2 % of total mucosal IAP on days 12 and 18 to 7 % and 14 % on days 24 and 32 respectively. In the jejunum, IAP activity decreased postnatally, but mRNA content was unaltered; only the 2.7 kb mRNA and 65 kDa IAP isoenzyme were present. Administration of cortisone or cortisone + thyroxine induced simultaneous appearance of the duodenal 3.0 kb mRNA and 90 kDa isoenzyme with an increased PhP/βGP ratio. Thus postnatal increase in duodenal IAP activity is related to the expression of a 90 kDa PhP-preferring isoenzyme encoded by the 3.0 kb mRNA. The low-PhP/βGP-ratio 65 kDa isoenzyme is expressed in the duodenum and in the jejunum and is encoded by the 2.7 kb mRNA.

INTRODUCTION

Rat intestinal alkaline phosphatase (IAP) (orthophosphoric-monoester phosphohydrolase, EC 3.1.3.1.) is one of the brush-border membrane proteins which undergo remarkable changes in the level of expression during postnatal development. The change in IAP expression is uniquely regulated along the horizontal axis of small intestine. IAP activity is low during the first 20 days of postnatal age and rises to adult levels on days 22–24 in the duoenum, but not in the jejunum or ileum (Moog and Yeh, 1973; Yeh and Moog, 1975a). Similar postnatal developmental changes also occur in mice (Moog, 1966). The developmental increase in duodenal IAP activity is accompanied by an increase in substrate preference toward phenyl phosphate (PhP) compared with β-glycerophosphate (βGP). These developmental changes have been proposed to occur either by enzyme activation, by differential isoenzyme expression, or both (Moog, 1966; Etzler and Moog, 1968; Moog and Yeh, 1973). IAP cDNAs have been cloned (Henthorn et al., 1988; Lowe et al., 1990) and are now available for clarification of the mechanisms underlying the developmental increase in duodenal IAP activity.

In adult rats, two IAP mRNA species are present in the intestine, and their cDNAs have been cloned (Eliakim et al., 1989; Strom et al., 1991; Engle and Alpers, 1992). The two mRNAs have about 70 % nucleotide sequence identity, with differences occurring along the entire length of the coding region, diverging completely at the C-terminus (Strom et al, 1991; Engle and Alpers, 1992). Thus these mRNA species are likely the products of two separate genes and might be individually regulated. Fat feeding increases duodenal content of the 3.0 kb mRNA more than the 2.7 kb mRNA species (Eliakim et al., 1990b; Engle and Alpers, 1992). Thyroxine (T_4) has been reported to increase the 3.0 kb IAP mRNA in adult jejunum (Hodin et al., 1992). The significance of differential changes in mRNA species remains unknown. Multiple IAP mRNA species are also present in human intestine and they are thought to encode only one IAP enzyme (Henthorn et al., 1988). However, data obtained from *in vitro* translation studies suggest that rat intestinal IAP mRNAs encode two distinct IAP isoenzymes (Sussman et al., 1986). To understand the possible functional significance of differing IAP mRNA expression, the IAP isoenzyme encoded by each mRNA species first must be identified. Because the postnatal developmental increase in duodenal IAP activity is accompanied by altered substrate specificity, we reasoned that analysis of this process might provide identification of IAP isoenzymes. In addition, because the pituitary–adrenal and pituitary–thyroid systems are known to modulate duodenal IAP expression during development (Yeh and Moog, 1975b), the induction of IAP expression after cortisone, T_4 or T_4 + cortisone administration could be examined to determine whether these hormones induce developmental changes in mRNA species and their putative encoding isoenzymes. The data might confirm the IAP isoenzyme identification obtained during normal postnatal development.

Thus the aims of the present study were to determine: (1) whether co-ordinated changes in IAP activity and mRNA content occur during postnatal development; (2) whether the postnatal increase in duodenal PhP-preferring IAP isoenzymes is the result of expression of a new mRNA species and its encoded isoenzyme; and (3) whether these developmental changes in IAP activity, IAP mRNA species and isoenzymes can be precociously induced by cortisone and T_4. The present study demonstrates the concomitant appearance of the 3.0 kb IAP mRNA and the 90 kDa IAP isoenzyme in the duodenum during postnatal development and also after cortisone or T_4 + cortisone administration. These data not only correlate the appearance of the 3.0 kb mRNA, the

Abbreviations used: (I)AP, (intestinal) alkaline phosphatase; PhP, phenyl phosphate; βGP, β-glycerophosphate; T_4, thyroxine.

90 kDa protein and the PhP-preferring IAP isoenzyme during normal or induced postnatal development, but also provide the first identification of the IAP isoenzyme encoded by each of the independent IAP mRNA species.

EXPERIMENTAL

Animal treatments

Sprague–Dawley rats from Charles River Breeding Laboratories (Wilmington, MA, U.S.A.) were mated and bred in animal rooms with a 12 h-light/12 h-dark cycle. Rats were fed Purina Laboratory Chow and water *ad libitum*. Litters were reduced to 12 at day 1, the second day after birth. For studies of IAP development, animals were killed by decapitation on postnatal days 12, 18, 24 and 32. The small intestine was removed and luminal contents were collected by flushing with a 3–6 ml of ice-cold normal saline (0.9 % NaCl) containing 0.1 mM phenylmethanesulphonyl fluoride, 1 μg/ml leupeptin and 1 μg/ml pepstatin. A 6 cm segment below the pylorus (duodenum) and the middle 10 cm segment of the small intestine (jejunum) were quickly removed for total RNA isolation according to the method of MacDonald et al. (1987). A 0.5 cm segment from the mid duodenum and jejunum was collected and the mucosa scraped free of underlying muscle layers for IAP assays. The luminal washes were centrifuged at 500 ***g*** to remove cells and fragments of brush borders (Eliakim et al., 1989) and the supernatant was stored at −70 °C. The luminal IAP is either secreted on a phospholipid-rich particle or released during tissue processing by contamination with serum containing glycosylphosphatidylinositol-anchor-specific phospholipase D (Eliakim et al., 1990a). Because neither of these IAP forms sediments at 500 ***g***, the supernatant fraction contains total luminally secreted IAP. In a separate experiment for the induction of a precocious increase of IAP activity, day-12 rats were separated into four groups and administered a single dose of vehicle, T_4 (1 μg/g body weight), cortisone (50 μg/g body weight) or T_4+cortisone respectively. Animals were killed 3 days later, and tissues were collected as described above. The hormone dose and schedule have been shown to induce precocious expression of intestinal sucrase-isomaltase (Yeh et al., 1989).

IAP activity and protein assays

IAP activity in tissues and luminal washes was determined with PhP and βGP substrates as described previously (Moog and Yeh, 1973). For PhP activity, a prewarmed 500 μl substrate solution consisting of 50 mM PhP, 10 mM $MgCl_2$ and 125 mM carbonate buffer, pH 9.8 was mixed with 125 μl of tissue homogenate (0.5–0.1 mg of tissue/ml). After incubation at 37 °C for 5 min, the reaction was stopped by adding 80 μl of 500 mM Folin–Ciocalteau reagent (Sigma, St. Louis, MO, U.S.A.) in 600 mM HCl. The released phenol in the reaction mixture was measured 10 min after adding 800 μl of 2 M Na_2CO_3 by the absorbance at 540 nm as described by King and Armstrong (1934). For activity against βGP, all assay conditions were the same as for PhP, except that the substrate was βGP and the optimal pH was 9.4. The enzymic reaction was terminated with 400 μl of 10 % trichloroacetic acid. The released phosphorus was measured 10 min after the addition of $FeSO_4$/molybdate reagent by the absorbance at 660 nm (Taussky and Shorr, 1953). The concentration of both substrates in the reaction mixture was optimal for maximal activity (Moog, 1961). The enzyme activity measured during the 5 min incubation period was proportional to the amount of IAP present. IAP specific activity is expressed as μmol of substrate hydrolysed/min per mg of protein. The PhP/βGP ratio was determined as PhP specific activity divided by βGP specific activity. Protein concentrations were determined by the method of Lowry et al. (1951), using BSA as standard.

Dot- and Northern-blot analyses

Aliquots of RNA were subjected to denaturing agarose/formaldehyde-gel electrophoresis (Eliakim et al., 1989). For determination of RNA species, 10 μg of total RNA was size-fractionated by electrophoresis on a denaturing 1.0 %-agarose/formaldehyde gel. The gel was stained with ethidium bromide to localize 28 S and 18 S rRNA, the RNA was transferred to a Nytran membrane by vacuum, and the RNA samples in the gel were stained with ethidium bromide and photographed. Only RNA samples with intact 28 S and 18 S bands were used for further analysis. The intensity of the 28 S rRNA band was scanned with a video densitometer and calculated with the 1-D Analyst II Data Analysis software (Bio-Rad, Richmond, CA, U.S.A.) to normalize total RNA quantity as previously documented (Eliakim et al., 1990b). For dot blots, total RNA was denatured at 65 °C for 30 min in 6 × SSC (1 × SSC is 0.15 M NaCl/0.015 M sodium citrate)/7.4 % formaldehyde, diluted in serial concentrations, applied to a cassette assembly and blotted on to a nitrocellulose membrane. RNA blots were air-dried, baked at 80 °C for 2 h and hybridized with labelled probes as described previously (Yeh et al., 1991a). The probe used was a *Pst*1 restriction segment of rat IAP cDNA encoding the region from nucleotide 190 to 599 (Lowe et al. 1990) and was labelled by the random-primer labelling method with [^{32}P]dCTP (Amersham Corp. Arlington Heights, IL, U.S.A.). Blots were processed by autoradiography at −70 °C overnight using Kodak X-OMAT AR Film with intensifying screens.

SDS/PAGE and Western blotting

Aliquots of duodenal and jejunal homogenate and luminal washes containing 10 μg of protein were mixed with the same volume of 2 × reducing sample buffer [120 mM Tris buffer (pH 6.8)/2 % SDS/20 % glycerol/10 % 2-mercaptoethanol], boiled for 5 min and subjected to SDS/PAGE and Western blotting. SDS/PAGE employed 4 % stacking and 7.5 % separating gels using the buffer system of Laemmli (1970). Prestained protein molecular-mass standards consisting of myosin (200 kDa), phosphorylase *b* (97 kDa), BSA (68 kDa), ovalbumin (43 kDa) and three other low-molecular-mass proteins (GIBCO BRL, Gaithersburg, MD, U.S.A.) were used. Proteins in SDS/PAGE were electrophoretically transferred to a nitrocellulose membrane, and IAP bands were detected by immunoblotting as described previously (Yeh et al., 1991b), using the monospecific rabbit anti-(rat IAP) antiserum characterized previously (Yedlin et al., 1981). The apparent molecular mass of an IAP isoenzyme was calculated from its mobility relative to standards.

Statistical analysis

One-way analysis of variance was performed to calculate the F value. When the F value was significant, the corrected Student's t test was used to test for differences among groups using Instat software (GraphPad Software, San Diego, CA, U.S.A.). P values of less than 0.05 were considered significant.

Table 1 Developmental changes in duodenal and jejunal IAP activity measured with PhP or βGP

IAP specific activity is expressed as μmol of substrate hydrolysed/min per mg of protein. For detailed assay conditions, see the Experimental section. The PhP/βGP ratio is the ratio of the specific activities toward the respective substrates. Results shown are means ± S.E.M.($n = 4$). P values refer to comparisons of data with the same superscript: $^{a,b}P < 0.01$ and $^{c,d}P < 0.05$.

	IAP specific activity					
	Duodenum			Jejunum		
Age (days)	PhP (units)	βGP (units)	PhP/βGP ratio	PhP (units)	βGP (units)	PhP/βGP ratio
12	$0.9 \pm 0.2^{a,b}$	1.8 ± 0.2^{c}	$0.48 \pm 0.04^{c,d}$	1.8 ± 0.2^{c}	2.2 ± 0.2^{c}	0.82 ± 0.05
18	1.1 ± 0.2	2.1 ± 0.3	0.50 ± 0.02	1.6 ± 0.3	1.6 ± 0.1	0.96 ± 0.11
24	7.7 ± 1.6^{a}	2.0 ± 0.3	2.65 ± 0.28^{c}	1.1 ± 0.2	1.2 ± 0.2	0.88 ± 0.03
32	8.4 ± 0.5^{b}	3.2 ± 0.1	2.58 ± 0.12^{d}	0.7 ± 0.2^{c}	0.9 ± 0.2^{c}	0.74 ± 0.06

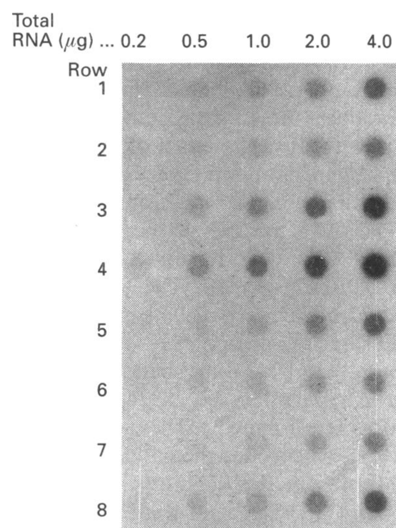

Figure 1 Dot blot of IAP mRNA content during postnatal development

The quantities of isolated total RNA samples were determined by the presence of intact 28 S rRNA after agarose/formaldehyde-gel electrophoresis and ethidium bromide staining. After normalization from the amount of 28 S rRNA, aliquots of RNA samples (values on the top) from the duodenum (rows 1–4) and jejunum (rows 5–8) of rats at 12 (rows 1 and 5), 18 (rows 2 and 6), 24 (rows 3 and 7), and 32 (rows 4 and 8) days of age were blotted on a nitrocellulose membrane and hybridized with the ^{32}P-labelled cDNA probe as described in the Experimental section.

RESULTS

Postnatal changes in duodenal and jejunal IAP activity

Duodenal IAP activity measured with PhP was low on days 12 and 18, increased 7-fold on day 24, and remained unchanged on day 32 (Table 1). When the IAP activity was measured with βGP, it increased gradually, but non-significantly, until day 32 (Table 1; $P < 0.05$ versus day 12). In contrast, jejunal IAP activity measured with both PhP and βGP declined steadily, but not significantly so, until day 32 (Table 1; $P < 0.05$ versus day 12). There was no change in the PhP/βGP ratio during the postnatal decline of jejunal IAP activity. The greater change in PhP activity than βGP activity during postnatal development in the duodenum is consistent with the expression of an IAP isoenzyme with high PhP/βGP ratios (Moog and Yeh, 1973).

Table 2 Postnatal changes in the relative levels of IAP mRNA in the duodenum and jejenum and of 2.7 kb and 3.0 kb mRNA in the duodenum

Duodenal and jejunal IAP mRNA content was determined by densitometry from dot blots shown in Figure 1. The quantity of 2.7 and 3.0 kb IAP mRNA was determined by densitometry from three separate set of Northern blots, as shown in Figure 2. The relative amounts of 2.7 kb and 3.0 kb mRNA are expressed as a percentage of that of day-12 rats and of day-24 rats respectively after normalization for 28 S rRNA content as described in the legend to Figure 1. Abbreviation: ND, not detectable. Results are means ± S.E.M.($n = 3$–4). P values refer to comparisons of data with the same superscript: $^{a,c}P < 0.05$ and $^{b}P < 0.01$.

	IAP mRNA content			
	Duodenum			Jejunum
Age (days)	Total	2.7 kb	3.0 kb	Total
12	100	100	ND	100
18	$82 \pm 10^{a,b}$	$92 \pm 10^{a,b}$	ND	76 ± 10
24	$337 \pm 47^{a,c}$	$195 \pm 22^{a,c}$	100^{a}	67 ± 7
32	$745 \pm 135^{b,c}$	$321 \pm 39^{b,c}$	229 ± 15^{a}	78 ± 11

Postnatal changes in duodenal and jejunal IAP mRNA levels and species

Quantification of total IAP mRNA by dot blots (Figure 1 and Table 2) showed that the duodenal IAP mRNA content did not change on day 18 relative to day 12, but was 4-fold higher on day 24 and 9-fold higher on day 32 than on day 18 (Table 2). The increase in duodenal mRNA content was relatively less than the 7-fold increase in PhP activity from day 18 to day 24 (Tables 1 and 2). Moreover, the approx. 2-fold increase in IAP mRNA content on day 32 compared with day 24 was not accompanied by any increase in IAP activity.

Northern-blot analysis revealed that only a 2.7 kb mRNA was present in the duodenum on postnatal day 12 and day 18 (Figure 2). An additional 3.0 kb mRNA was detected on days 24 and 32 (Figure 2). The steady-state content of the 2.7 kb transcript in the duodenum was the same on days 12 and 18 after birth, but increased about 2-fold on day 24 and 3-fold on day 32 (Table 2). The 3.0 kb mRNA transcript appeared simultaneously with the increase in the PhP/βGP ratio of IAP activity (Table 1).

In contrast with the rise of duodenal IAP mRNAs, jejunal mRNA content did not change in any of the samples taken between day 12 and 32 (Figure 1 and Table 2). Northern-blot analysis showed that only one mRNA (2.7 kb) was present in the

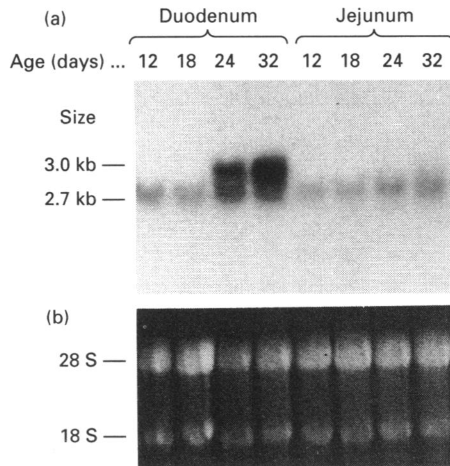

Figure 2 Northern blot of IAP mRNA species during postnatal development

(**a**) Each lane contained 10 μg of total RNA from the duodenum and jejunum of day-12, -18, -24, and -32 rats. The ^{32}P-labelled *Pst*I IAP fragment was used as the probe, as described in the Experimental section. The 2.7 kb IAP is present in all lanes, and the 3.0 kb IAP species is present only in the duodenum of day-24 and -32 rats. Jejunal 2.7 kb mRNA levels did not differ among age groups. RNA samples in the same gel stained with ethidium bromide prior to transfer are shown in (**b**). The 28 S and 18 S bands are indicated at the left.

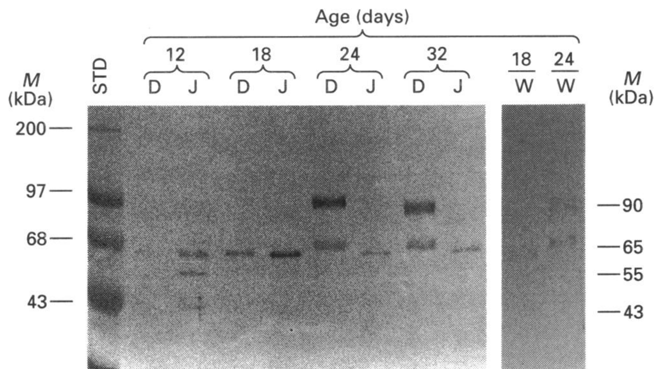

Figure 3 Western blot of changes in IAP isoenzymes during postnatal development

Duodenal (D) or jejunal (J) homogenate containing 10 μg of protein were subjected to SDS/PAGE under reducing conditions for immunoblotting using monospecific rabbit anti-IAP antiserum. Molecular-mass (*M*) standards (STD) are shown on the left. The molecular masses of the 90, 65, 55 and 43 kDa IAP bands are indicated on the right.

jejunum, and the transcript level was not significantly higher on days 24 and 32 than on days 12 and 18 (Figure 2). Expression of the 3.0 kb mRNA transcript in the jejunum does occur later in life, because a low content of this mRNA has been reported in adult rats (Eliakim et al., 1990b; Hodin et al., 1992).

Postnatal expression of IAP isoenzymes

The possibility that the 3.0 kb and 2.7 kb mRNAs might encode different IAP isoenzymes was examined by Western blotting. In the duodenum, only one IAP band with an apparent molecular mass of 65 kDa was present on days 12 and 18, but an additional band of apparent mass 90 kDa was first detected on day 24 (Figure 3). Moreover, the 65 kDa isomer was apparently converted into one with an apparent molecular mass of 67 kDa, although no such change was seen in the jejunum. By 32 days, the larger isomer size had changed to an apparent mass of 88 kDa. Thus the 65kDa IAP appeared to be encoded by the 2.7 kb mRNA species, which was the only species expressed in the duodenum on days 12 and 18. The 90 kDa IAP isoenzyme appeared to be encoded by the 3.0 kb mRNA species, because these events developed concomitantly (Figures 2 and 3).

In the jejunum, the 65 kDa IAP band was present in all four rats in each age group on days 12, 18, 24 and 32, and the 90 kDa band was not detected (Figure 3). These data also imply that the 2.7 kb mRNA species encodes the 65 kDa IAP isoenzyme with low PhP/βGP ratios in the jejunum. Additionally, a distinct 55 kDa and a faint 43 kDa band were present on day 12, but were not detectable in older animal groups (Figure 3). Because no other mRNA species were found, these bands might represent partially degraded forms of the 65 kDa IAP. It is known that distal intestine contains a high concentration of soluble IAP (Seetharam et al., 1977). The 55 and 43 kDa bands detected may represent the soluble IAP produced after cleavage by phospholipases and perhaps also by proteinases (Eliakim et al., 1990a).

Table 3 Postnatal increase in total small-intestinal and luminal IAP activity

Small-intestinal contents were flushed with saline and centrifuged at 500 ***g*** for 5 min at 4 °C to remove coarse particles. The supernatant designated as luminal wash was collected to determine total luminal PhP and βGP activities. A unit of PhP activity is expressed as 1 μmol of PhP hydrolysed/min. Results are means ± S.E.M. ($n = 4$). *P* values refer to comparisons of data with the same superscript: $^{a}P < 0.05$; $^{b}P < 0.01$; $^{c,d}P < 0.001$.

Age (days)	Small intestine: Weight (mg)	Small intestine: Total PhP (units)	Luminal wash: Total PhP (units)	Luminal wash: % of tissue activity	Luminal wash: PhP/βGP ratio
12	604 ± 9^{a}	107 ± 2^{a}	1.7 ± 0.4^{b}	1.6 ± 0.4	0.8 ± 0.1
18	$1112 \pm 30^{a,c}$	$274 \pm 17^{a,c}$	$5.7 \pm 0.5^{b,c}$	1.9 ± 0.3^{c}	1.0 ± 0.1^{b}
24	$2730 \pm 246^{c,d}$	$889 \pm 20^{c,d}$	$65.8 \pm 2.1^{c,d}$	$7.4 \pm 0.3^{c,d}$	3.4 ± 0.5b,a
32	4910 ± 201^{d}	1827 ± 107^{d}	247.2 ± 7.0^{d}	13.6 ± 0.5^{d}	3.7 ± 0.2^{a}

Release of mucosal IAP into the small-intestinal lumen

Intestinal secretion or release of IAP could be one mechanism underlying the disproportional changes in duodenal PhP activity relative to duodenal IAP mRNA content on postnatal day 32 compared with day 24. Total luminal PhP activity increased on day 18 compared with day 12, and this increase paralleled total small intestinal PhP activity (Table 3). On day 24, total luminal PhP activity was 12-fold higher than day 18; this increase was disproportionately greater than the 3.2-fold increase in total tissue PhP activity (Table 3). A further 3.8-fold increase in luminal PhP activity occurred from 24 to 32 days of age; this increase also was significantly greater than the 2-fold increase in total tissue PhP ($P < 0.001$). Luminal IAP activity had a higher PhP/βGP ratio than duodenal mucosa in both day 24 and day 32 rats (Tables 1 and 3). Western blots showed that the luminal wash contained both 65 kDa and 90 kDa isoenzymes on postnatal day 24 or 32, but contained only the 65 kDa isoenzyme on day 12 or 18 (Figure 3).

Table 4 Effect of cortisone and/or T_4 on duodenal and jejunal IAP activity

Day-12 rats were separated into four groups and were given saline (control), T_4 (1 μg/g body weight), cortisone (C, 50 μg/g) or T_4+C and were killed 3 days later. Results are means ± S.E.M. (n = 5–6). P values refer to comparisons of data with the same superscript: $^{a,b,c}P < 0.001$, $^{d,e}P < 0.01$ and $^{f}P < 0.05$.

	IAP activity					
	Duodenum			Jejunum		
Group	PhP (units)	βGP (units)	PhP/βGP ratio	PhP (units)	βGP (units)	PhP/βGP ratio
Control	$1.2 \pm 0.1^{a,b}$	$1.2 \pm 0.1^{a,d}$	$1.08 \pm 0.11^{a,d}$	1.7 ± 0.1^{f}	3.3 ± 0.3^{f}	0.54 ± 0.03
$+T_4$	1.5 ± 0.1	1.3 ± 0.3	1.24 ± 0.07	1.7 ± 0.2	2.6 ± 0.1	0.66 ± 0.09
+C	$6.9 \pm 1.5^{a,c}$	$2.9 \pm 0.2^{d,e}$	2.39 ± 0.45^{d}	1.5 ± 0.2	2.2 ± 0.3	0.65 ± 0.03
$+T_4$+C	$20.5 \pm 2.2^{b,c}$	$5.1 \pm 0.4^{a,e}$	4.00 ± 0.12^{a}	1.2 ± 0.1^{f}	1.7 ± 0.2^{f}	0.72 ± 0.03

Table 5 Effect of T_4 and cortisone (C) on duodenal and jejunal IAP mRNA levels

The quantity of IAP mRNA was determined by densitometry from dot blots and of 2.7 kb and 3.0 kb RNA species determined from Northern blots shown in Figure 4. Values are expressed as a percentage of the control after normalization for 28 S rRNA. The relative amount of the 3.0 kb RNA is expressed as a percentage of that of +C rats. Abbreviation: ND, not detectable. See the legend to Table 4 for animal treatments. Results are means ± S.E.M. (n = 6). $^{*}P < 0.05$ and $^{**}P < 0.01$ versus control or $+T_4$.

	IAP mRNA content			
	Duodenum			Jejunum
Group	Total	2.7 kb	3.0 kb	Total
Control	100	100	ND	100
$+T_4$	98 ± 26	97 ± 8	ND	95 ± 6
+C	$424 \pm 89^{*}$	$212 \pm 34^{*}$	100	150 ± 21
$+T_4$+C	$588 \pm 76^{**}$	$316 \pm 42^{**}$	228 ± 30	124 ± 26

Effect of thyroxine (T_4) and/or cortisone on IAP expression

T_4 and cortisone are known to modulate intestinal expression of the PhP-preferring isoenzyme (Yeh and Moog, 1975b). Thus administration of T_4, cortisone or T_4 + cortisone should induce simultaneous expression of the 3.0 kb mRNA and the 90 kDa isoenzyme, and an increase in the PhP/βGP ratio, if these events were related. T_4 injection alone did not change IAP expression (Table 4). A single dose of cortisone on day 12 did induce on day 15 precocious elevation of duodenal IAP activity to the levels expected on postnatal day 24. This increase was accompanied by an increasing PhP/βGP ratio (Table 4) and total IAP mRNA content (Figure 4 and Table 5). Northern-blot analysis showed that cortisone also induced precocious expression of the 3.0 kb species and increased the 2.7 kb transcripts (Figure 4). The appearance of the 3.0 kb mRNA was accompanied by the production of the 90 kDa IAP isoenzyme (Figure 5). When cortisone and T_4 were administered together, they stimulated duodenal PhP activity about 2-fold higher than cortisone alone (Table 4). This induction was accompanied by increased PhP/βGP activity ratios, appearance of the 90 kDa isoenzyme,

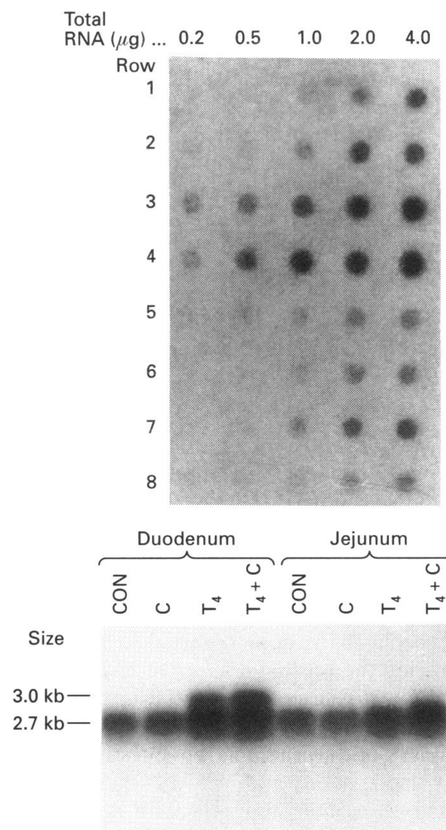

Figure 4 Dot and Northern blots of intestinal IAP mRNA content after T_4 and/or cortisone administration

For dot blots, defined amounts of total RNA (as in Figure 2) from the duodenum (rows 1–4) and jejunum (rows 5–8) of control (rows 1 and 5) rats and in rats treated with T_4 (rows 2 and 6), cortisone (C) (rows 3 and 7) and T_4 + cortisone (rows 4 and 8) were used (see the Experimental section for details). For Northern blots, total RNA (10 μg) from the duodenum of control (CON) rats, and of rats after T_4, cortisone or T_4 + cortisone administration were used as described in Figure 2. The 2.7 kb mRNA is present in all animal groups, and the 3.0 kb species is induced in cortisone- or T_4 + cortisone-treated rats.

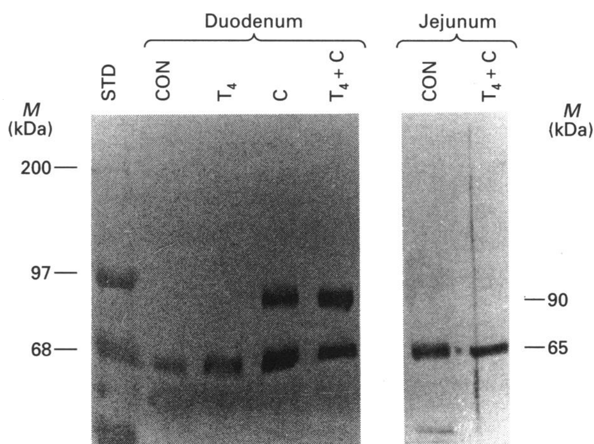

Figure 5 Western-blot analysis of the effect of T_4 and/or cortisone on IAP isoenzyme expression

The duodenal homogenate containing 10 μg of protein from a control (CON) rat and rats treated with T_4, cortisone (C) or T_4 + cortisone and jejunal homogenate from control and T_4 + cortisone treated rats were subjected to SDS/PAGE under reducing conditions followed by immunoblotting as described in Figure 3. The values on the right indicate the molecular masses (M) of the IAP isoforms.

and greater expression of the 3.0 kb transcript (Figures 4 and 5 and Tables 4 and 5). The administration of 2–4 times more cortisone had no additive effect (results not shown), suggesting that the T_4 enhancing effect was not mediated by increasing endogenous production of adrenocorticoids. Administration of cortisone or T_4+cortisone did not induce jejunal PhP or βGP activity. T_4+cortisone even decreased jejunal βGP activity (Table 4). Jejunal IAP mRNA levels also were not altered after cortisone, T_4 or cortisone+T_4 administration (Table 5). Only the 65 kDa IAP isoenzyme was detected in the jejunum in all groups (Figure 5).

DISCUSSION

At least four major AP isoforms are encoded by separate genes in humans: intestinal (IAP), placental, placental-like (germ-cell) and tissue-non-specific AP (Harris, 1982; Berger et al., 1987; Weiss et al., 1988; Henthorn et al., 1988; Millán and Manes, 1988). The tissue-non-specific AP is ubiquitously expressed in many tissues, including liver, bone and kidney. Three genes encoding different AP isoforms (intestinal, placental-like and tissue-non-specific) have been reported in mice (Manes et al., 1990). Following the postnatal surge of IAP activity, duodenal epithelium is the tissue containing the largest amount of this enzyme. Postnatal increase of IAP activity has been reported only in mammals born with immature intestines, such as rats and mice (Moog, 1953; Moog and Yeh, 1973). The increase of IAP activity that occurs concomitantly with accelerated postnatal intestinal growth (Yeh and Moog, 1975a; Yeh, 1977) may have functional significance, presumably related to hydrolysis and absorption of phosphorylated compounds.

The present data support the notion that two distinct IAP isoenzymes are expressed in rat intestine and are encoded by different mRNAs. Developmental expression of these two IAP isoenzymes is differentially regulated with regional specificity (Figures 2–5). The 90 kDa isoenzyme appears to be the product of the 3.0 kb mRNA, whereas the 65 kDa is encoded by the 2.7 kDa mRNA. These two mRNAs have been shown to have about 70% sequence identity, and the sequence differs over the entire length of the molecule (Strom et al., 1991; Engle and Alpers, 1992). These divergent structures encode only 79% identical amino acid residues, certainly enough to account for different substrate specificities. The role of two separate duodenal IAPs is unknown, but they have structurally less sequence identity than human placental AP and intestinal AP (Henthorn et al., 1987).

The observation that a 2-fold higher duodenal IAP mRNA level on day 32 than day 24 was not accompanied by an increase in IAP content and activity suggests post-transcriptional regulation. This mechanism of regulation has been reported for many other enterocyte proteins, including apolipoproteins and lactase-phlorizin hydrolase (Alpers, 1990; Freund et al., 1991; Yeh et al., 1991a), but has not been reported previously for IAP (Eliakim et al., 1991). Luminal IAP was found to be disproportionately greater on day 32 than day 24, implying increased mucosal IAP turnover rate. Since luminal IAP shows characteristics of duodenal IAP, it appears to originate mainly from the duodenum. This interpretation is consistent with the reports that (1) duodenal explants have been shown to release 20-times more IAP activity than ileal explants in organ culture (Koyama et al., 1987) and (2) the increase in the release of phospholipid membrane-bound IAP after fat feeding in adult rats occurred mostly in the proximal intestine (Eliakim et al., 1991). The release of duodenal IAP into the lumen could be by epithelial-cell secretion of IAP on a phospholipid-rich membranous particle (Eliakim et al., 1989), by brush-border-membrane shedding (Beaudoin and Grondin, 1991) or both. It is unlikely that IAP is released in a soluble form, since nearly all luminal IAP is membrane-bound when serum contamination of the lumen with glycosyl-phosphatidylinositol-anchor-specific phospholipase D is prevented (Eliakim et al., 1990a).

Previously, we reported that T_4+cortisone stimulates IAP activity more than cortisone alone in hypophysectomized rats (Yeh and Moog, 1975b). The present data agree and indicate further that T_4+cortisone stimulates greater expression of the 3.0 kb mRNA and the 90 kDa isoenzyme than cortisone alone. Enhanced 90 kDa isoenzyme expression is associated with increased PhP/βGP ratio. These data support the suggestion that the 90 kDa protein is the PhP-preferring isoenzyme. An alternative possibility (not mutually exclusive) is that catalytic activity of the 65 kDa IAP has been altered. The 65 kDa isomer in the duodenum of day 12 and 18 rats and the jejunum of all ages appeared to migrate faster than that in the duodenum of day 24 and 32 rats (Figure 3). Similarly, this IAP band migrated faster (estimated difference in the molecular mass of 2 kDa) in the duodenum of control and T_4-treated than in the cortisone- and T_4+cortisone-treated rats (Figure 5). Because IAP contains about 20% (w/w) carbohydrate (Saini and Done, 1970; Yedlin et al., 1981), it is likely that changes in mobility are related to the extent of glycosylation. It remains to be determined if glycosylation affects IAP activity and substrate preference for this 65 kDa isoenzyme.

This work was supported in part by National Institutes of Health Research Grants DK-33916, AG-01625 and AM-14038, and by the Center for Excellence in Cancer Research Treatment and Education, LSU Medical Center, Shreveport, LA, U.S.A.

REFERENCES

Alpers, D. H. (1990) Digestion **46**, 50–58
Beaudoin, A. R. and Grondin., G. (1991) Biochim. Biophys. Acta **1071**, 203–219
Berger, J., Garanttini, E., Hua, J. C. and Udenfriend, S. (1987) Proc. Natl. Acad. Sci. U.S.A. **84**, 695–698
Eliakim, R., DeSchryver-Kecskemeti, K., Nogee, L., Stenson, W. F. and Alpers, D. H. (1989) J. Biol. Chem. **264**, 20614–20619
Eliakim, R., Becich, M. J., Green, K. and Alpers, D. H. (1990a) Am. J. Physiol. **259** (Gastrointest. Liver Physiol. **22**), G618–G625
Eliakim, R., Seetharam, S., Tietze, C. C. and Alpers, D. H. (1990b) Am. J. Physiol. **259** (Gastrointest. Liver Physiol. **22**), G93–G98
Eliakim, R., Mahmood, A. and Alpers, D. H. (1991) Biochim. Biophys. Acta **1091**, 1–8
Engle, M., and Alpers, D. H. (1992) Clin. Chem. **38**, 2506–2509
Etzler, M. E. and Moog, F. (1968) Biochim. Biophys. Acta **154**, 150–161
Freund, J.-N., Duluc, I. and Raul, F. (1991) Gastroenterology **100**, 388–394
Harris, H. (1982) Harvey Lect. **76**, 95–123
Henthorn, P. S., Raducha, M., Edward, Y. H., Weiss, M. J., Slaughter, C., Lafferty, M. A. and Harris, H. (1987) Proc. Natl Acad. Sci. U.S.A. **84**, 1234–1238
Henthorn, P. S., Raducha, M., Kadesch, T., Weiss, M. J. and Harris, H. (1988) J. Biol. Chem. **263**, 12011–12019
Hodin, R. A., Chamberlain, S. M. and Upton, M. P. (1992) Gastroenterology **103**, 1529–1536
King, E. J. and Armstrong, A. R. (1934) Can. Med. Assoc. J. **31**, 376–381
Koyama, I., Arai, K., Sakagishi, Y., Ikezawa, H. and Komoda, T. (1987) J. Chromatogr. **420**, 275–286
Laemmli, U. K. (1970) Nature (London) **227**, 680–685
Lowe, M. O., Strauss, A. W., Alpers, R., Seetharam, S. and Alpers, D. H. (1990) Biochim. Biophys. Acta **1037**, 170–177
Lowry, O. H., Rosebrough, N. J., Farr, A. L. and Randall, R. J. (1951) J. Biol. Chem. **193**, 265–275
MacDonald, R. J., Swift, G. H., Przybyla, A. E. and Chirgwin, J. M. (1987) Methods Enzymol. **152**, 219–227
Manes T., Glade, Y. Y., Ziomek, C. A. and Millán, J. L. (1990) Genomics **8**, 541–554
Millán, J. L. and Manes, T. (1988) Proc. Natl. Acad. Sci. U.S.A. **85**, 3024–3028
Moog, F. (1953) J. Exp. Zool. **124**, 329–346
Moog, F. (1961) Dev. Biol. **3**, 153–174

Moog, F. J. (1966) Exp. Zool. **161**, 353–367
Moog, F. and Yeh, K. Y. (1973) Comp. Biochem. Physiol. **44B**, 657–666
Saini, P. K. and Done, J. (1970) FEBS Lett. **7**, 86–89
Seetharam, B., Yeh, K. Y., Moog, F. and Alpers, D. H. (1977) Biochim. Biophys. Acta **470**, 424–436
Strom, M., Krisinger, J. and DeLuca, H. F. (1991) Biochim. Biophys. Acta **1090**, 299–304
Sussman, N. L., Seetharam, S., Blaufuss, M. C. and Alpers, D. H. (1986) Biochem. J. **234**, 563–568
Taussky, H. H., and Shorr, E. (1953) J. Biol. Chem. **202**, 675–685
Weiss, M. J., Ray, K., Henthorn, P. S., Lamb, B., Kadesch, T. and Harris, H. (1988) J. Biol. Chem. **263**, 12002–12010

Received 12 August 1994/18 February 1994; accepted 7 March 1994

Yedlin, S. T., Young, G. P., Seetharam, B., Seetharam, S. and Alpers, D. H. (1981) J. Biol. Chem. **256**, 5620–5626
Yeh, K. Y. (1977) Anat. Rec. **188**, 69–76
Yeh, K. Y. and Moog, F. (1975a) Dev. Biol. **47**, 156–172
Yeh, K. Y. and Moog, F.(1975b) Dev. Biol. **47**, 173–184
Yeh, K. Y., Yeh, M. and Holt, P. R. (1989) Am. J. Physiol. **256** (Gastrointest. Liver Physiol. **19**), G604–G614
Yeh, K. Y., Yeh, M., Montgomery, R. K. Grand, R. J. and Holt, P. R. (1991a) Biochem. Biophys. Res. Commun. **180**, 174–180
Yeh, K. Y., Yeh, M. and Holt, P. R. (1991b) Am. J. Physiol. **260** (Gastrointest. Liver Physiol. **23**), G371–G378