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Evidence that Constitutive Androstane Receptor Interacts with Glucocorticoid Receptor Interacting Protein-1 in Domain- and Ligand-Dependent Manner

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Phenobarbital (PB) induction of CYP2B genes is mediated by translocation of the constitutively active androstane receptor (CAR) to the nucleus. In this study, the interaction of CAR with the p160 coactivator glucocorticoid receptor-interacting protein 1 (GRIP1) was examined. Binding of GRIP1 to CAR was shown by glutathione S-transferase (GST) pull-down and affinity DNA binding. Full-length GRIP1 was bound to GST-CAR, whereas little or no binding of GRIP1 to GST was observed. The CAR antagonist androstenol had little effect on the binding, whereas the agonist TCPOBOP increased the binding. N- or C-terminal fragments of GRIP1 that contained the central receptor-interacting domain bound to GST-CAR, but the presence of ligand increased the binding to GST-CAR of only the fragments containing the C-terminal region. To determine whether GRIP1 could bind to a CAR-RXR-DNA complex, CAR-RXR bound to biotinylated DNA containing four copies of the CYP2B1 NR1 was incubated with ^{35}S-GRIP1. Specific binding of ^{35}S-GRIP1 was observed as an increase in binding when both CAR and RXR were present. Binding was further increased if the agonist TCPOBOP was added to the reaction, and the antagonist androstenol modestly reduced the binding. In gel shift analysis, binding to CAR was observed only with GRIP1 fragments containing the C-terminal region, and the binding was increased by a CAR agonist and decreased by a CAR antagonist. These results suggest that the C-terminal region is required for stable binding of GRIP1 to CAR-RXR-DNA complexes but not for stable binding to CAR alone. Furthermore, the influence of the C-terminal region of GRIP1 on binding is modulated by ligand binding.