Experimental studies of steroids and azoles binding to cytochrome P450 7B1

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Cytochrome P450 (CYP) 7B1 is involved in many metabolic processes including androgen metabolism, synthesis of bile acids from cholesterol in liver, modification of dehydroepiandrosterone neurosteroids in the brain. The enzyme has been implicated to play a role in diseases such as prostate cancer, atherosclerosis and spastic paraplegia.
To establish structural characteristics of the enzyme active site, the binding of azoles and steroids to recombinant CYP7B1 was examined. Among investigated 17 azoles only miconazole, 4-phenylimidazole, bifonazole, clotrimazole, ketoconazole, cyproconazole, tebuconazole and econazole effectively bind with CYP7B1. The Kd of values estimated from spectral titration are in the range of 10-8 to 10-6 M for different azoles.
Screening of a large group of steroids by spectral titration, including cholesterol, 22(R)-hydroxycholesterol, 22(S)-hydroxycholesterol, 25-hydroxy- or 27-hydroxycholesterol, androstenedione, 5α-cholestan-3β,17β-diol, DHEA, pregnenolone, progesterone, 16α-hydroxyprogesterone, 16α-hydroxypregnenolone, 17α-hydroxypregnenolone, 21-hydroxypregnenolone indicated that only 5α-cholestan-3β,17β-diol, DHEA, pregnenolone, 25-hydroxy- and 27-hydroxycholesterol interact with CYP7B1 inducing a type I spectral shift, and so being potential substrates for this hemeprotein. We tested hydroxylation of these steroids in reconstituted hydroxylation system, containing CYP7B1, CPR and NADF(H). Analysis of the reaction products with LC-MS with APCI source and ion-trap type mass analyzer confirms hydroxylation of 25- and 27-hydroxycholesterol based on formation of two intensive peaks of product ions in mass spectrum at 401, 383 m/z (substrates peaks 403, 485 m/z). The activity of CYP7B1 to these substrates was about 10-15 μmol per μmol of enzyme per min. Much less activity CYP7B1 showed with 5α-cholestan-3β,17β-diol, pregnenolone and DHEA. Thus, the difference in activity of CYP7B1 with respect to various steroids match with its multiple functions in different tissues and may be connected with the physiological activity of hydroxylation products.