SUPPLEMENTARY DATA

XOR activity and uric acid detection

Tissue samples were homogenized in ice cold potassium phosphate buffer (50 mM) containing 100 µM EDTA, and protease inhibitor cocktail (Sigma). The homogenate was centrifuged at 21,000 x g, 4°C, for 30 min. The supernatant was subjected to size exclusion chromatography to remove endogenous purines and low molecular weight inhibitors. Uricase was inhibited by oxonic acid (100 µM) to avoid an underestimation of enzyme activity. Total XOR activity was determined on the basis of the rate of uric acid production in the presence of xanthine (75 µM) and nicotinamide adenine dinucleotide (NAD^+, 0.5 mM). After 60 min of incubation at 37°C, the reaction was terminated by protein precipitation with cold acetonitrile. The uric acid content of protein-free samples was determined by a HPLC-based electrochemical technique as described below. Allopurinol (100 µM), an inhibitor of XOR, was used in parallel samples to confirm that urate formation was specific. One unit of activity (U) is defined as 1 µmole/min urate formed at 37°C and pH 7.4. Total protein concentration was determined prior to and following gel filtration with a modification of the bicinchoninic acid method (BCA, Pierce). Measurements were carried out blindly without knowledge by the operator of the animal treatment groups. Uric acid was measured by electrochemical detection (ESA Coul-Array System) coupled to reverse-phase HPLC using a Phenomenex column (Luna 3µm C18(2) 100 A, 150x4.6 mm) and isocratic mobile phase (50 mM sodium dihydrogen phosphate, 4 mM dodecyltrimethylammonium chloride, 2.5% methanol, pH 7.0). Potentials for 8 channels were as follows: 0, 0, 390, 350, 300, 180, 90, -100 mV. Quantitation/integration was performed on the dominant peak. In the case of XOR activity in plasma, 10 µL of plasma was used in lieu of tissue and processed with the same protocol as above without gel filtration. XOR activity in plasma utilized volume (U/mL) as a denominator rather than protein.