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MINERALOCORTICOID RECEPTOR GENE EXPRESSION IN THE GASTROINTESTINAL TRACT: DISTRIBUTION AND ONTOGENY

PETER J. FULLER* and KAREN VERITY
Medical Research Centre, Prince Henry's Hospital, St Kilda Road, Melbourne 3004, Australia

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Summary—The gastrointestinal tract is a well characterized target tissue for aldosterone, where it regulates electrolyte transport, particularly in the descending colon. Previous studies have demonstrated the presence of aldosterone receptors in gastrointestinal tissues. We have used specific cRNA probes for the rat mineralocorticoid receptor to explore both the distribution and ontogeny of mineralocorticoid receptor gene expression in the gastrointestinal tract.

Mineralocorticoid receptor gene expression is found throughout the small and large intestine, but is absent from the stomach. The highest levels are observed in the distal colon, and significant expression is found in the duodenum; in both tissues levels of expression are higher than those in kidney. In both the developing duodenum and colon, mineralocorticoid receptor gene expression precedes the development of the full physiological response to aldosterone. These findings emphasise the colon as an important target tissue for aldosterone, and raise the question of potential roles for aldosterone in the duodenum.

INTRODUCTION

Adrenocortical steroid hormones are known to regulate electrolyte transport in the gastrointestinal tract [1]. These corticosteroids act via two closely related receptors [2]: mineralocorticoid or Type I receptors (MR) and glucocorticoid or Type II receptors (GR). These receptors are members of a larger family of ligand-dependent transcription activation factors which includes not only the receptors for steroid hormones but also those for thyroid hormone and retinoic acid [3].

In 1976, Pressley and Funder [4] reported the presence of both classes of corticosteroid receptor in the rat gastrointestinal tract. Subsequent studies have confirmed and extended these observations [5–11]. In the case of the mineralcorticoid receptor such studies have been hindered by both a lack of specificity and by receptor instability [9]. Despite the use of more robust assays [12] and of more specific synthetic steroids as receptor ligands [6, 7, 9], neither the distribution of mineralocorticoid receptors throughout the gastrointestinal tract, nor receptor ontogeny, has been adequately defined. The recent cloning of a rat MR cDNA clone [13] has thus enabled us to define the distribution of MR gene expression in the gastrointestinal tract and to contrast this distribution with that of GR gene expression. In addition, we have examined the ontogeny of MR and GR gene expression in these tissues.

*To whom correspondence should be addressed.

EXPERIMENTAL

Tissue preparation

Male Sprague–Dawley rats weighing 120–180 g, from a pathogen-free colony in the Central Animal House, Monash University, were used in all experiments. Rats were maintained on water and standard rat chow *ad libitum*. For the ontogeny study, tissues were collected and pooled from 18 day fetal, newborn, 3, 7, 10, 12, 15, 18, 21, 25 and 30 day post-natal rats, and from adults rats. Animals were killed by cervical dislocation, and the intestinal specimens removed, washed in normal saline, snap frozen in liquid nitrogen and stored at −80°C.

mRNA analysis

Total RNA was isolated by the method of Chirgwin *et al.* [14]. The amount of RNA was measured at OD_{254}, and by inspection of ethidium bromide stained gels. Northern blot analysis was performed as previously described [15, 16]. Briefly, 12.5 μg of total RNA was denatured in 1 M glyoxal–50% dimethylsulphoxide, electrophoresed in a 0.8% agarose-gel and transferred to Hybond nylon membranes (Amersham, England) [17]. The membranes were baked at 80°C for 2 h, u.v.-crosslinked for 10 min, prehybridized at 42°C and hybridized at 68°C in the hybridization solution of Leiter *et al.*[18] [50% formamide, 5 × SSPE, 0.15 M Tris–HCl, pH 8.0, 1% sodium dodecylsulphate (SDS) and heparin 500 mg/ml] for cRNA probes. For the cDNA probe, prehybridization and hybridization was in a

conventional hybridization solution [15, 16] at 42°C. The blots were then washed once at room temperature in 2 × SSC (1 × SSC is 0.15 M sodium chloride–0.015 M sodium citrate, pH 7.0), 0.1% SDS and then twice for 20 min at 68°C (cRNA probes) or 50°C (cDNA probe) in 0.2 × SSC, 0.1% SDS.

Membranes were then blotted dry and exposed to Kodak X-AR (Eastman Kodak, Rochester, N.Y.) or Fuji (Fuji Photo Film, Japan) X-ray film with a Cronex Lightening-Plus intensifying screen (DuPont, Wilmington, Del.) at −80°C.

Prior to rehybridization with a different probe, blots were placed in boiling distilled water for 2 min, re-exposed to determine the adequacy of removal of the previous probe, and then prehybridized and hybridized as before.

Probes

The plasmid $prMR_{EH}$ containing a 513 bp rat MR cDNA [13] was linearised with BamHI enabling synthesis of the ^{32}P-labelled mineralocorticoid receptor cRNA probe [19] with SP6 polymerase (Promega) and [α-^{32}P]UTP (>400 ci/mmol; BRESA, Adelaide, South Australia). The GR cRNA probe was constructed by subcloning a 620 bp AvaI-EcoRI fragment of the full length rat GR cDNA [20] corresponding to the steroid binding domain, into pGEM-4Z (Promega). The glucagon, cholecystokinin (CCK) and kallikrein cRNA probes, which are used as tissue specific controls in the ontogeny study, have been described previously [16]. The rat tubulin cDNA probe [21] was labelled by nick translation (Amersham Nick Translation Kit, Amersham, England) with [α-^{32}P]dCTP (1800 Ci/mmol; BRESA).

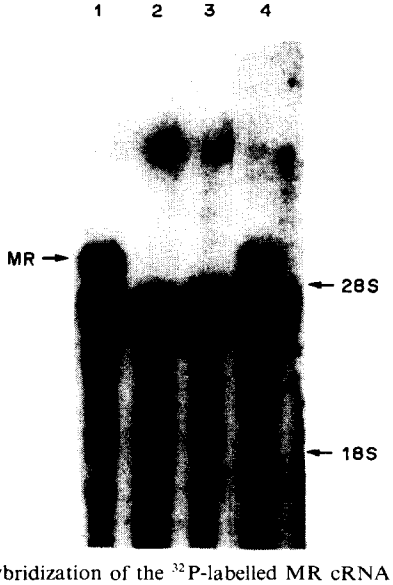

Fig. 1. Hybridization of the ^{32}P-labelled MR cRNA probe to a Northern blot of 12.5 μg of total RNA from descending colon (lane 1), liver (lane 2), thymus (lane 3) and kidney (lane 4). The position of the predominant MR transcript is indicated, as are the positions of the 28S and 18S ribosomal RNA.

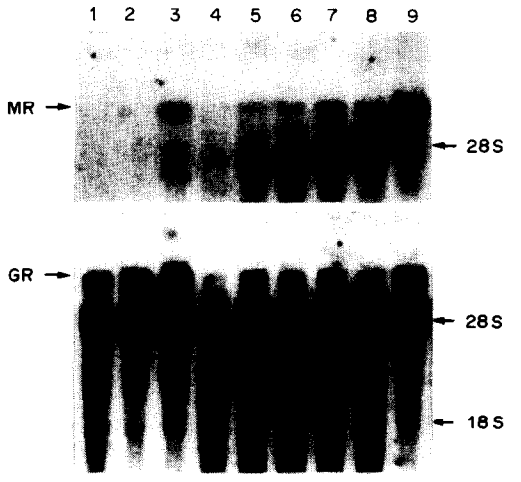

Fig. 2. Hybridization of the ^{32}P-labelled MR and GR cRNA probes to a Northern blot of 12.5 μg of total RNA from corpus (lane 1), antrum (lane 2), duodenum (lane 3), jejunum (lane 4), ileum (lane 5), caecum (lane 6), ascending colon (lane 7), transverse colon (lane 8) and descending colon (lane 9). The positions of the MR, GR and 28S RNA are indicated.

RESULTS

Figure 1 shows the expected hybridization of the MR cRNA probe to kidney RNA, strongly positive hybridization to colonic RNA and no hybridization with either liver or thymic RNA. Strong hybridization is also seen to the 28S ribosomal RNA under these conventional hybridization conditions, so that transcripts smaller than the ~6.0 kb transcript are difficult to detect. When both the hybridization solution and conditions are altered, from hybridization at 60°C in a conventional hybridization solution [16] to 68°C in the hybridization solution of Leiter *et al.*[18], a transcript more precisely defined as 6.0 kb and a much less abundant smaller transcript are also observed (Fig. 2).

A survey of the various gastrointestinal tract tissues shows widespread MR gene expression in both the small and large bowel but not to RNA from either the corpus or the antrum of the stomach (Fig. 2). The highest levels are seen in the descending colon; note also the relatively high level of expression in the duodenum. Conversely, GR gene expression is ubiquitous, with significant levels of GR mRNA throughout the gastrointestinal tract.

In order to further characterize MR gene expression in the descending colon, MR mRNA levels were examined in the upper and lower halves of the descending colon (Fig. 3); no significant difference was observed. In contrast to previous reports [13, 22], in our studies the colon rather than the hippocampus clearly has the highest relative level of MR gene expression (Fig. 3). Examination of poly $(A)^+$-enriched RNA reveals an identical pattern of hybridization, including the smaller less abundant transcript (data not shown).

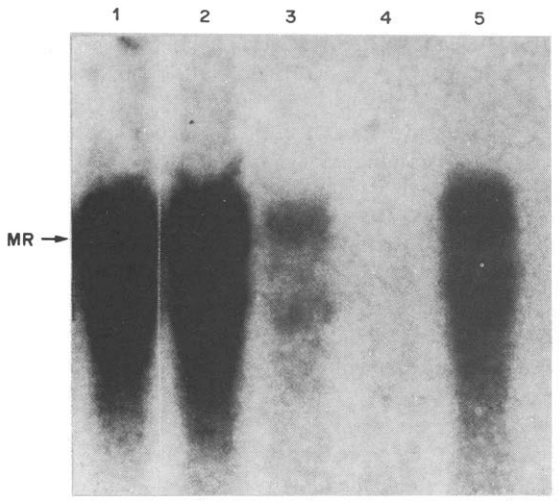

Fig. 3. Hybridazation of the ^{32}P-labelled MR cRNA probe to a Northern blot of 12.5 μg of total RNA from descending colon (lane 1), sigmoid colon (lane 2), kidney (lane 3), liver (lane 4) and hippocampus (lane 5).

The ontogeny of MR gene expression in the colon and duodenum was compared both with that in the kidney, and also with that of GR gene expression in the gastrointestinal tract. Hybridization with the other tissue specific (glucagon, CCK and kallikrein) or non-specific (tubulin) probes provides a control for the integrity of the RNA and contrasting patterns of developmental expression. In the colon, MR gene expression is already present at birth and changes

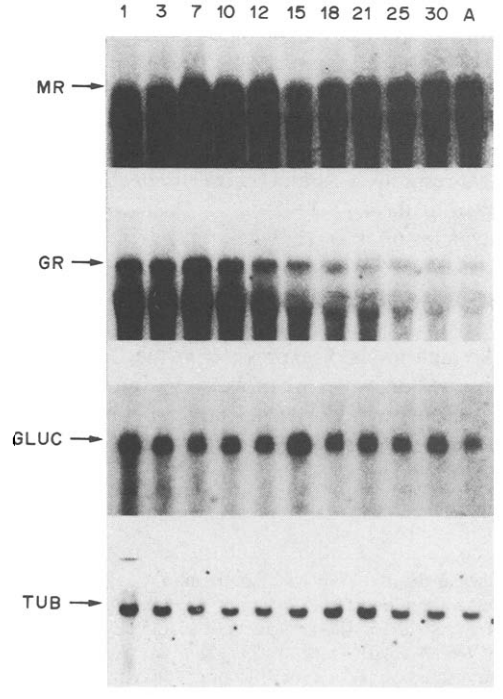

Fig. 4. Hybridization of ^{32}P-labelled MR, GR and glucagon (GLUC) cRNA and tubulin (TUB) cDNA probes to Northern blot of 12.5 μg of total colonic RNA from postnatal (days 1, 3, 7, 10, 12, 15, 18, 21, 25, 30) and adult rats.

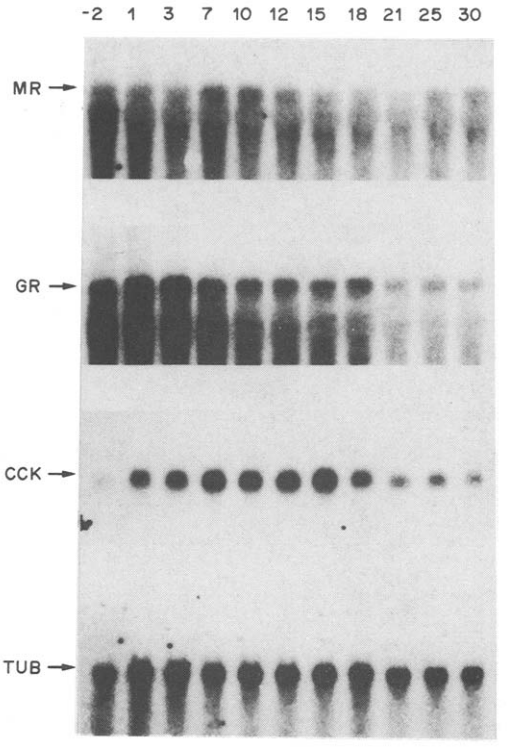

Fig. 5. Hybridization of the ^{32}P-labelled MR, GR and CCK cRNA and tubulin cDNA probes to a Northern blot of 12.5 μg of total duodenal RNA from prenatal (−2) and postnatal (days 1, 3, 7, 10, 12, 15, 18, 21, 25, 30) rats.

very little thereafter; a similar pattern is seen for tubulin and glucagon gene expression, whereas GR mRNA levels show a modest decrease (Fig. 4). These patterns are in marked contrast to the dramatic post-natal increase in Na,K-ATPase subunit gene expression which occurs in the colon [23]. The ontogeny of MR, GR and tubulin gene expression in the duodenum parallels that in the colon; in contrast, CCK mRNA levels increase markedly at birth, further increase gradually until weaning and then fall somewhat (Fig. 5). In the kidney, MR gene expression is low in the fetus and neonate, but increases thereafter to reach adult levels only well after weaning; GR mRNA levels, however, show a reciprocal pattern (Fig. 6). Kallikrein gene expression has a similar pattern to that of the MR gene.

DISCUSSION

Using ligand binding techniques, various studies have demonstrated the presence of both mineralocorticoid and glucocorticoid receptors in gastrointestinal tissues [4–11]. These studies have largely focused on the colon where corticosteroids of both classes are known to regulate electrolyte transport [1]. In 1987 Arriza *et al.*[22] cloned and sequenced the human MR cDNA and used this cDNA in turn to isolate a rat MR cDNA close [13]. These authors [13, 22] have conducted a limited tissue survey of MR gene expression with particular emphasis on the hippocampus.

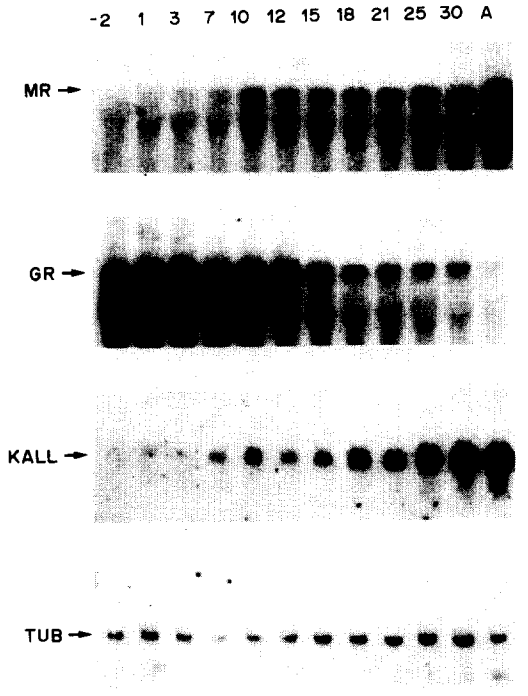

Fig. 6. Hybridization of ^{32}P-labelled MR, GR and kallikrein (KALL) cRNA and tubulin cDNA probes to a Northern blot of 12.5 μg of total kidney RNA from prenatal (−2) and postnatal (days 1, 3, 7, 10, 12, 15, 18, 21, 25, 30) rats.

The present data extends these studies by defining both the distribution and ontogeny of the expression of the MR gene in the gastrointestinal tract. These patterns of MR gene expression clearly contrast with those of GR gene expression.

The relatively low abundance of the mRNA species, and the large size of the transcript which renders them susceptible to partial degradation, make such studies difficult. The problem of partial degradation of larger transcripts is particularly well demonstrated in Figs 4–6, where the shorter transcripts (glucagon, CCK, kallikrein and tubulin) yield discrete bands on autoradiography, whereas the receptor transcripts show significant partial shortening. Some workers have avoided this problem by using short probes in RNAase protection assays [13]; however, despite the partial degradation, both receptor mRNAs are clearly demonstrated.

The distribution of MR gene expression observed in the gastrointestinal tract correlates with the known pattern of responsivity to administered aldosterone. The effects of aldosterone on gastrointestinal electrolyte transport are seen both in the distal small bowel and colon, with the most marked effects in the distal colon. The level of MR gene expression in the distal colon is greater than that observed in either the kidney, the classical mineralocorticoid target tissue, or in the hippocampus which has been proposed to have the highest levels of expression [13, 22].

Though MR gene expression is low or absent from the stomach, it is high in the duodenum. The work of Suzuki *et al.*[24, 25] suggests that duodenal MR may mediate an effect of aldosterone on Mg^{2+}-HCO_3-ATPase and carbonic anhydrase activities, so that the predominant effect of the hormone may be on acid–base balance, as it is in the medullary collecting duct [26]. The finding that the aldosterone antagonist spironolactone blocks not only the side-effects but also the desired therapeutic effect of carbenoxolone sodium on the healing of peptic ulcers has suggested a role for MR mediated effects in the upper gastrointestinal tract [27]. It is thus possible that aldosterone induces an increase in the capacity of the duodenal enzymes which buffer gastric acid.

The MR gene is expressed in both the duodenum and colon of the neonatal rat. This precedes development of the full mineralocorticoid response in both colon [28] and kidney [29], so that appearance of the MR would not appear to be rate limiting. It is possible that the MR subserves some other function in development, perhaps by responding to corticosterone as occurs in the adult hippocampus [2], in which case the appearance of 11β-hydroxysteroid dehydrogenase activity might be the critical developmental determinant of aldosterone responsivity [30]. The developmental pattern of corticosteroid receptor gene expression in the gastrointestinal tract differs from the hippocampus where Type I binding appears at post-natal day 8 and Type II binding though present at birth is low, rising only slowly to achieve adult levels [31]. MR gene expression in the kidney is also low initially, and increases steadily over the period of study; this profile is slightly at variance with a previous report of aldosterone binding where maximum levels were found at day 15 [29]. It should be noted, however, that the only earlier time point in this study was day 7. These authors demonstrated a renal response to aldosterone (antinatriuresis) at days 7–9 but this response was much lower than the response observed at days 13–15 [29].

In conclusion, this study defines the distribution of adrenocortical steroid hormone receptor gene expression in the gastrointestinal tract and the ontogeny of this gene expression. Particularly striking are the high levels of expression in the distal colon, an observation which reinforces the importance of the colon as an aldosterone target tissue, and the significant levels of mineralocorticoid receptor in the duodenum where its physiological role remains to be determined.

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