P.673

THE 14-DAY CARBAMAZEPINE TREATMENT INCREASES EXPRESSION OF KAT1 (CCBL1) AND KAT2 (AADAT) GENES IN RAT BRAIN CORTEX

T. Kocki, Ł. Pyzia, I. Zakrocka, K. Komsta-Wicha, Pabiś Sz., W. A. Turski

Chair and Department of Experimental and Clinical Pharmacology, Medical University of Lublin, Poland

Introduction: Kynurenic acid (KYNA), a tryptophan metabolite formed along kynurenine pathway, was shown to display potent neuroprotective and anticonvulsive properties and was implicated in the pathogenesis of epilepsy and neurodegenerative disorders including Huntington's, Alzheimer's, and Parkinson's diseases and psychiatric disturbances such as depression and schizophrenia. KYNA is the only known until now endogenous broad-spectrum antagonist of excitatory amino acid receptors, displaying the highest affinity towards the glycine site of N-methyl-D-aspartate (NMDA) receptor. KYNA is synthesized mainly within astrocytes by kynurenine aminotransferases (kat1 and kat2) from its bioprecursor, L-kynurenine.

Objectives: Our previous studies indicated that 14-day CBZ (40 mg/kg; ip) treatment of rats increases KYNA concentration in brain cortex. In the present study the influence of long-term administration of antiepileptic drug carbamazepine (CBZ) on gene expression level of kat1 (Ccbl1) and kat2 (Aadat) in rat brain cortex was investigated.

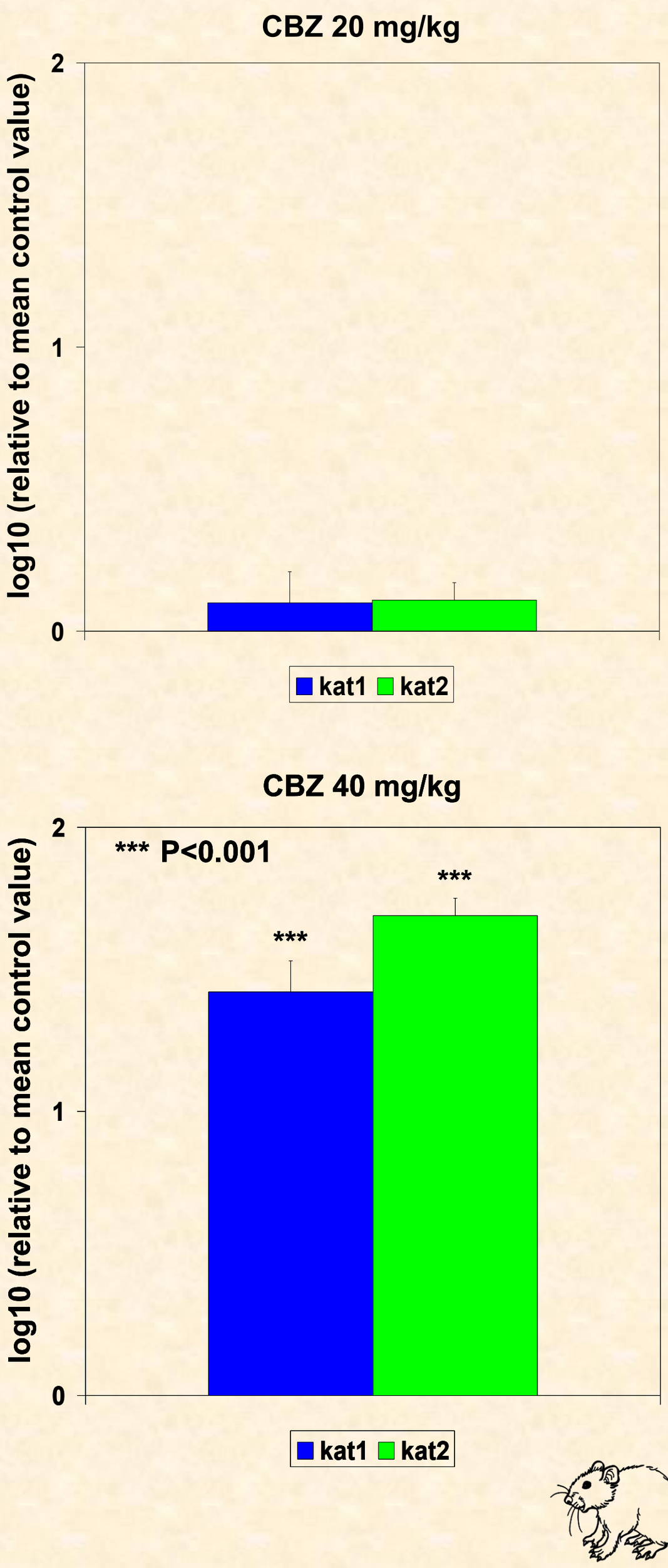
Materials and methods: The experiments were carried out on male Wistar rats (180-220g) kept under standard laboratory conditions with free access to food and water.

Experimental procedures have been approved by the I Local Ethical Committee and are in agreement with European Communities Council Directive on the use of animals in experimental studies.

Three groups of adult male Wistar rats were injected intraperitoneally (ip) once a day for 14 days with either saline, or CBZ (20 mg/kg and 40 mg/kg). Animals were decapitated 24 hrs after the last injection and the brain was removed and placed in RNA*later* Stabilization Solution (Thermo Fisher Scientific, MA, USA). Samples were stored at -80 C for further analysis.

RNA extraction, was performed using fully automated sample preparation spin-column kit (QIAcube, Qiagen, Germany). Quantity and quality of the extracted RNA and the integrity of RNA samples were determined by gel-based electrophoresis (The Experion automated electrophoresis station, Bio-Rad, CA, USA). RNA was stored at -80 C for further analysis. Synthesis of single-stranded cDNA from total RNA was performed using High-Capacity cDNA Reverse Transcription Kits based on Applied Biosystem protocols.

Probe sets of TaqMan Gene Expression Assays and buffers (ThermoFisher Scientific, MA, USA) with FAM-NFQ markers and oligonucleotide primers for rat genes: Ccbl1 gene (cytoplasmic cysteine conjugate-beta lyase) kat1; Aadat gene (aminoadipate aminotransferase) Kat2. Two reference genes were tested: *Gapdh (*glyceraldehyde-3-phosphate dehydrogenase) *and Actb (*β-actin). Amplification and quantification of *kat1*, *kat2* genes carried out using QuantStudio 12K Flex Real-Time PCR System (ThermoFisher Scientific, MA, USA). Data were analyzed using the program ExpressionSuite Software (ThermoFisher Scientific, MA, USA).



Conclusion: Our data revealed that chronic CBZ increases the brain expression of kat1 and kat2 and thus suggests the novel mechanism of action of CBZ.

Disclosure statement: *Supported by the grant from Medical University of Lublin, No DS 457*