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STUDY CYTOCHROME P450IAI GENE EXPRESSION BY RTPCR.

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To investigate the mechanism of the regulation of cytochrome P450IA1 gene expression, ethoxyresorufin deethylase(EROD) and benzo(a)pyrene hydroxylase in B6 mouse liver, in isolated perfused rat liver system, and in B6 mouse hepatocyte Hepa-I cells were examined. In C57BL/6N mouse, 3-methylcholan- threne(3MC) treatment have resulted in the stimulation of EROD activity based on fluorometry by 2.79 fold comparirng with that of control. Measurement of mRNA of cytochrome P450 was carried out by either nothern blot or dot blot analysis. Findings are similar to that of studies with enzymes. Furhtermore, when RTPCR method was applied to detect mRNA in Hepa I cell and liver tissues the results were more clear. Cytochrome P450IA1 upstream DNA containing CAT construct was transfected into Hepa-1 cells. After transfection of CAT construct, 3MC and flavonoids, such as, chrysin, hesperetin, kaempferol, morin, myricetin and aminoyrine were treated. 48 Hours after treatments, cells were harvested and assayed for CAT mRNA by RTPCR. 3MC treatment to hepa I cells transfected with trout P450IA1-CAT construct increased CAT mRNA by 2.81 fold when it was compared with that of control. This increase CAT mRNA was decreased by concomitantly treated flavonoids and aminopyrine. The level of CAT protein was 29.2-58.0% of 3MC stimulated CAT protein. Results of this study suggested that RTPCR seems to be a very good method to study regulation of gene expression in liver tissue or Hepa cells.

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