Species Differences in Tissue Distribution and Enzyme Activities of Arylacetamide Deacetylase in Human, Rat, and Mouse

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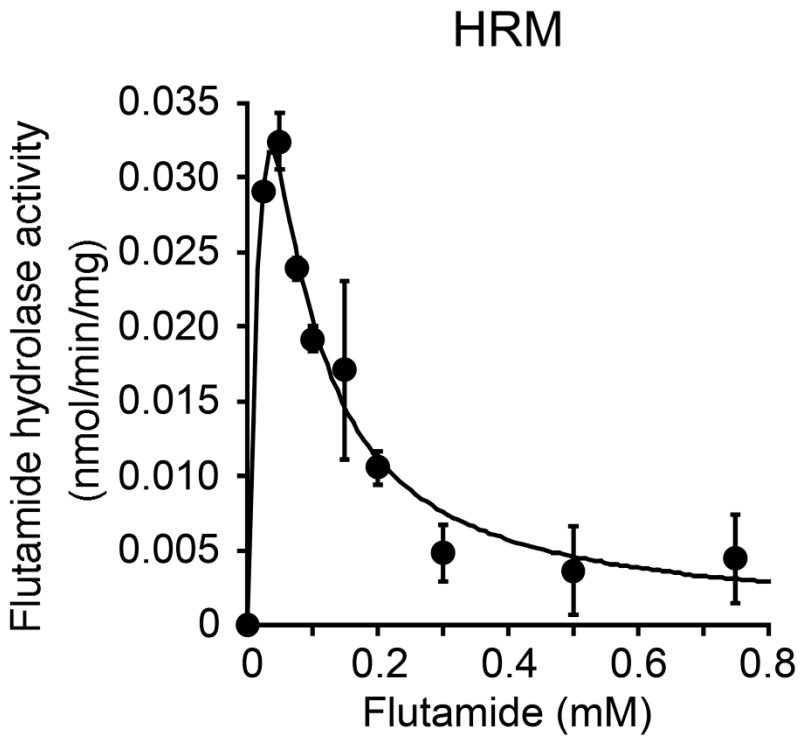
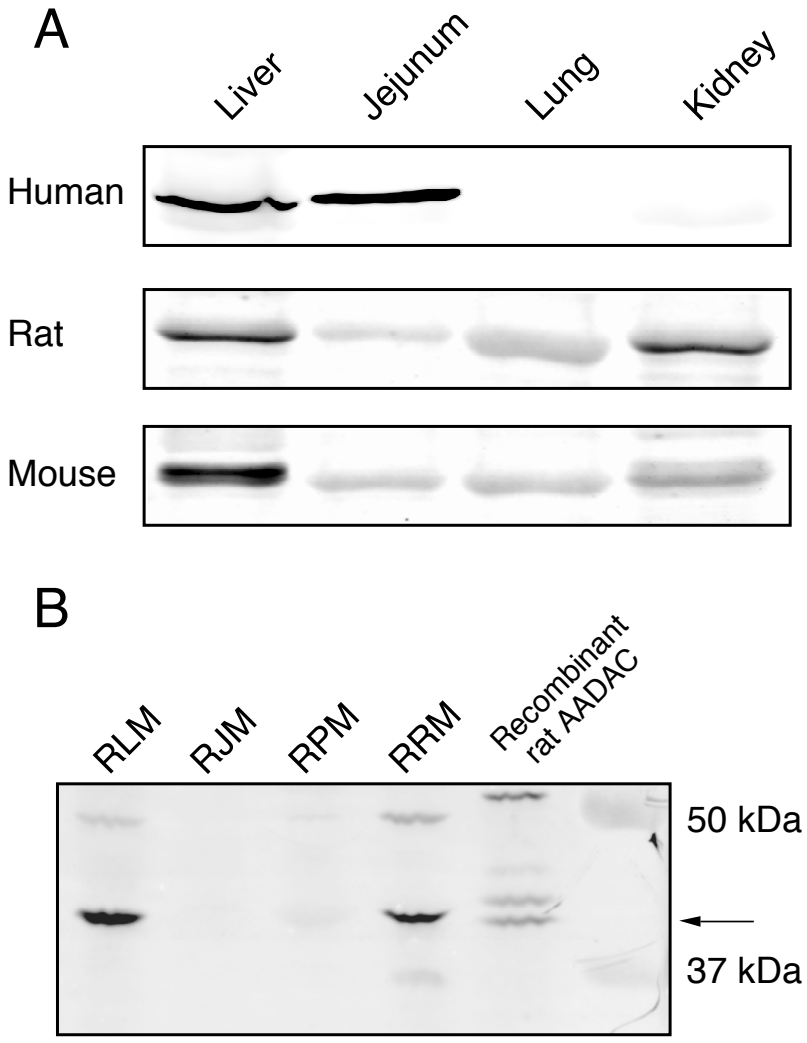


Supplemental Fig. 1. Kinetic analysis of flutamide hydrolase activities in HRM. HRM (0.4 mg/ml) was incubated with 0.025–0.75 mM flutamide. Each data point represents the mean ± SD of triplicate determinations.



Supplemental Fig. 2. (A) Immunoblot analysis after SDS-PAGE of AADAC protein in liver, jejunum, pulmonary, and renal microsomes of human, SD rat, and C57BL/6 mouse. Enzyme sources (30 μg) were separated by electrophoresis using 10% SDS-polyacrylamide gel and the membrane was probed with anti-human AADAC antibody. (B) Immunoblot analysis after native PAGE of recombinant rat AADAC and AADAC protein in liver, jejunum, pulmonary, and renal microsomes of SD rat. Enzyme sources (rat tissue microsomes: 30 μg; recombinant AADAC: 20 μg) were solubilized with PBS containing 1% Triton X-100 and 0.5% SDS on ice for 60 min and were centrifuged at 13,000g for 30 min. Solubilized proteins were separated by electrophoresis using Perfect NT Gel M (5 – 15% gradient) (DRC, Tokyo, Japan) at 10 mA for 5 hr at 4 ˚C. The separated proteins were transferred to a PVDF membrane and probed with anti-human AADAC antibody.