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EXPRESSION OF ACTIVE ALPHA-3 SUBUNIT OF RAT BRAIN Na^+,K^+-ATPASE FROM THE MESSENGER RNA INJECTED INTO XENOPUS OOCYTES

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Cloned cDNA encoding the so-far uncharacterized alpha-3 subunit of rat brain Na^+,K^+-ATPase (Hara et al. (1987) J.Biochem. 102, 43-58, Shull et al. (1986) Biochemistry 25, 8125-8132) was incorporated into a vector carrying the SP6 promoter. The mRNA produced *in vitro* was injected into Xenopus oocytes with the mRNA encoding the Na^+,K^+-ATPase beta subunit of Torpedo electroplax. Increased Na^+,K^+-ATPase activity in the oocyte membrane was observed. This newly expressed activity was inhibited by ouabain ($Ki = 1.5x10^{-7}M$), suggesting that the alpha-3 subunit of rat brain Na^+,K^+-ATPase is a highly ouabain-sensitive catalytic subunit. © 1989 Academic Press, Inc.

Na^+,K^+-ATPase consists of two kinds of subunits, alpha and beta. The alpha-subunit is known to be catalytic, but the function of the beta subunit remains unclear. Three kinds of alpha-subunits have been found. The alpha-1 subunit occurs in all cell membranes in higher animals and shows lower sensitivity to ouabain. The alpha-2 subunit, which is also known as alpha-plus, exists in the central nervous system, cardiac tissue and adipocytes and shows higher ouabain-sensitivity. The third type of alpha subunit, alpha-3, was recently found by cDNA cloning by our group (1) and others (2,3). From Northern blot analysis, alpha-3 was suggested to exist in the brain, spinal cord and neonatal cardiac tissues (4-5). An alpha-3 peptide was detected immunologically in rat brain axolemmal fraction, but the ATPase activity was not detected conclusively (6). We introduced the alpha-3 cDNA into cultured mammalian cells and succeeded in establishing stably transformed cell lines. From the ouabain-sensitivity of the newly expressed Na^+,K^+-ATPase activity in the transfected cells, the alpha-3 subunit was suggested to be a highly ouabain-sensitive catalytic subunit of rat brain Na^+,K^+-ATPase (7). However, there were some difficulties in the experiment. First, the transfected cells had only twice as much Na^+,K^+-ATPase activity as wild-type cells. Secondly, the newly expressed activity might be due to some change in endogenous Na^+,K^+-ATPase and not to the alpha-3 subunit, because the newly expressed protein had not

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been identified. Third, ouabain-sensitivity of the cells is known to increase with transfection with non-Na^+,K^+-ATPase genes, including *ras*-oncogenes (8). In order to rule out these possibilities, another method for expressing the enzymatic activity from the cDNA is necessary. Recently, expression of active Na^+,K^+-ATPase from mRNAs in Xenopus oocytes was achieved (9). In this work, mRNAs that were produced from the alpha-3 cDNA *in vitro* were injected into oocytes, and the expressed Na^+,K^+-ATPase was analyzed. Compared to the control, the injected oocytes showed twice the activity of Na^+,K^+-ATPase, and the newly expressed activity was found to be highly ouabain-sensitive.

METHODS

The cDNA encoding the alpha-3 subunit of rat brain Na^+,K^+-ATPase (pRNKA131) was described earlier. A DNA fragment AvaI (-71)/BamHI(3492) was excised from pRNKA131 (numbers in parentheses indicate the 5'-terminal nucleotide generated by cleavage). The fragment containing 71 bp of 5'-noncoding, 3039 bp of coding, 378 bp of 3'-noncoding, 46 bp of poly A tail and 29 bp of plasmid vector sequences was inserted into a vector having the SP6 promoter (pGEM4Z Promega Biotec). The plasmid pSPRNKA3 thus obtained, carried the entire coding sequence for the rat brain Na^+,K^+-ATPase alpha-3 subunit. The plasmid pSPNKAB carrying the *Torpedo californica* Na^+,K^+-ATPase beta subunit was constructed as described earlier (9). mRNAs were synthesized *in vitro* using HindIII-cleaved pSPRNKA3 and SalI-cleaved pSPNKAB as templates. The sizes of the mRNAs were determined by gel electrophoresis. These mRNAs were injected into *X. laevis* oocytes as shown in previous paper (9). ATPase activities were measured at 37°C using the NaSCN-treated microsomes of oocytes. Reaction mixtures contained 140 mM NaCl, 14 mM KCl, 5 mM $MgCl_2$, 1 mM ATP, 50 mM imidazole-HCl (pH 7.5) and 20-80 μg of microsomes with various concentrations of ouabain. Protein concentration was determined by the method of Lowry et al. using bovine serum albumin as a standard (10).

RESULTS AND DISCUSSION

The alpha and beta subunit-specific mRNAs were injected into oocytes (0.5 μg/μl each). After incubating for around 60 hours at 19°C, the microsomal fraction was obtained from the cells and treated with NaSCN. Na^+,K^+-ATPase in the microsomes from injected or uninjected cells was measured. When both rat alpha-3 and Torpedo beta mRNAs were injected, the specific activity of Na^+,K^+-ATPase was 2.6 μmoles Pi/mg/h, which was twice that in the case of no injection (1.35 μmoles Pi/mg/h). The injection of Torpedo alpha-1 mRNA with the Torpedo beta mRNA yielded the specific activity of 6.5 μmoles Pi/mg/h. Earlier results (9) showed that expression of the Na^+,K^+-ATPase in Xenopus oocytes requires the injection of both alpha and beta mRNAs. This indicates that the amount of the oocyte beta subunit may not be sufficient and/or the endogenous beta subunit can not associate with the Torpedo alpha-1 subunit. In our case, the cell injected with both rat alpha-3 and Torpedo beta mRNAs showed lower specific activity of Na^+,K^+-ATPase than the cells injected with both the Torpedo alpha-1 and the Torpedo beta mRNAs, suggesting two possibilities. First, the Torpedo beta subunit might not bind well to rat alpha-3 subunit, and second, the efficiency of translation of the alpha-3 mRNA might not be as high as that of the Torpedo alpha-1 mRNA in the oocytes.

The ouabain-sensitivities of the expressed Na^+,K^+-ATPases were examined. The activity of the uninjected cells was subtracted from those of injected cells in Fig. 1. Calculated Ki values for ouabain in the cells with the rat alpha-3 and Torpedo beta mRNAs and the cells with Torpedo alpha-1 and Torpedo beta mRNAs were each around 1.5×10^{-7}M. The Ki value for the uninjected

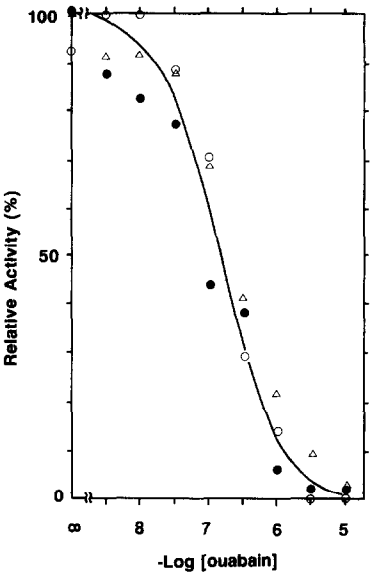

Fig.1 Ouabain inhibition of the Na^+,K^+-ATPases in oocyte microsomes: Torpedo alpha and Torpedo beta mRNA-injected cells (△), Torpedo beta and rat alpha-3 mRNA-injected cells (●), and uninjected cells (○). The curve was drawn based on the Ki value of $1.5x10^{-7}$M.

cells was also $1.5x10^{-7}$M as shown in Fig.1. It is well known that rat alpha-1 has low sensitivity to ouabain, and its Ki is $1x10^{-3}$-$1x10^{-4}$M. Thus, a Ki of $1.5x10^{-7}$M for ouabain represents very high sensitivity. High ouabain-sensitivity of rat brain alpha-3 subunit was suggested on the bases of its amino acid sequence (1). An analysis of the expressed Na^+,K^+-ATPase from the alpha-3 cDNA in 3T3 cells also suggested high sensitivity of the alpha-3 subunit to ouabain (7). Moreover, highly ouabain-sensitive activity was reported to remain after trypsin treatment of rat brain Na^+,K-ATPase, although the alpha-1 and alpha-2 subunits were destroyed by this treatment (11). The results shown here and in the previous paper (7) strongly suggest that the rat alpha-3 subunit is a highly ouabain-sensitive catalytic subunit of Na^+,K^+-ATPase. Northern blot analysis confirmed that the alpha-3 mRNA exists in rat brain and spinal cord. Although there has been no evidence for a relationship between high ouabain-sensitivity and the function of the sodium pump, the strict tissue-specific expression of alpha-3 indicates that the high sensitivity for ouabain might be related to some regulatory function of Na^+,K^+-ATPase in the central nervous system (12).

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