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Meprin mRNA in rat intestine during normal and glucocorticoid-induced maturation: divergent patterns of expression of α and β subunits

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Abstract Meprin is a zinc-metalloendopeptidase expressed in intestinal epithelial cells. In rat jejunum collected from postnatal day 4 (P4) through P25 meprins α mRNA exhibited uniform levels for the first three postnatal weeks and then declined, whereas meprin β mRNA showed a biphasic pattern with high levels in the first postnatal week followed by low levels from P7 through P19 and then a marked rise at P22 and P25. Dexamethasone treatment beginning at P10 had no significant effect on levels of meprins α mRNA, whereas this treatment caused a precocious increase in expression of meprin β mRNA. These divergent patterns of expression of meprins α and β mRNA suggest distinct roles for the two subunits during the suckling and weaning phases of rodent intestinal development.

Key words: Metalloendopeptidase; Meprin; Development; mRNA expression; Glucocorticoid; Rat intestine

1. Introduction

Meprins are metalloendopeptidases found on the apical membranes of epithelial cells from both kidney and small intestine [1–3]. All mammalian meprins studied to date have been found to have two subunits, α and β which are derived from distinct transcripts [4–9]. Analysis of primary structures reveals that the two subunits are approximately 45% identical and 60% similar. Between mouse, rat and human the respective subunits are approximately 80% identical [10]. They have a very similar multi-domain structure featuring the actual protease domain, adhesive domains, an EGF-like domain and a C-terminal membrane-spanning domain. Based on an extended zinc-binding motif in the protease domain, the meprins have been classified as belonging to the astacin family of metalloendopeptidases [11]. The protein subunits can associate to form both homo- and hetero-dimers; these in turn may associate in various combinations to yield tetramers [7,12,13]. The mature β subunit is an integral membrane protein whereas the α subunit, after proteolytic cleavage of the C-terminal anchor, is entirely extracellular and is associated with the β subunit through disulfide and non-covalent bonds [9,13,14]. Although both subunits display proteolytic activity, there are differences in their catalytic specificity [15]. Most notably, the α subunit appears to favor biologically active peptides. Despite extensive study, the precise role of these metalloendopeptidases has remained elusive. Based on other members of the astacin family, proposed functions include degradation of extracellular matrix proteins [16–18], modification of growth factors [19] and regulation of differentiation and development [20,21].

The developing rodent intestine offers fertile ground for the study of factors involved in both growth and differentiation. During the first three postnatal weeks, the intestinal epithelium undergoes dramatic growth and significant functional changes. Moreover, the luminal substrates change markedly during this period as the offspring make the transition from the suckling period (first 2 weeks) through the weaning period (third week) and finally to the fully-weaned stage (fourth week). Accompanying these dietary changes there are extensive changes in digestive and absorptive capacities of the small intestine [22]. In general, hydrolases required for the digestion of milk components (such as lactase-phlorizin hydrolase), display high activity during the suckling period and then decline, whereas those associated with digestion of components of the solid diet (such as sucrase-isomaltase) display the converse pattern with little or no activity during the suckling period and a dramatic rise during the third postnatal week [22,23]. In recent years these changes in hydrolytic activities have been shown to be reflected by changing levels of expression of the respective mRNAs [24]. The factors responsible for these marked changes in gene expression in the developing rodent intestine have not been fully identified. It is clear that maturation is driven in part by an intrinsic timing mechanism but various hormones have also been implicated [23–25]. Amongst the latter, the most compelling evidence points to a role for glucocorticoid hormones. Circulating concentrations of endogenous glucocorticoid rise steeply at the end of the second postnatal week and when exogenous glucocorticoid is administered at earlier ages, the ontogenic changes in enzyme activities are elicited precociously [22–24]. As with normal development, the effects of glucocorticoid on enzyme activities are reflected by changes at the mRNA level [24,26,27].

For the meprins, both α and β mRNAs have been detected in adult rat and mouse intestine [5,28]. However, to date there have been no studies of the expression of the two subunits in the developing intestine. Thus, the goals of the current study were to quantitate the expression of meprin α and β mRNAs during both normal and glucocorticoid-induced intestinal maturation in rats.

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Abbreviations: P, postnatal day; EF, elongation factor-1α; ANOVA, analysis of variance

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2. Materials and methods

2.1. Animals and tissue collection

Adult males and timed-pregnant dams of the Sprague-Dawley strain (Charles River Crl:CD(SD)BR) were obtained from Charles River Breeding laboratories (Portage, MI, USA) and were maintained as described elsewhere [29]. Two males (age 48 days) were used for a detailed collection of tissue along the length of the intestinal tract. The developmental pattern of meprin expression was studied by collecting jejunum from three rat pups (from three different litters) at various ages from postnatal day 4 (P4) through postnatal day 25 (P25). To study the effect of exogenous glucocorticoid, dexamethasone was chosen because it does not bind to corticosteroid-binding globulin and thus yields circulating concentrations unaffected by developmental changes of the latter [30]. Starting at P10, littermates received either dexamethasone (0.4 μg/g body weight) or vehicle injections on a daily basis and were killed after 1 or 4 days of treatment. In all experiments, intestinal segments were flushed with cold saline (0.9% NaCl) prior to being frozen in liquid nitrogen.

2.2. RNA isolation and Northern blotting

Total cellular RNA from the above tissue samples was isolated using guanidine isothiocyanate extraction and pelleting through a caesium chloride cushion as described elsewhere [26,29]. Northern blots were generated according to routine procedures using 20 μg total RNA per lane. Blots were probed with ^{32}P-labeled cDNAs using hybridization and washing conditions described elsewhere [26]. The probe used for meprin α was a 230 bp fragment from human meprin α (nucleotide 1808–2037) which has no homology to meprin β and has 76% identity to rat meprin α; that used for meprin β was a 435 bp fragment of human meprin β (nucleotide 1449–1883) with low homology to meprin α and with 80% identity to rat meprin β. Blots were probed first with meprin α, then stripped and reprobed with meprin β, then stripped and reprobed with elongation factor-1α (EF) as a constitutive marker [31].

2.3. Quantitative analyses

Signals from Northern blots were quantified by phosphorimaging using a box spanning the central region of each band. Background subtraction was achieved using a box of the same size from a representative region of the blot above the bands. To correct for loading variations, these data were expressed as a ratio of the hybridization signal of the band of interest (meprin α or β) to that of the constitutive marker EF. These values are termed 'relative RNA abundance' and are shown graphically as means ± S.E.M. for the number of animals in each experimental group. Statistical analysis was by analysis of variance (ANOVA). A P value of $P < 0.05$ was considered significant, and post-hoc Fisher's pairwise comparisons were then performed when appropriate.

3. Results

3.1. Longitudinal distribution of meprin mRNAs in adult rats

Because many intestinal mRNAs display marked gradients of expression along the length of the intestinal tract, we performed a preliminary study using adult rats to examine the expression of the meprin RNAs in five regions of the small intestine as well as two regions of the large intestine. The results can be seen in Fig. 1. Both α and β mRNAs were detected in all regions. The estimated sizes of the transcripts were 3.7 kb and 2.7 kb for meprin α mRNA and meprin β mRNA, respectively. The sizes are in good agreement with those reported in the literature for both rats and mice [5,28]. From these qualitative data it is apparent that both α and β mRNAs have relatively uniform expression throughout most of the small intestine (specifically from the proximal jejunum to the distal ileum). For meprin α mRNA there is a modest reduction in the duodenum and in both regions of the colon. A similar but more pronounced pattern is seen for meprin β mRNA. On the basis of these results the entire jejunum was utilized in all subsequent studies.

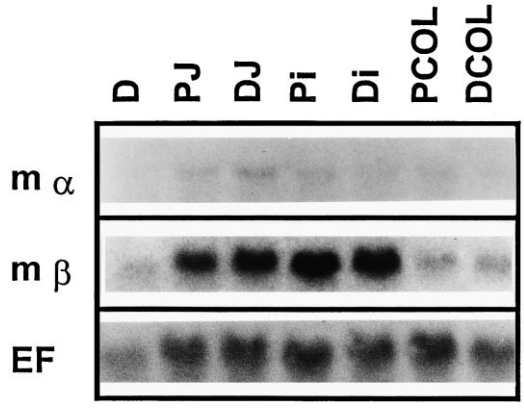

Fig. 1. Expression of meprin α and β mRNAs in different regions of the intestinal tract of adult rats. Representative Northern blot showing signal for meprin α mRNA (mα), meprin β mRNA (mβ) and for the constitutive marker EF. Intestinal segments are labeled as follows: D = duodenum, PJ = proximal jejunum, DJ = distal jejunum, Pi = proximal ileum, Di = distal ileum, PCOL = proximal colon and DCOL = distal colon.

3.2. Expression of meprin mRNAs during normal development

Jejunal RNA was prepared from rat pups from postnatal day 4 (P4) through postnatal day 25 (P25). This time period encompasses the suckling period (first 2 weeks), the transitional or weaning period (third week) and the fully weaned stage (fourth week). Results (Fig. 2) showed distinct patterns of expression for the two meprin mRNAs. Levels of meprin α mRNA were relatively constant from P4 through P22 and then declined by P25. ANOVA for meprin α showed the overall effect of age to be not significant ($P = 0.11$). However, Fisher's pairwise comparisons showed that the value at P25 was significantly less than those at P4, 7, 13 and 16. In contrast, meprin β mRNA displayed a biphasic pattern of expression with high levels seen in the neonatal period, a marked decline by the end of the first postnatal week, consistent low levels through P19 and then a sharp increase by P22 which was maintained through P25. In this case, the overall effect of age was highly significant ($P < 0.001$ by ANOVA) and individual comparisons showed that the levels of meprin β mRNA at P4 as well as at P22 and P25 were significantly elevated over those seen from P7 through P19.

3.3. Effects of glucocorticoid administration during the suckling period

In order to assess whether the changes observed for meprin α and β mRNA during normal development can be precociously elicited by exogenous glucocorticoid, dexamethasone was administered to rat pups starting at P10. Jejunal RNA was prepared at P11 and P14, i.e. after 1 and 4 days of treatment, respectively. As can be seen in Fig. 3A dexamethasone had little or no effect on meprin α mRNA but caused a distinct increase in the levels of meprin β mRNA. The quantitative analysis (Fig. 3B) showed that for meprin α mRNA there was no statistically significant effect of either age or treatment (by two-way ANOVA). In contrast for meprin β mRNA the effects of both age and treatment were highly significant ($P < 0.005$ and $P < 0.001$, respectively). In addition, in the two-way ANOVA the interaction between age and treatment

was significant ($P < 0.03$) indicating that age influenced the response to treatment. More specifically, the dexamethasone-induced elevation of meprin β mRNA was significantly greater after 1 day of treatment (age 11) than after 4 days of treatment (age 14).

4. Discussion

Studies in adult rats have reported that both meprin α mRNA and meprin β mRNA are more abundant in the small intestine than the large intestine [5]. As there was no information regarding the distribution of these two mRNAs along the length of the small intestine of the rat, our preliminary experiment examined five different regions from the duodenum to the distal ileum. Our findings reflected those of the enzyme activity in the small intestine of adult rats with a rise in mRNA from proximal duodenum to jejunum. Throughout the jejunum and ileum we found a relatively constant expression of both meprin α and meprin β mRNA. On this basis, we could have chosen to use either jejunum or ileum for our subsequent studies. As there are considerably more data on developmental patterns of mRNA expression in the jejunum [24,26,27], we chose this region for comparative purposes.

The developmental pattern observed for meprin α mRNA, with high levels throughout the suckling period and then a decline at weaning is similar to that reported for the mRNA for lactase-phlorizin hydrolase [32,33], as well as various other digestive and absorptive functions that are required for the assimilation of the milk diet [24]. This raises the possibility of a unique function for meprin α during the suckling period. Obvious possibilities are a nutritional role via the hydrolysis of milk proteins or a protective role via the hydrolysis of one or more of the many biologically active peptides found in milk. A role in either protein or peptide hydrolysis during the suckling period is particularly cogent in view of the fact

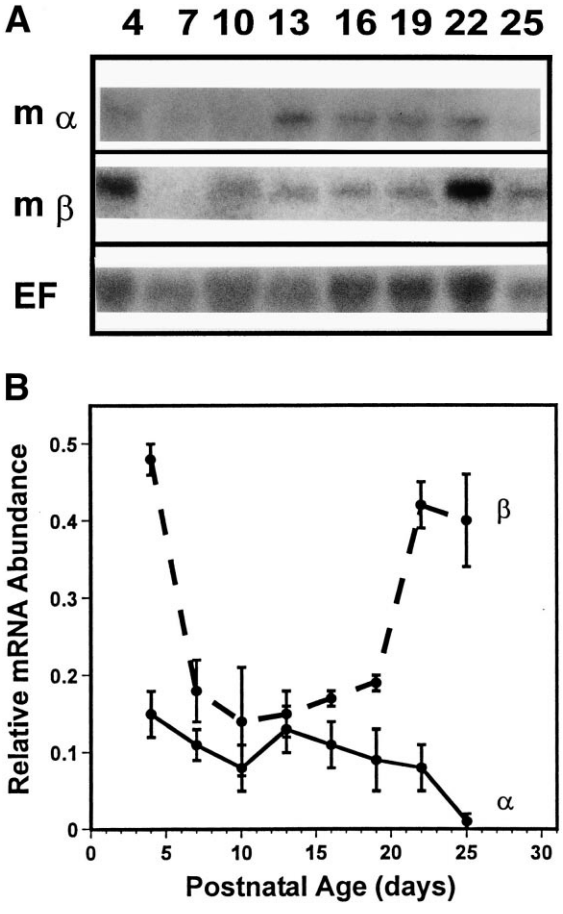

Fig. 2. Postnatal development of meprin α and β mRNA in rat jejunum. A: Representative Northern blot of jejunal RNA collected from individual rats every third day from P4 through P25. Postnatal ages (days) are shown above each lane. Labels for RNAs are as in Fig. 1. B: Relative abundance of meprin α and β mRNA shown (i.e. ration of each with EF) as means ± S.E.M. for three animals

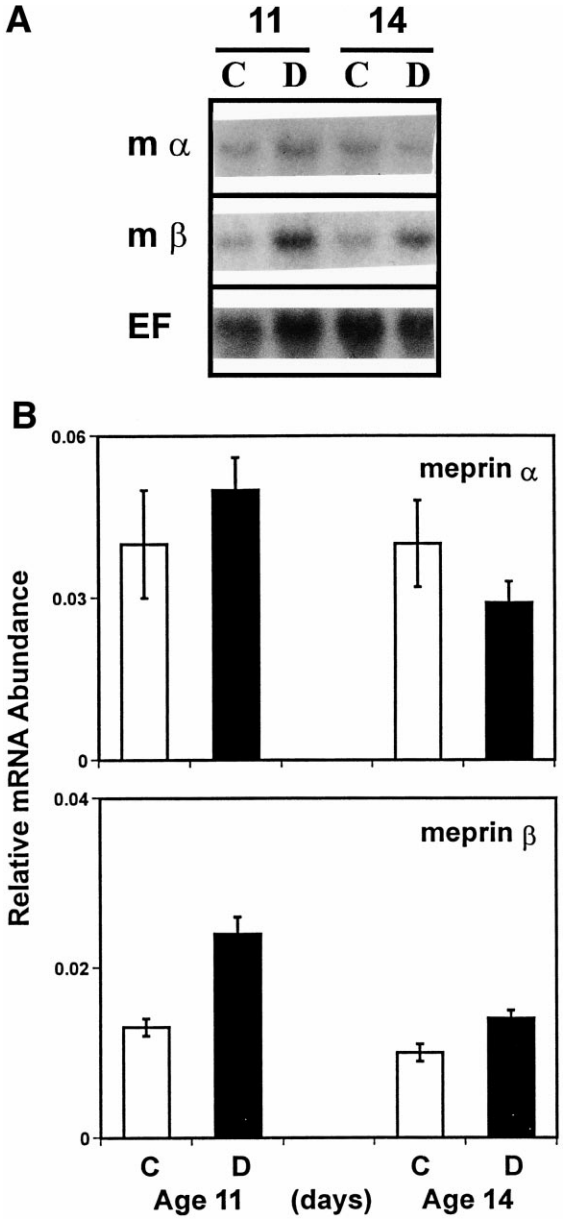

Fig. 3. Effect of exogenous glucocorticoid on meprin α and β mRNA expression in rat jejunum. Data are shown for control (C) and dexamethasone treated (D) rats at P11 and P14 receiving either vehicle or dexamethasone treatment starting at P10. A: Representative Northern blots for meprin α (mα) and β mRNA (mβ) as well as the constitutive marker (EF). B: Relative abundance of meprin α and β mRNA (i.e. ration of each with EF) shown as means ± S.E.M. for three animals.

that secretion of pancreatic proteases is minimal at this time [22].

For meprin β mRNA, from P7 through P25, the developmental pattern is analogous to those of the α-glucosidase family of brush-border hydrolases, specifically sucrase-isomaltase [29] and trehalase [27]. These are hydrolases that are required for digestion of the carbohydrate components of solid food. Similarly, we would propose that the marked increase of meprin β expression at the time of weaning may suggest a specific role for this protease in the digestion of proteins found in the solid diet. The fact that the proteolytic capacity of meprin β is enhanced in the presence of trypsin [15,34] also fits with a physiological role at the time of weaning when there is a surge in the luminal concentrations of trypsin [22].

The high levels of expression of meprin β mRNA in the first postnatal week raise the possibility of a different role for the β subunit at this time. Given the minimal secretion of trypsin in the neonatal rat [22], it would seem that the protease activity of meprin β would remain latent [15,34]. Under these circumstances, rather than function as an enzyme, meprin β could serve as a membrane receptor or docking protein. The two adhesive domains MAM [35] and MATH [36] represent potential domains that may mediate interactions with other proteins. Human meprin β but not α contains two potential phosphorylation sites (Tyr-682 and Ser-688) as well as a protein kinase C phosphorylation site motif [9]. The two phosphorylation sites are also present in the rat β subunit [5]. This distinct cytoplasmic domain of meprin β raises the possibility that the subunit serves in a signal transduction capacity.

Given that meprin α expression declines at the time of weaning, whereas that of meprin β increases, the effects of glucocorticoid treatment were consistent with the literature on other brush-border hydrolases. Specifically, the lack of effect of glucocorticoid on meprin α mRNA is congruent with reports that lactase expression does not decline precociously following glucocorticoid administration to suckling rats [24]. In contrast, for the brush-border hydrolases that show increased expression at the time of weaning, there is a large body of evidence showing that their expression can be activated precociously by glucocorticoids [23,24], and that such activation is reflected at the mRNA level [26,27,29]. Thus, in our studies, the ability of dexamethasone to cause a precocious increase in the levels of meprin β mRNA is consistent with the general pattern for hydrolases showing increased expression in the weaning period (e.g. the α-glucosidases). On the other hand, the relatively rapid response of meprin β mRNA following dexamethasone treatment (with higher levels observed after 1 day than after 4 days of treatment) is quite different from that reported for α-glucosidase mRNAs. Specifically, both sucrase-isomaltase mRNA [26,29] and trehalase mRNA [27] display slower increases with higher levels observed after 4 days of treatment, than after 1 day. The sucrase-isomaltase temporal pattern has been ascribed to the action of the hormone on the cells in the upper regions of the crypt, followed by a slow emergence onto the villi where sucrase-isomaltase begins to be expressed [23,24]. The different temporal pattern observed for meprin β mRNA in response to dexamethasone administration to suckling rats raises the possibility that this gene may be directly activated by glucocorticoids in cells already on the villus. This suggestion is in agreement with the finding that meprin β mRNA (in human intestine) appears to be expressed earlier along the crypt/villus axis than does sucrase-isomaltase mRNA [37].

In summary, the divergent pattern of expression of meprin α and β mRNA during development as well as in response to glucocorticoid is a clear indication for distinct roles for the two subunits. Identification of hormone recognition sites in the promoter region of the two subunits may shed more light on the regulation of expression and provide clues as to the different functions of meprin α and β.

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