# Plasma-membrane calcium-pump isoforms in human and rat liver

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Plasma-membrane $Ca^{2+}$-pumping ATPases (PMCAs) extrude $Ca^{2+}$ from the cytoplasm of all cells. Some previous studies of ATP-dependent $Ca^{2+}$ transport by liver membranes suggested there exist specific properties of the hepatic PMCA, including regulation by hormones which affect calcium signalling. Multiple PMCA isoforms are now known to result from expression of four different genes (known as PMCA 1–4) and alternative RNA splicing at three possible sites (A, B and C). We investigated which isoforms are expressed in adult human and rat liver RNA using reverse-transcription polymerase chain reaction with mixed primers designed to amplify parts of all the known PMCA transcripts. In human liver, products were identified by sequencing from PMCA1, PMCA2 and PMCA4, but not from PMCA3 or from any new gene. In rat liver, by contrast, only PMCA1 and PMCA2 were detectable, although we confirmed that the primers were able to amplify from rat lung a new sequence which is part of rat PMCA4. Of the alternatively spliced variants, at site A in the PMCA2 sequences, all the exons were included in both adult and fetal human liver. In human liver, the exon at site B was excluded in some products from PMCA1 and PMCA4, and at site C, only PMCA1b and one form of PMCA4 were found. Blots of human liver RNA showed PMCA1 and PMCA4 were abundantly expressed, unlike PMCA2. On blots of rat liver RNA, PMCA1 was more abundant than PMCA2, and purified rat parenchymal cell RNA gave similar findings. In summary, no new hepatic PMCA isoforms have been demonstrated, but differences between the predominant human and rat isoforms may have consequences for $Ca^{2+}$ signalling or the response to liver cell injury.

## INTRODUCTION

Plasma-membrane $Ca^{2+}$-pumping ATPases (PMCAs) extrude $Ca^{2+}$ from all cells and so determine the total intracellular [$Ca^{2+}$]. Along with other intracellular $Ca^{2+}$ pumps which sequester $Ca^{2+}$ in the endoplasmic or sarcoplasmic reticulum, they maintain the low cytoplasmic free [$Ca^{2+}$] ($10^{-7}$ M) used in $Ca^{2+}$ signalling in response to hormones [1]. In polarized cells such as the intestinal enterocyte, differential expression in apical and basolateral membranes is necessary for transepithelial $Ca^{2+}$ transport.

In the liver, $Ca^{2+}$ signalling has been studied in detail and liver PMCAs have been clearly demonstrated. ATP-dependent $Ca^{2+}$ transport in purified rat liver plasma-membrane vesicles has been shown by several groups and has been differentiated from the activity of other $Ca^{2+}$-dependent ATPases [2]. PMCA activity in the rat liver is affected by certain hormones, including glucagon, parathyroid hormone and vasopressin [3], which use $Ca^{2+}$ as an intracellular messenger and achieve their effects directly on the PMCA through G-proteins [4]. Furthermore, differences in PMCA activities in basolateral and canalicular membrane fractions could have a role in the secretion of $Ca^{2+}$ into bile [5]. In general, the properties of the PMCA in liver are similar to those of PMCAs located elsewhere. For instance, $Ca^{2+}$-dependent phosphorylation of proteins from ATP gave a product of ~ 135 kDa [6] and an antibody which detects other PMCAs (mAb-5F10) cross-reacted with epitopes in liver membranes [7]. However, some properties of the rat liver PMCA, especially the interactions with calmodulin, appear to differ from other PMCAs and a limited amino acid sequence has suggested that this might represent a new isoform [7].

Multiple isoforms of PMCA have been described [1,8] and it is likely that the different properties of PMCA in various tissues are due to differential expression of these forms. In humans, four genes (ATP2B1 to ATP2B4) encode the $Ca^{2+}$ pumps known as PMCA1, PMCA2, PMCA3 and PMCA4 [9–12]. In rats, the complete sequences of the homologues PMCA1, PMCA2 and PMCA3 have been determined [13,14], whereas only partial sequences of rat PMCA4 have been described [15–17]. Further diversity is produced by alternative splicing of transcripts from all these genes at three possible sites (A, B and C) as described in Table 1. Site A is located near the putative phospholipid regulatory domain and alternative splicing has been reported in PMCA2, PMCA3 and PMCA4. Transcripts differing at site B have been detected in human PMCA1 and PMCA4, and multiple splicing variants occur in all genes at site C and affect the calmodulin-binding domain (see Table 1 for references).

In our previous studies of the forms of PMCA in intestine we described a partial 3′ sequence for rat PMCA4 but could not detect this transcript in liver [17]. The purpose of the present study was to determine which of the known forms of PMCA were present in human and rat liver, and whether any new gene or particular splicing variant could account for the properties of the liver $Ca^{2+}$ pump.

## EXPERIMENTAL

### RNA preparation

Normal human liver was obtained from two patients having partial hepatectomy for tumours; rat liver was obtained from young animals. Tissue was snap-frozen in liquid nitrogen. For some experiments, fetal human tissue [20], rat lung, cerebral cortex or purified hepatic cells [21] were used. Total RNA was prepared by acid guanidinium isothiocyanate phenol/chloroform extraction [22].

Abbreviations used: EtBr, ethidium bromide; PMCA, plasma-membrane $Ca^{2+}$-pumping ATPase.

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The partial sequence of rat PMCA4 has been submitted to EMBL and Genbank Databases under the accession no. X76452.

**Table 1 PMCA isoforms applicable to this paper**

ND, not detected (no alternative splicing described in any tissue); NGP, no gene product detected in liver; NR, not reported; NS, not studied.

| Gene product... | PMCA1 | | | PMCA2 | | | PMCA3 | PMCA4 | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Alternative splicing site... | A | B | C | A | B | C | | A | B | C |
| Human: | | | | | | | | | | |
| References | 9, 12, 18 | 9, 17 | 8, 9, 18, 19 | 11, 18 | NR | 11, 18, 19 | 18 | 10, 18 | 8, 10, 17 | 10, 17–19 |
| Additional bases | ND | 0/+108 | 0/+87/+114/+152/+154 | 0/+42/+135 | | 0/+227 | NGP | 0/+36 | 0/+108 | 0/+178 |
| Nomenclature | 1x | | 1b/1c/1d/1e/1a | 2z/2x/2w | | 2b/2a | | 4z/4x | 4g/– | 4b/4a |
| PCR primers used | 875X, 874X | 685V, 607 | 664, 666 | 875X, 874X | NS | NS | NS | 875X, 874X | 686V, 607 | 665, 608 |
| cDNA probes used | HASA1 | PLC-29* | PLC-29* | HASA2 | H2-3′ | H2-3′ | NS | HASA4 | PLC-204* | PLC-204* |
| Rat: | | | | | | | | | | |
| References | 13 | 13, 17 | 13, 16, 24 | 13, 24 | NR | 13, 16, 24 | 14, 16 | NR | 16, 17 | 16 |
| Additional bases | ND | ND | 0/+87/+114/+152/+154 | 0/+42/+93/+135 | | 0/+172/+227/+229 | NGP | | ND | 0/+175 |
| Nomenclature | | | 1b/1c/1d/1e/1a | 2z/2x/2y/2w | | 2b/2c/2a | | | | 4b/4a |
| PCR primers used | 875X, 874X | NS | NS | 875X, 874X | NS | NS | NS | 875X, 874X | NS | NS |
| cDNA probes used | RASA1 | R1-3′* | R1-3′* | RASA2 | R2-3′ | R2-3′ | NS | RASA4 | R4-3′* | R4-3′* |

* The amplification of these cDNA probes with mixed primers 607 and 611 is described in refs. [17] and [23].

## PCR

Mixed-primer PCR was used to attempt to amplify all PMCA sequences in the liver RNA from the two species. Previous studies had concentrated on regions around the splicing sites B and C [17], and to extend these the present work used primers in the 5′ half of the coding sequence, which included splice site A. Peptides conserved in all four known human and rat PMCA sequences were identified. These were coded for by bases 790–806 and 1396–1412 in human PMCA1 [9] and the corresponding sequences in the other genes. Two degenerate mixed primers (875X and 874X) were then synthesized (Oswell DNA Service; Edinburgh); these were designed to amplify all the possible codons and incorporated *Eco*R1 sites at their 5′ ends for subsequent cloning. The sequences were: 875X, 5′-CGCGAATTCACNCAYGTNATGGARGG-3′; 874X, 5′-CGCGAATTCGCNGTNGCRTTNCCCAT-3′. The primers used to study alternative splicing at sites B and C (607, 608, 611, 685V, 686V, 664, 665, 666) have been detailed previously [17].

Amplification conditions followed the methods described before [17,23]. Briefly, reverse transcription was performed with 1 $\mu$g of poly(A)RNA, random hexamers and avian myeloblastosis virus reverse transcriptase. Approximately 10% of the reaction product was used as template cDNA in a 'hot-start' PCR with 500 ng of each primer.

### Analysis of products

The products of the PCR reaction were separated on agarose gels and visualized by ethidium bromide (EtBr) staining. Bands of products previously cut with *Eco*R1 were excised from the gel, eluted, and cloned into M13 or pBluescript for sequencing by the dideoxynucleotide method. Blots were probed with species- and gene-specific cDNA probes generated in this study from the cloned and sequenced PCR products. These are known as HASA1 (for human PMCA1 sequence near alternative splice site A), HASA2, HASA4, RASA1, RASA2 and RASA4 (see Table 1). Probes were labelled with [$\alpha$-$^{32}$P]dCTP [17].

### Northern blots

Total RNA (25 $\mu$g), dissolved in formamide/formaldehyde-containing Mops buffer, was electrophoresed, blotted and incubated with equivalently labelled cDNA probes as described before [17]. Equal loading and integrity of the RNA was assessed by EtBr staining. Probes used in Northern blots included those amplified and sequenced in this study around alternative splice site A as described above, and also those directed towards a more 3′ region near splice sites B and C studied previously [17,23]. Table 1 indicates these probes to human PMCA1 (PLC-29), rat PMCA1 (R1-3′), human PMCA4 (PLC-204) and rat PMCA4 (R4-3′), and similar probes to human PMCA2 (H2-3′) and rat PMCA2 (R2-3′) also amplified with mixed primers 607 and 611. These cDNA probes are all gene- and species-specific under the hybridization and washing conditions used.

## RESULTS

### PCR products and identification

Amplification of human liver cDNA with the mixed primers 875X and 874X gave three major bands upon gel electrophoresis ranging in size from ~ 500 to ~ 700 bp. Rat liver cDNA also produced multiple bands; the two smallest were similar in size to the two larger bands in humans (Figure 1).

The PCR products from human liver were identified by

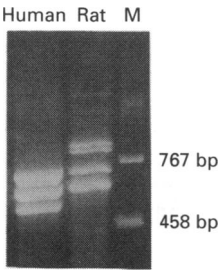


**Figure 1 Gel electrophoresis of PCR products**

An EtBr-stained 1.4% (w/v) agarose gel with PCR products obtained with mixed primers 875X and 874X and cDNA prepared from human or rat liver RNA is shown. The positions of two DNA markers (M) at 767 and 458 bp are shown.

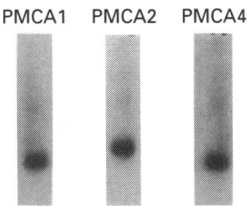


**Figure 2 Northern blots of gels of human liver PCR products**

PCR products amplified from human liver cDNA as in Figure 1 were blotted on to membranes, and hybridized with probes to PMCA1 (HASA1), PMCA2 (HASA2) or PMCA4 (HASA4).

```
   T    C              A    T TG   C T            C T
 1 CTCTGGACGGATGGTGGTGACTGCTGTGGGAATCAATTCCCAGACTGGAATCATCTTCAC   60
    S  G  R  M  V  V  T  A  V  G  I  N  S  Q  T  G  I  I  F  T

   T    G   TC       T  C  A GG
61 CCTCTTAGGGGCTAATGAGGAAGAAGACGATGAGAAAAAGAAGAAAGGTAAAAAACAAGG  120
    L  L  G  A  N  E  E  E  D  D  E  K  K  K  K  G  K  K  Q  G

     C                    G    A  C        C
121 AGTCTCTGAAAATCGCAACAAAGCAAAAACCCAGGATGGAGTGGCCTTGGAAATCCAGCC  180
    V  S  E  N  R  N  K  A  K  T  Q  D  G  V  A  L  E  I  Q  P

                  AA   C A          C    G  GT    TG
181 ACTCAACAGCCAGGAGGGGCTCGATAGTGAGGAAAAGGAGAAGAAAGCAAGCAAGGGACC  240
    L  N  S  Q  E  G  L  D  S  E  E  K  E  K  K  A  S  K  G  P

   T A          A  G             G  T          T    T
241 CAAGAAGGAGAAGTCGGTCCTGCAGGGCAAGCTCACACGCCTGGCTGTGCAGATCGGGAA  300
    K  K  E  K  S  V  L  Q  G  K  L  T  R  L  A  V  Q  I  G  K

   A  C      C         GCT     G  TT                 T  A
301 GGCAGGTCTGATCATGTCTATCCTCACAGTCCTCATCCTGATTCTATACTTCGTGGTTGA  360
    A  G  L  I  M  S  I  L  T  V  L  I  L  I  L  Y  F  V  V  D

     C  T     A  T   A AC       C        T    CA C
361 CAATTTCGTGATACAGCGCCGGGCATGGCTTCCTGAGTGCACTCCTGTGTACATCCAGTA  420
    N  F  V  I  Q  R  R  A  W  L  P  E  C  T  P  V  Y  I  Q  Y

   C                  CA          G
421 TTTTGTCAAGTTCTTCATCATCGGAGTCACTGTACTGGTAGTGGCTGTGCCAGAGGGGCT  480
    F  V  K  F  F  I  I  G  V  T  V  L  V  V  A  V  P  E  G  L

     T          A   AC          G                A    T
481 GCCACTGGCTGTCACCGTCTCGTTGGCCTACTCTGTAAAGAAAATGATGAAGGACAACAA  540
    P  L  A  V  T  V  S  L  A  Y  S  V  K  K  M  M  K  D  N  N

    C A A          T  T       C
541 CTTGGTCCGGCACTTGGACGCCTGTGAGACG  571
    L  V  R  H  L  D  A  C  E  T
```

**Figure 3 Partial sequence of rat PMCA4**

The nucleotide sequence is shown of a product sharing sequence similarity with human PMCA4 amplified from rat lung, but not from rat liver, using the mixed primers 875X and 874X (primer sequences not shown). Bases which differ in human PMCA4 are shown above the rat sequence commencing at nucleotide 795 of the sequence reported by Strehler et al. [10]. The rat amino acid sequence is shown in single-letter code for the open reading frame homologous to human PMCA4.

sequencing clones obtained from the different bands. Products of expected sizes were found for human PMCA1 and PMCA4 in clones from the middle band, and for PMCA2 in the largest band. No sequences were obtained from PMCA3 or from any new isoform. The smallest band of PCR products was shown to be the Z form of $\alpha$1-antitrypsin, which was amplified as parts of the sequence share significant sequence similarity with the primers. The identity of the bands was further confirmed by probing blots with labelled inserts from clones in which the sequences had been confirmed to be those of PMCA1, PMCA2 and PMCA4 (Figure 2).

In the rat liver PCR products, sequencing showed the expected products for only PMCA1 (smallest band) and PMCA2 (middle band). No sequences derived from PMCA4 were detected despite sequencing over 30 clones. As in the human products, no clones representing PMCA3 or any new PMCA isoform were found, but an unidentified product, which shared no significant sequence similarity with any PMCA except in the primer region, was also amplified and accounted for the largest bands on blotting. Blots confirmed that PMCA2 was found in the middle band, and PMCA1 was in the smallest band (results not shown).

The absence of products from rat PMCA4 in liver was further investigated. To confirm that the primers were able to amplify rat PMCA4, which had not previously been sequenced in this region, cDNA was prepared from rat lung tissue and amplified using the same conditions as liver. From lung PCR products, clones of rat PMCA4 were readily identified upon sequencing. The new partial sequence is shown in Figure 3 and shares 85% nucleotide identity and 88% amino acid identity with human PMCA4 in this region. In contrast, the identity values with the other genes are lower, the highest being 76% with rat PMCA3. Hence, the inability to amplify PMCA4 by PCR from rat liver is because of the absence of this transcript in rat liver and is not due to incorrect primers.

## Alternatively spliced variants

Alternative splicing of three exons at site A in the 5′ region of both rat and human PMCA2 is reported to produce at least three different transcripts with 0, 42 or 135 bp of additional sequence [11,24] (Table 1). Splicing variants at site A have also been described for PMCA3 and recently for PMCA4 [18]. In our experiments, we found no evidence for alternative splicing in liver at site A, as only the originally described sequences were amplified for human PMCA1 [9], PMCA4 [10] and rat PMCA1 [13]. With PMCA2, only the splicing variant with all 135 bp (2w) was found in sequences of clones from adult human or rat liver and fetal human liver. In blots of PCR products, a single band was found with fetal liver which was the same size as that found in adults (Figure 4a). Thus we did not confirm the presence of the variant with only 42 bp additional sequence (2z) previously described in fetal human liver [11].

Amplifications across site B using human liver cDNA and the primers described before [17] gave some PCR products of PMCA1 and PMCA4 which lacked the alternatively spliced exons. These were apparent in both the EtBr-stained agarose gels of the products and when blots of these were probed with gene-specific cDNA (Figure 4b) as described before [17]. As previously shown in intestine, these alternative products were less abundant than those which included the exon. In similar studies of the splicing variants of PMCA1 and PMCA4 at site C, only one band for each, i.e. PMCA1b and PMCA4b, were visible on gels of the PCR products; the blots of these gels are shown in Figure 4(c). Neither of these isoforms include the alternatively spliced exons.

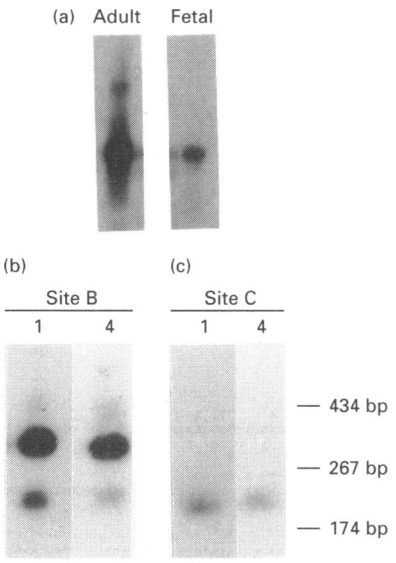


**Figure 4 Analysis of alternatively spliced variants in human liver**

(**a**) PMCA2 variants at site A were amplified with primers 875X and 874X blotted and probed with PMCA2 (HASA2). The size of product seen in human adult and fetal liver corresponds to the variant with 135 bp of additional sequence. (**b**) Products of PCR amplifications across site B using primers 685V and 607 for PMCA1 and 686V and 607 for PMCA4 were probed with PLC-29 and PLC-204 respectively [17]. Products of ~ 300 and ~ 200 bp are seen for both genes. (**c**) Products of amplifications across site C using primers 664 and 666 for PMCA1 (1) and 665 and 608 for PMCA4 (4) were probed with PLC-29 or PLC-204. In each case, a single band of ~ 200 bp is seen.

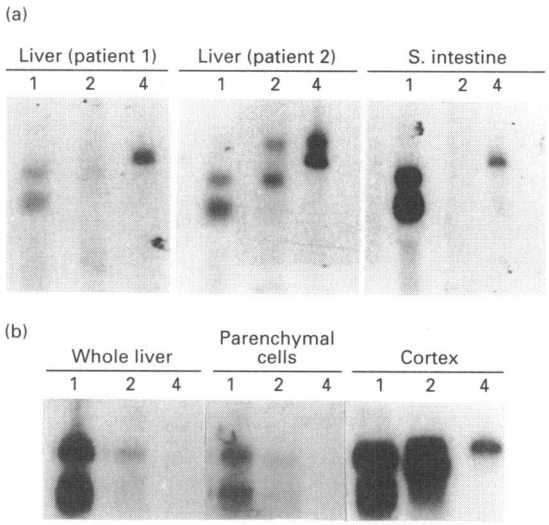


**Figure 5 Blots of RNA probed with isoform-specific probes**

Total RNA (25 $\mu$g) was loaded in each lane and hybridized with gene- and species-specific cDNA probes for PMCA1 (1), PMCA2 (2) or PMCA4 (4). The probes were those amplified and sequenced in this study (HASA1, HASA2, HASA4 for human tissue and RASA1, RASA2, RASA4 for rats). These were labelled so that their specific activities were equivalent to one other. Washing and autographic exposures were performed in parallel. (**a**) RNA from two sources of adult human liver (patient 1 and patient 2) compared with RNA from human small-intestinal mucosa (s. intestine). (**b**) RNA prepared from rat whole liver tissue or from purified liver parenchymal cells compared with RNA from rat cerebral cortex.

### PMCA transcripts on Northern blots

The PCR results described above do not provide accurate quantification of the relative amounts of the different genes detected on amplification. This was better achieved by probing RNA blots with equivalently labelled isoform-specific probes to the three genes (Figure 5). In blots of human liver, transcripts for PMCA1, PMCA2 and PMCA4 were confirmed; PMCA4 and PMCA1 were the most abundant. Two sizes of transcripts ( ~ 5.5 and ~ 7.5 kb) were found as expected for PMCA 1, resulting from different polyadenylation sites in the 3′-untranslated region. Blots probed for PMCA4, which in previous studies of different tissues from 12 individuals has always given a single band at ~ 8 kb, resulted in an additional larger band in liver RNA prepared from one patient. We have not been able to study sufficient liver samples to ascertain the frequency of this variation in liver PMCA4 transcript size. Although the relative amounts differed, PMCA2, with two sizes of transcripts ( ~ 7.5 and ~ 9.5 kb), was always the least abundant. For comparison, Figure 5(a) also shows RNA from human proximal intestinal mucosa probed in parallel and demonstrates the preponderance of mRNA for PMCA1, a lesser amount of PMCA4 but no clearly detectable PMCA2. Similar results were found with probes to the region around splice site A amplified in this study (HASA1, HASA2, HASA4) and with the 3′ probes amplified as described before (PLC-29, H2-3′ and PLC-204) [17].

In blots of RNA from rat liver, PMCA1 was the most abundant transcript, PMCA2 was much less abundant and, as in the PCR experiments, no PMCA4 was detectable (Figure 5b). When RNA prepared from parenchymal cells was probed, a similar pattern was found, suggesting that the low level of expression of PMCA2 is not confined to one of the less abundant cell types such as Kupffer cells. Rat cerebral cortex studied in parallel demonstrated expression of PMCA1, PMCA2 and PMCA4. Again, similar results were found with the 5′ probes (RASA1, RASA2 and RASA4) and the 3′ probes (R1-3′, R2-3′ and R4-3′).

## DISCUSSION

This paper describes the first comprehensive study of the expression of PMCA genes in human and rat liver tissue. Despite earlier suggestions [7] we found no evidence to support the expression of a new gene in liver. Human liver contains transcripts for PMCA4, PMCA1 and PMCA2 in decreasing order of abundance. In contrast, rat liver contains PMCA1 and, much less abundantly, PMCA2, but PMCA4 appears not be expressed in this tissue in rats.

PMCA1 and PMCA4, but not PMCA2 or PMCA3, were thought to be widely or even ubiquitously expressed based on the results of other studies of various tissues [16,18,19]. Our inability to detect PMCA4 in rat liver in this present paper by both PCR and on Northern blots supports the findings on blots shown by Keeton et al. [16] and in our previous paper [17]. In that paper, which used a different PMCA4 probe derived from a more 3′ region of the rat gene (R4-3′), we also showed that rat small intestinal mucosa did not express PMCA4. It is always possible that another form of PMCA exists in the liver which would not amplify, as it does not share sequence similarity with the primer sequences used in our mixed PCR reaction; however, this possibility becomes increasingly remote as different pairs of primers and different cDNA probes are used.

Expression of PMCA2 in both human and rat liver is clearly demonstrable, though at much lower levels than for the other genes. The results with purified parenchymal cells suggest that this expression occurs in the majority of the hepatocytes rather than in a minor cell type such as the lipocytes, endothelial or

Kupffer cells. Whether the expression of PMCA2 might be linked to a particular function in the parenchymal cells, for example the excretion of $Ca^{2+}$ into bile at the canalicular membrane [5], cannot be determined from the present experiments.

We have also defined which alternatively spliced variants are found in human liver. Splicing at site A in PMCA2 resulted in the product which possessed all the additional bases and was the same in both fetal and adult liver, in contrast with the findings of the previous report [11]. We were able again to demonstrate alternative sequences at site B in transcripts from both PMCA1 and PMCA4, but, as previously discussed [17], this does not necessarily imply that functioning PMCA proteins with the altered transmembrane topology are found. At site C, we found only the variants which are most widespread, i.e. PMCA1b and PMCA4b. This is similar to the situation reported in human fetal liver [19].

An important inference that can be drawn from our results is that the specific properties previously described for $Ca^{2+}$ transport and ATPase activity in rat liver membranes are almost certainly those of PMCA1. These include the very high affinity for calmodulin and the resultant difficulty in showing stimulation by exogenous calmodulin [7]. Furthermore, based on the descriptions of significant inhibition of the rat liver $Ca^{2+}$ pump by hormones including glucagon-(19–29)-peptide, calcitonin and parathyroid hormone [3,4,25], a direct interaction of the G-protein $G_s\alpha$ with PMCA1b seems likely. Additional studies on this interaction in liver and in other tissues will provide an exciting link between these two aspects of the response to hormones.

In human liver, expression of PMCA4 mRNA is equal to or slightly greater than that of PMCA1. Hence the effects of various agents an overall plasma-membrane $Ca^{2+}$-pump activity may be dissimilar to those in the rat. PMCA4 does not have the consensus cyclic-AMP-phosphorylation site near the C-terminus in PMCA1 [26], though both are likely to be phosphorylated by protein kinase C [27]. It remains to be explored how these differences in PMCA expression between the species affect intracellular $Ca^{2+}$ concentrations after hormonal stimulation. Additionally, there may be differential susceptibility to the various forms of liver cell injury that have been shown to be a result of chemicals which target thiol groups in the PMCA in rat liver [28,29].

PMCA1 is the overwhelmingly predominant form of the plasma membrane $Ca^{2+}$ pump in rat liver, as it is in intestinal mucosa from both human and rats [17,23] and in human osteoblasts [30]. Although it is apparently expressed ubiquitously, in the above tissues it clearly functions in more than a purely housekeeping role and thus will be the subject of considerable further study. There are no reports of successful expression of this protein in a cell-line where its specific properties can be studied in isolation from those of other isoforms; however, experiments with easily available rat liver will in effect provide much of that data.

Received 17 February 1994/9 May 1994; accepted 16 May 1994

This work was supported by a grant from the Medical Research Council to J. R. F. W. We are indebted to Denis Calnan for preparing the isolated rat parenchymal cells and to Dr. Clare Selden for gifts of fetal liver RNA. Preliminary results were presented at the 44th Annual Meeting of the American Association for the Study of Liver Diseases and published in Hepatology (1993) **18**, 166A.

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