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Original Contribution

MOLECULAR CLONING OF TWO RAT Na^+/Pi COTRANSPORTERS: EVIDENCE FOR DIFFERENTIAL TISSUE EXPRESSION OF TRANSCRIPTS

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Abstract—Recently, Na^+/Pi cotransport activity has been demonstrated in rat liver hepatocytes. Here, we report the isolation of two Na^+/Pi cotransporter cDNAs (RNaPi-1a and RNaPi-1b) from a rat liver cDNA library. The two cDNAs have the same coding but different 5′-untranslated regions. The rat cDNAs encode a polypeptide of 465 amino acids, having 62% and 66% identity with the rabbit NaPi-1 and human kidney Na^+/Pi cotransporter, respectively. Northern blot analysis showed that a RNaPi-1a–specific probe detected two major transcripts (2.3 and 1.8 kb), whereas a RNaPi-1b–specific probe hybridized with one transcript (1.8 kb) in rat kidney, liver, and hepatocytes in primary culture. Rat liver expressed much higher levels of RNaPi-1a than RNaPi-1b, whereas the converse was true for rat kidney. Low levels of RNaPi-1 mRNAs were also detected in rat heart, brain, and skeletal muscle. These findings indicate that there are at least two isoforms of RNaPi-1 transcripts expressed in liver and kidney and that the levels of expression of the RNaPi-1a and RNaPi-1b may be controlled by tissue-specific factors.

Keywords—Na^+/Pi-cotransporter, cDNA cloning, Rat hepatocytes, Tissue-specific expression, Kidney

INTRODUCTION

Cytosolic Pi plays a central role in oxidative and substrate-level formations of high-energy phosphates, regulates the activities of a variety of enzymes, and is essential for cell growth and replication. It is now clear that cytosolic Pi concentrations are rigidly regulated and that a variety of mechanisms are involved in this process (Biber and Murer, 1993; Bonjour and Caverzasio, 1984; Raymond et al., 1991; Wehrle and Pedersen, 1989). After the original discovery that Pi transport across the luminal brush-border membrane of mammalian kidney proximal tubule cells and intestinal cells was carrier-mediated and Na^+ dependent (for reviews, see Bonjour and Caverzasio, 1984), Na^+/Pi cotransport activity has been found in many different cells, including hepatocytes (Ghishan et al., 1993; Younus and Butterworth, 1993), cardiac myocytes (De Young and Scarpa, 1991; Escoubet et al., 1989; Jack et al., 1989), and skeletal muscle cells (Polgreen et al., 1994).

Recently, a number of cDNAs for Na^+/Pi cotransporters have been isolated from kidney and other tissues. Based on sequence similarities, they can be divided into three groups. The first group includes NaPi-1, isolated from rabbit kidney using expression cloning in oocytes (Werner et al., 1991), and NaPi-1–related isolates, including the human analogue NPT1 (Chong et al., 1993; Miyamoto et al., 1995) and a brain-specific isolate BNPI (Ni et al., 1994). The second group includes NaPi-2 isolated from rat kidney (Magagnin et al., 1993), NaPi-3 from human kidney, NaPi-4 from opossum kidney cells (Sorribas et al., 1994), and a mouse analogue (Collins and Ghishan, 1994). The third group includes two retrovirus receptors (Ram-1 and Glvr-1) (Kavanaugh et al., 1994; Olah et al., 1994). Because of our interest in studies on Na^+/Pi cotransport in rat tissues, we used the rabbit NaPi-1 cDNA as probe and isolated two previously unknown rat analogues of this transporter (RNaPi-1a and RNaPi-1b) by screening a rat liver cDNA library. We characterize these RNaPi-1 isoform transcripts in both liver and kidney and demonstrate that the two transcripts of the RNaPi-1 are differentially expressed in these organs.

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