Carboxylesterases (CESs) play an important role in hydrolytic metabolism of many ester-type prodrugs. CESs are classified into four families, in which CES1 and CES2 families are mainly involved in the metabolism of prodrugs in humans. In contrast, little is known about the individual role of different families for drug metabolism in experimental animals. Previously we reported that rat CESs are induced by several glucocorticoid hormones. In the present study, we cloned dexamethasone (DEX)-inducible rat CES isozyme and characterized it using baculovirus-mediated expression system. When the rats were treated with DEX, methylprednisolone 21-hemisuccinate (MP-21HS) hydrolase activity was 25-fold increased in liver microsomes. Immuno-blot analysis with specific antibodies against CES2 isozymes indicated that one of CES2 isozymes is induced by the treatment of rats with DEX. This CES isozyme was tentatively designated as CES RL4. Then we cloned the cDNA encoding the rat CES isozymes from rat liver cDNAs. The deduced amino acid sequence of this clone was found to be very similar to those of human CES2 isozymes. Together with immunochemical studies, these findings suggest that CES RL4 belongs to CES2 family. In addition, we expressed this clone in Sf9 cells and showed that recombinant CES RL4 exhibits considerable hydrolytic activity towards irinotecan-HCl and MP-21HS. These results also suggest that rat CES2 isozyme significantly contributes to the MP-21HS hydrolase activity induced by glucocorticoid hormones.