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Distribution of naturally occurring D-aspartic acid and D-glutamic acid in the rat brain and periphery

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[Introduction] It was long believed that only L-amino acids were present in higher animals. However, the D-forms were observed in mammals in recent years and thought to be the candidates of novel physiologically active substances and/or biomarkers of diseases. Among them, D-aspartic acid (D-Asp) and D-glutamic acid (D-Glu) were reported to have the activities in hormonal secretion and neuronal transmission. In the present study, the distributions of naturally occurring D-Asp and D-Glu in the rat brain and periphery were investigated.

[Methods] Brain and peripheral tissues of Wistar rats were homogenized in MeOH, and the amino acids were derivatized with 4-fluoro-7-nitro-2,1,3-benzoxadiazole (NBD-F). The NBD-Asp and NBD-Glu were fractionated by a microbore-monolithic-ODS column and their enantiomers were separated using a narrowbore-Sumichiral OA-2500S column.

[Results and Discussion] By using the micro-2D-HPLC system, simultaneous determination of Asp and Glu enantiomers were completed within 150 min without interference from the intrinsic substances in the tissues and physiological fluids. The lower limits of detection of D-Asp and D-Glu were 2 fmol and 1 fmol, respectively, and the RSD values of within-day and day-to-day precision were less than 6.8%. The amounts of D-Asp and D-Glu in 8 brain tissues, 10 peripheral tissues, plasma and urine were determined.

D-Asp is mainly distributed in the endocrine tissues (>100 nmol/g), and the highest amount was observed in the pineal gland (2030 nmol/g). On the other hand, the amounts of D-Glu were extremely small in all the tissues tested and only trace amounts (0.5-9.0 nmol/g) were observed. Further works using various animal samples and clinical samples to elucidate the origins and physiological meanings of D-Asp and D-Glu are currently in progress.