EFFECT OF PROBENECID IN THE LEVELS OF KYNURENIC ACID AND CATECHOLAMINES IN DIFFERENT BRAIN REGIONS

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Introduction
Kynurenic acid (KYNA) is an antagonist for the α7-nicotinic acetylcholine receptor (nAChR) and the glycine co-agonist site of the N-methyl-D-aspartate receptor at nanomolar concentrations. KYNA is synthesized in the brain from kynurenine (KYN) through irreversible transamination by kynurenine aminotransferases (KAT). In turn, KYNA can be synthesized within the brain from L-tryptophan, or transported across the brain-blood barrier (BBB) by the large neutral amino acid uptake system. It has been estimated that 40% of KYNA is synthesized in the brain, while the remaining comes from the periphery. Its activity on excitatory amino acid receptors and nAChR might account for its neuromodulatory and neuroprotective effects in the CNS. Thus, fluctuations in brain KYNA levels under physiological and pathological conditions could modulate the activity of cholinergic and glutamatergic transmission. Furthermore, KYNA, at nanomolar concentrations, can decrease the extracellular level of dopamine in the striatum of rats. Neuroprotective effects of KYNA have been addressed through pharmacologic manipulations leading to increased brain concentrations of KYNA, which have shown anti-epileptic effects. Probenecid (PROB), a drug widely used for the treatment of the gout, inhibitor of the organic acids transporter (OAT), and found in the BBB, has been previously shown to increase brain concentrations of KYNA. PROB has shown to prevent OAT-mediated transport of KYNA towards the cerebrospinal fluid, the blood torrent and finally, out of the circulatory system as urine in experiments in which its precursor KYN was co-administered. However, there is no direct evidence showing if the increase in KYNA concentrations by PROB action, without its precursor, is dose-dependent, and if this accumulation also differs through the time and brain region.

Materials and methods
Animals
Male Wistar bred-in-house rats (270 - 300 g) were used throughout the study. Animals (6 per group) were randomly assigned to six experimental groups as follows:

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<td>CONTROL</td>
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<td>PROB</td>
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After the time provided, striatal, hippocampal, and the cortex tissues were collected by decapitation and immediately dissected out on ice and preserved for limited times at ~70 °C. During dissection, all efforts were made to minimize animal suffering.

Measurement of KYNA levels
KYNA content was measured in fresh tissues homogenates obtained from animals of experimental groups, and determined according to established high performance liquid chromatography (HPLC) procedures, with a mobile phase containing 200 mM of zinc acetate and 5% acetic acid (pH 6.2) was used for KYNA content and detected fluorimetrically (excitation/emission wavelength 344/380 nm).

Measurement of DA levels
DA levels were measured in freshly obtained tissues from control and experimental groups, according to previous reports. The brain DA tissue content was analyzed by HPLC with electrochemical detection, as previously described. All samples were analyzed in duplicate; the concentrations of DA and its metabolites were first calculated as μg per gram of wet tissue.

KYNA Immunohistochemical Assay
Polyclonal antibody against KYNA (Sigma-Aldrich, Inc. St. Louis, MO, USA) was used to examine the cellular location in rat brain sections. The expression of KYNA was examined using an immunohistochemical approach. This assay was performed after 1, 2, 3 hours after PROB (30 mg/kg, i.p.) Formalin-fixed, paraffin-embedded archived tissues were sectioned (thickness of 4 μm). The sections were then dewaxed, rehydrated and goat serum albumin-blocked. Following blocking, the sections were incubated with the antibody for 2 h at room temperature (1:100 dilution). The slides were incubated with a secondary FITC-labeled antibody (1:1000 dilution). Finally, the slides were VectaShield-mounted. Images were obtained in an inverted microscope (Leica Microsystems, Heidelberg, Germany) using an ARKX laser at 488 and 520 nm to define the intracellular location of KYNA.

Conclusions
Our data show a time-dependent increase of KYNA levels in cortex and striatum after administration of PROB, effect not observed in hippocampus, this increase is inversely related to DA levels in time. Clearly, PROB in C57 has effect on the DAH resulting in an accumulation of KYNA, and may be a valid therapeutic tool for regulating the glutamatergic and cholinergic transmission.