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ADDITIONS AND CORRECTIONS

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Expression of multiple forms of cytochrome P450 and associated mono-oxygenase activities in rat brain regions☆

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The abstract should be modified to indicate that the cDNA synthesized from rat brain poly(A)RNA was double stranded, which can easily hybridize with the cDNA from rat liver P450. The revised abstract, therefore, should read:

Further to our earlier results which depicted reverse Southern blot analysis using double-stranded cDNA, synthesized from rat brain mRNA using oligo-dT primers, the constitutive expression of CYP2B and CYP2E in rat brain has now been demonstrated by northern blot analysis of rat brain mRNA.

We had demonstrated the constitutive expression of CYP2B and CYP2E in rat brain using reverse Southern blot analysis (Fig. 2 original paper). In that analysis, the hybridization was based on authentic CYP2B1 and CYP2E1 cDNAs and cDNA synthesized from rat brain mRNA using oligo(T) primers. We have now performed 2 northern blot analysis using mRNA prepared from untreated rat brain cortex and hybridized with digoxigenin-labelled riboprobe (both sense and antisense) transcribed from cDNA to CYP2B1 and CYP2E1.

Total RNA was isolated from liver and cortical region of brain from male Wistar rats (3 months old) as described by Chomczynski and Sacchi [1]. Poly(A)RNA-enriched preparation was isolated from total RNA of rat brain cortex using oligo(dT) cellulose chromatography. Total RNA from liver (3 μg) and poly(A)RNA from rat brain cortex (3 μg) were separated electrophoretically and transferred to positively charged nylon membrane [2]. After UV cross-linking, the blot was hybridized with digoxigenin-labelled sense or antisense riboprobe prepared using cDNA for CYP2B1 or CYP2E1, at 55° overnight. The membrane was washed, then incubated with anti-digoxigenin Fab fragments conjugated with alkaline phosphatase, and the bands were visualized using nitroblue tetrazolium and 5-bromo-4-chloro-3-indolyl phosphate.

The cDNA for CYP2B1 was obtained from Dr. Milton Adesnik (Genbank accession No. J00719) and subcloned into pBluescript II SK(+). The cDNA for CYP2B1 along with the T3 and T7 promoters was excised from the vector by restriction enzyme digestion with *Bss*HII followed by gel purification. This insert was used for preparing the sense and antisense transcripts using T3 or T7 RNA polymerase. The cDNA for CYP2E1 was obtained from Dr. B. J. Song [3], and riboprobes (sense and antisense) were synthesized after linearizing the plasmid using restriction enzymes.

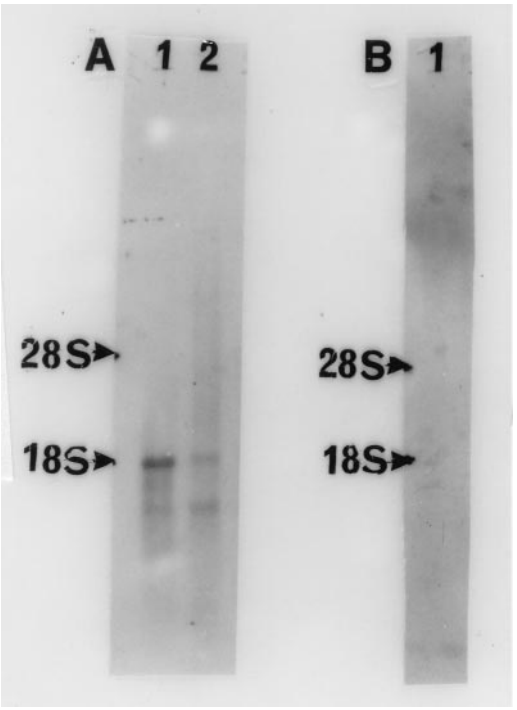

FIG. 1. Northern blot analyses of rat liver and brain RNA using the (A) antisense and (B) sense riboprobes transcribed from cDNA for CYP2B1. (A) Rat liver total RNA (3 μg, lane 1) and poly(A)RNA from rat brain cortex (3 μg, lane 2) were subjected to electrophoresis, transferred to nylon membrane, and hybridized with digoxigenin-labelled antisense riboprobe to CYP2B1. The location of the 18S and 28S RNA is indicated. (B) Rat liver total RNA (3 μg, lane 1) was subjected to electrophoresis, transferred to nylon membrane, and hybridized with digoxigenin-labelled sense riboprobe to CYP2B1. The location of the 18S and 28S RNA is indicated.

The constitutive expression of CYP2B in rat brain cortex was seen on northern blot analysis of the poly(A)RNA (Fig. 1A). The molecular mass of the transcript was 1.6 kb, which was similar to that seen in the liver (lane 1; Fig. 2A). In the blots hybridized with the sense digoxigenin-labelled transcript to CYP2B1, no bands were observed (Fig. 1B). Constitutive expression of CYP2E in rat brain cortex was clearly discernible on northern blot analysis of rat brain poly(A)RNA (Fig. 2A). The molecular mass of the transcript was 1.6 kb, which was also similar to that seen with liver RNA. No bands were visible in the blots hybridized with the sense transcript (Fig. 2B).

☆ PII of original article S0006-2952(98)00036-7.

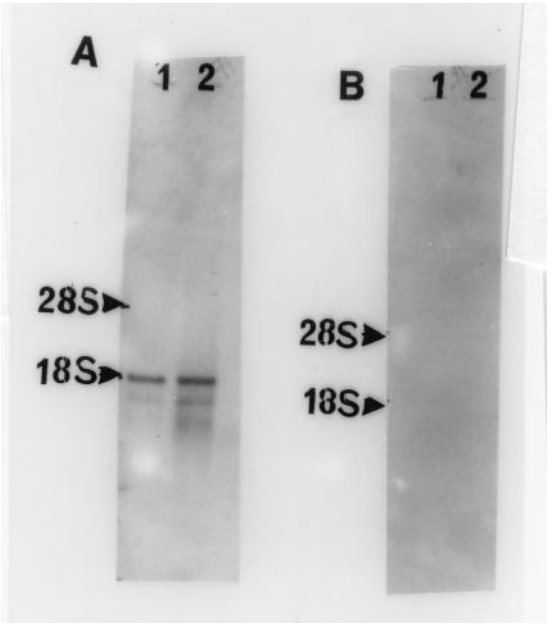

FIG. 2. Northern blot analyses of rat liver and brain RNA using the (A) antisense and (B) sense riboprobes transcribed from cDNA for CYP2E1. (A) Rat liver total RNA (3 μg, lane 1) and poly(A)RNA from rat brain cortex (3 μg, lane 2) were subjected to electrophoresis, transferred to nylon membrane, and hybridized with digoxigenin-labelled antisense riboprobe to CYP2E1. The location of the 18S and 28S RNA is indicated. (B) Rat liver total RNA (3 μg, lane 1) and poly(A)RNA from rat brain cortex (3 μg, lane 2) were subjected to electrophoresis, transferred to nylon membranes, and hybridized with digoxigenin-labelled sense riboprobe to CYP2E1. The location of the 18S and 28S RNA is indicated.

Northern blot analyses using poly(A)RNA from rat brain cortex show the constitutive expression of CYP2B and CYP2E in rat brain, further confirming earlier results by the reverse Southern blot analysis published in the original paper. Northern blot analyses depicted here conclusively demonstrate the constitutive expression of CYP2B and CYP2E in rat brain.

References

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