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Identification of a Novel Constitutively Active Receptor (CAR) and its cDNA Sequence in an Aquatic Mammal, Baikal Seal (*Phoca sibirica*)

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The cytochrome P450 (GYP) 2B is a member of GYP superfamily involved in the metabolic activation/detoxification of xenochemicals, and in the biotransformation of endogenous signaling molecules such as steroid hormones. CYP2B genes are induced by phenobarbital, *ortho*-chlorine substituted PCB congeners and a large number of structurally diverse xenochemicals, primarily due to the enhanced transcription. Recent studies have shown that gene transcription of CYP2B in rodent can be activated or repressed by a novel nuclear receptor CAR (constitutively active receptor or constitutive androstane receptor) in response to both endogenous and exogenous compounds. While the molecular details of CYP2B regulation in mouse and rat have been largely known, the occurrence of CYP2B genes and the regulatory mechanisms involved in the GYP induction in other mammalian species are not well understood. Aquatic mammals such as seal and dolphin are at the top position of the food chain in the hydrospheric ecosystem and are facing the risk of accumulating hydrophobic endocrine disrupters.

Our previous study revealed that antibody to rat CYP2B1 protein immunochemically recognized a CYP2B-like protein in free-ranging largha and ribbon seals. Interestingly, the expression level of CYP2B-like protein in the seal species was not correlated with the blubber residue level of *ortho*-chlorine substituted PCB congeners, while there was a significant correlation between the levels of seal CYP1A protein and non-/mono-*ortho* chlorine substituted PCBs. Based on these results, the lack of response to PB-type inducers in seal species indicates the presence of differential regulatory mechanisms for seal CYP2B-like genes from those of rodent CYP2B, not the lack of structural gene(s) coding for CYP2B homologues.

The present study therefore attempted to provide more information on the novel nuclear receptor CAR in seal, which might also be a transcriptional factor of putative seal CYP2B. To understand the function of seal CAR, we initially cloned the cDNA encoding CAR of the Baikal seal (*Phoca sibirica*), which accumulates high levels of *ortho*-chlorine substituted PCBs, and is thought to be one of sensitive species to chemical pollution as suggested by mass mortality in 1987-88.

The Baikal seal CAR cDNA had an open reading frame of 1047 bp that encodes a protein of 349 amino acids. Comparison of CAR amino acid sequences showed sequence identities with human CAR (83%), rat CAR (76%), mouse CAR1 (73%) and mouse CAR2 (58%), revealing a high conservation in the DNA binding domain and a low homology in the ligand binding/dimerization domain. These results indicate the occurrence of CAR homologue at least in the seal species, and that there may be a species-specific response of CAR potentially activated by a variety of endocrine disrupters.