Both sodium channel II and IIA alpha subunits are expressed in rat brain

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Complementary DNAs encoding four distinct isoforms of the large (α) subunit of the voltage sensitive sodium channel have been isolated from rat brain mRNA (1,2,3). Two of these, II and IIA, differ in only 36 nucleotides within the coding region and in only 6 amino acids (1, 3). Because of the possible functional significance of electrophysiological differences between isoforms, it is important to establish definitively that the sequence differences are not due to cloning artifacts and to estimate the relative prevalence levels of the two mRNAs in rat brain.

Using the fact that the two cDNAs are divergent in sequence upstream of nucleotide (nt) position −52, we have estimated the amounts of the two mRNAs by PCR and by RNAase protection asays. For PCR, sense oligo nt primers (C −95 to −76) and A (−108 to −69) are specific for the II and IIA cDNAs respectively (1, 2); antisense primer B (82−101) and probe F (−39 to −20) are common to both. Reverse transcription and PCR were carried out as described (4) on total brain RNA from 14 day old rats (Sprague-Dawley). The amount of RNA used was 1 μg (lanes 1 and 4 in part I of figure), 0.1 μg (lanes 2 and 5), or 0.01 μg (lanes 3 and 6). Specific products were detected by hybridization of gel blots with probe F. Bands of the expected length of 196 nt for II (lanes 1−3) and 209 nt for IIA (lanes 4−6) were observed in relative quantities which indicate that the II and IIA subtypes mRNAs are expressed in the rat brain sample in a ratio of about 10:1. For RNAase protection assays, total brain RNA from adult Wistar rats was hybridized to an excess of antisense transcript probe containing some vector sequences and nts 565 to −135 of the rat IIA sequence. After RNAase digestion as described (5), gel analysis showed bands of the expected lengths 620 (II) and 700 (IIA) (since II mRNA diverges from the IIA sequence at −52) (Part II in Figure). The figure shows that the intensity ratio of the II to IIA bands is *ca.* 10 to 20:1, in agreement with the PCR assay.

Our conclusion is that the two mRNAs occur in rat brain in a ratio of 10 to 20:1 at the ages indicated. Further studies must address the questions of differences in regional distribution of the two gene products, and whether they are derived by alternate splicing from a single chromosomal gene or from transcription of two different genes.

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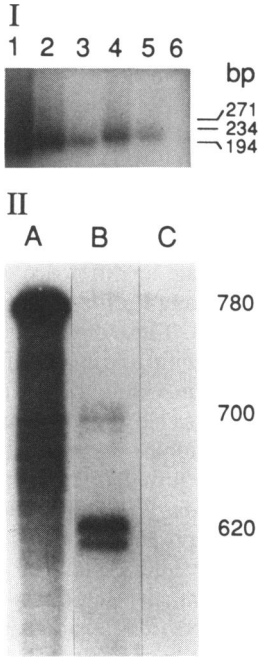

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