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Sensitive Two-Dimensional Determination of D-Amino Acids in Mammals and the Study on Their Functions

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D-Amino acids are the enantiomers of widely observed L-amino acids and it is increasingly recognized that the D-isomers are also present in mammals having inherent specific functions. In the present investigation, a sensitive and selective two-dimensional HPLC system combining micro-reversed phase and chiral columns has been established. This HPLC procedure enables the sensitive determination of trace levels of D-amino acids in mammalian tissues as their NBD-derivatives. Using this procedure, the presence of various D-amino acids of small amount such as D-Ala, D-Leu and D-Pro has been demonstrated in mammals. These D-amino acids have particular tissue distributions; D-Leu and D-Pro are localized in the pineal and pituitary glands in the brain. Distribution of D-Ala in mammals has been investigated in the brain and peripheral tissues, and found that D-Ala is localized in the anterior lobe of pituitary gland and pancreas. To obtain the further information on its function, a specific monoclonal antibody against D-Ala has been raised, and the cellular localization has been investigated by the immunohistochemical staining. The double staining results with various hormones in the tissues demonstrated that D-Ala is localized to the ACTH secreting cells in the anterior pituitary gland, and to the insulin secreting cells in the pancreatic Langerhans islet. These results strongly indicate that these D-amino acids are the candidates of novel physiologically active substances and the marker molecules of diseases in mammals.