ceptor for 1α, $25(OH)_2D_3$ is known to be present in osteoblast and absent in osteoclast cell lines, no systematic study has been carried out on the callus tissue which is formed during fracture-healing. Therefore I investigated a 1α, $25(OH)_2D_3$ receptor/binding protein for all cullus fractions: nuclear, postnuclear membrane, and high speed cytosol fraction of the callus tissue of a tibial fracture. The binding of 1α, $25(OH)_2D_3$ observed in the nuclear fraction was not saturable. Saturable binding was observed in the callus membrane and the cytosol fractions where the K_D/B_{max} values were 0.83 ± 0.35 nM/35.8 ± 5.28 fmol/mg protein and 0.66 ± 0.38 nM/9.8 ± 1.4 fmol/mg protein, respectively. These receptor-ligand kinetics values were clearly different from those of the membrane receptor for 24R, $25(OH)_2D_3$.

Thus I confirmed the presence of a membrane binding protein for 24R, $25(OH)_2D_3$, which is distinct from the 1α, $25(OH)_2D_3$ receptor and also from DBP. This implies that 24R, $25(OH)_2D_3$ may generate biological responses via a signal transduction pathway(s) separate and distinct from that of 1α, $25(OH)_2D_3$. Collectively, my results suggest that 24R, $25(OH)_2D_3$ is a functionally important vitamin D_3 metabolite in bone biology and may function to generate biological responses through interaction with the membrane receptor indicated in the present study.

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Environmental Monitoring Using Wildlife as a Biomarker: Inhabiting Environment Differentially Changes P450 Isozyme Specific Activities in Wild Rodents

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Summary

In order to estimate the suitability of using accumulation of pollutants in wildlife as an indicator of environmental pollution, I investigated the residue levels of organochlorine compounds (OCs) and their accumulation patterns in 8 species of terrestrial mammals and 10 species of birds. The accumulation of OCs to environment has been of great concern, because of their persistent and less degraded properties.

OCs accumulated in terrestrial mammals and birds were mostly in the order of polychlorinated biphenyls (PCBs)>dichlorodiphenyltrichloroethane compounds (DDTs)>hexachlorocyclohexane isomers (HCHs)>hexachlorobenzene (HCB). The accumulation levels of OCs in terrestrial mammals were lower than those in birds. The contamination levels of OCs were found to be higher in omnivorous mammals than in herbivorous ones, and in fish-eating ones and

raptors than in omnivorous birds. In fox and dog, PCB-180 (2, 2', 3, 4, 4', 5, 5'-heptachlorobiphenyl) was the most dominant PCB congener, while in the other species PCB-153 (2, 2', 4, 4', 5, 5'-hexachlorobiphenyl) was the most persistent. This phenomenon may be caused by the species differences in metabolic preferences of cytochrome P450 (CYP) toward PCB congeners.

The residual levels of environmental pollutants depend on the balance between the levels of uptake and excretion of these chemicals. CYP multiple enzyme system plays a major role in oxidative metabolism of a broad variety of endogenous and exogenous compounds, including sterols, fatty acids, drugs and xenobiotics. The differences of the characteristics of CYP isozymes or their relative abundance lead to the differences in accumulation of environmental pollutants. Some CYP isozymes are known to be induced by xenobiotics, including environmental pollutants. I thought this property of CYP isozymes could be used as an indicator of environmental pollution. I collected *Clethrionomys rufocanus* (Cr) from three different sampling sites, a forest, urban and agricultural area, and investigated CYP -dependent enzyme activities in hepatic microsomes of Cr.

All of these CYP dependent enzyme activities in liver microsomes from Cr in the forest area were the lowest as compared with those from Cr in other sampling sites and were comparable to those from laboratory grown control animals.

Cr in the urban area had moderately induced levels of a CYP isozyme (the isozyme corresponding to rat's CYP1A) and related activities which are also induced by polycyclic aromatic hydrocarbons and dioxins. The animals in the agricultural area had high levels of CYP isozymes (the isozyme corresponding to rat's CYP1A and CYP2B) and related activities which are induced by agricultural chemicals. And Cr in agricultural area also had high levels of CYP2C11, CYP2E1, and CYP2D.

These results indicated that CYP-dependent enzyme activities in hepatic microsomes from Cr are rely upon their inhabiting environment (urban, agricultural and forest area). Cr in Sapporo showed the polymorphism in CYP2D dependent activities, in imipramine 2-hydroxylation (IH) and bunitrolol 4-hydroxylation (BH). Cr collected from the different origin were bred in the laboratory and raised under the same condition to elucidate if there are differences in genetic-makeups. The CYP dependent enzyme activities showed almost the same levels regardless of their parents' origin. Nowadays, there are huge numbers of chemicals in environment, however, most of them are unidentified and the effect of those chemicals on living organisms are not known. In order to estimate such chemicals' effects on the ecosystem of the environment, *Clethrionomys rufocanus* may be used as good indicator animals, because their CYP-dependent enzyme activities are differently changed with inhabiting environment. In this study, I was able to show that Cr can be used as a good indicator animal to assess the level of environmental pollution, and CYP dependent enzyme activities can be used as biomarkers of environmental pollution.

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