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Prolyl oligopeptidase inhibition decreases extracellular acetylcholine levels in rat hippocampus and prefrontal cortex

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HIGHLIGHTS

- We examined the effects of PREP inhibition on rat hippocampal and cortical ACh levels.
- Brain PREP was inhibited with KYP-2047 and JTP-4819 (15–50 μmol/kg i.p.).
- PREP inhibition decreases the extracellular levels of ACh in hippocampus and cortex.
- Cognition enhancement by PREP inhibitors is not likely to be mediated through cholinergic system.

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ABSTRACT

Several investigative prolyl oligopeptidase (PREP) inhibitors have been shown to improve learning and memory in various preclinical trials but the mechanism of action behind these effects remains unclear. Since hippocampal and cortical acetylcholine (ACh) is known to play an important role in cognitive processes, the effects of two potent PREP inhibitors, JTP-4819 and KYP-2047, on extracellular ACh levels in hippocampus and medial prefrontal cortex were assessed using in vivo microdialysis. Conscious rats were treated with a single dose (15 or 50 μmol/kg i.p.) of JTP-4819, KYP-2047 or vehicle, and extracellular ACh levels were monitored for 5 h after treatment. In hippocampus, KYP-2047 had no significant effect on the ACh levels, although a trend towards decreased levels was observed at the higher dose. JTP-4819 had no significant effect on the hippocampal ACh levels at the lower dose (15 μmol/kg), but the higher dose (50 μmol/kg) significantly decreased ACh levels in hippocampus by about 25%. In cortex, the smaller dose (15 μmol/kg) of KYP-2047 decreased ACh levels maximally by 25%, and a similar (ns) effect was also observed after the higher dose. JTP-4819 had no effect at the lower dose, but the higher dose decreased ACh levels maximally by about 30%. In conclusion, the present results suggest that the cognition-enhancing effects of investigative PREP inhibitors are not due to enhanced cholinergic transmission in hippocampus or cortex.

1. Introduction

Prolyl oligopeptidase (PREP; EC 3.4.21.26) is a cytosolic serine protease that hydrolyzes short proline containing peptides in vitro [4]. It is an abundant enzyme and widely distributed throughout the mammalian body. Despite intensive research, the physiological function and the general potential of PREP as a drug target have remained obscure. Nevertheless, PREP has attracted considerable pharmaceutical interest since its inhibitors have been shown to improve learning and memory in various preclinical models (for review, see Ref. [13]). Encouraged by these results, hundreds of PREP inhibitors have been synthesized by academic research groups and the pharmaceutical industry with the principal aim of generating antiamnesic drugs. At least three of these compounds have even entered clinical trials, and a PREP inhibitor S-17092 also displayed some cognition-enhancing effects in humans. However, the mechanism of action behind these effects has not been confirmed. In addition to cognition-enhancing effects, PREP inhibitors may have therapeutic potential in the treatment of Parkinson's disease or chronic obstructive pulmonary disease [7,15]. Before PREP inhibitors are further developed, a better understanding is needed on the physiological functions of PREP and on the pharmacodynamic effects of currently available PREP inhibitors.

Acetylcholine (ACh) is an important transmitter in cognitive functions (for review, see Ref. [14]), and cholinergic treatments such as cholinesterase inhibitors represent the standard treatment

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for cognitive deficits such as Alzheimer's disease. The cholinergic system in prefrontal cortex regulates attention and working memory functions and depletion of cortical ACh impairs memory performance in primates and rodents. The hippocampal cholinergic system has a critical role in spatial and declarative memory, i.e. in the capacity to remember everyday events [14].

It has been hypothesized that PREP inhibitors are able to increase brain ACh release by elevating the levels of certain neuropeptides (e.g. substance P, arginine-vasopressin, thyroliberin) that are involved in the regulation of brain ACh turnover [21]. However, recent reports have not provided support for any neuropeptide-increasing effects of PREP inhibitors [2,16,19,20]. Furthermore, the data on the effects of PREP inhibitors on brain ACh levels is scarce. Our recent study revealed that PREP inhibitors decrease striatal extracellular ACh levels in rats [10]. To further elucidate the role of PREP in central ACh release and to clarify whether modulation of ACh levels is associated with the previously reported cognition-enhancing effects of PREP inhibitors, this in vivo microdialysis study was designed to investigate the effects of two highly potent and brain penetrating PREP inhibitors, JTP-4819 and KYP-2047, on extracellular ACh levels in rat prefrontal cortex and hippocampus.

2. Materials and methods

2.1. Animals

Male Han/Wistar rats were supplied by the Laboratory Animal Centre of the University of Eastern Finland (Kuopio, Finland). The rats were housed in stainless steel cages and kept on a 12-h light/12-h dark cycle at an ambient temperature. The animals were 9 weeks old and weighed approximately 280 g at the beginning of the studies. Animals had free access to pelleted food (Teklad 2016S, Harlan Laboratories Inc, Boxmeer, Holland) and tap water. All procedures with the animals were performed according to appropriate European Community Guidelines and "Principles of Laboratory Animal Care" (NIH publication #85-23, revised in 1985). The procedures were reviewed and approved by the Finnish National Animal Experiment Board (license ESAVI-2012-3726).

2.2. Treatment

Two PREP inhibitors, JTP-4819 {(S)-2-[[(S)-2-(hydroxyacetyl)1-pyrrolidinyl]carbonyl]-N(phenylmethyl)-1-pyrrolidinecarboxamide} and KYP-2047 (4-phenylbutanoyl-L-prolyl-2(S)-cyanopyrrolidine), were used at doses of 15 and 50 μmol/kg i.p. The selected doses inhibit rodent brain PREP for up to 12 h [8,9]. Both compounds were synthesized in the University of Eastern Finland as previously described [11,24]. JTP-4819 was dissolved in saline, and KYP-2047 was dissolved in saline containing 5% Tween® 80. Controls received 10 ml/kg i.p. saline containing 5% Tween® 80.

2.3. In vivo microdialysis

First, the rats were anaesthetized with a mixture of ketamine (60 mg/kg; Intervet Int., Boxmeer, Holland) and medetomidine (0.4 mg/kg; Orion Pharma, Espoo, Finland). Then, the rats were placed into a stereotaxic frame (Stoelting Co., Wood Dale, Illinois, USA) and intracerebral guide cannulas (AgnTho's AB, Lidingö, Sweden) were implanted stereotaxically into the medial prefrontal cortex (MAB 9.7.IC, final coordinates from bregma AP +2.4 mm; L +0.6 mm; DV −1.0 mm) and hippocampus (MAB 9.9.IC, final coordinates from bregma AP −6.0 mm; L −5.0 mm; DV −3.2 mm) and fixed to the skull using anchor screws and dental cement. Single subcutaneous doses of buprenorphine (0.02 mg/kg; Schering-Plough, Belgium) and karprofen (5 mg/kg; Pfizer Animal Health SA, Belgium) were given to relieve any postoperative pain, and the animals were woken up by a single injection of atipamezole (1 mg/kg s.c.; Orion Pharma, Espoo, Finland). Finally, the animals were allowed to recover from the surgery for 5–6 days in individual cages.

Twelve hours before the start of the microdialysis experiments, the animals were moved to microdialysis bowls (CMA 120, CMA Microdialysis, Solna, Sweden), and the microdialysis probes (MAB 9.7.4 in cortex and MAB 9.9.4 in hippocampus; both probes had 4 mm exposed membrane, 6 kDa cut-off and membrane outer diameter of 0.6 mm, AgnTho's AB) were carefully inserted into the measurement sites. The probes were perfused with Ringer's solution (138 mM NaCl, 1.3 mM $CaCl_2$, 5 mM KCl, 1 mM $MgCl_2 \cdot 6H_2O$, 11 mM $NaHCO_3$, 1 mM $NaH_2PO_4 \cdot H_2O$, and 11 mM D-glucose, pH 7.4) [1] at a flow rate of 0.5 μl/min.

After a 12 h wash out period, the perfusate was changed into Ringer's solution containing 100 nM of the acetylcholinesterase (AChE) inhibitor, neostigmine (Sigma Chemical Co, St. Louis, MO, USA) and the flow rate was increased to 1.5 μl/min. Twenty min baseline samples were collected for 180 min to ensure stable baselines. Only the last four baseline samples were used to calculate each animal's individual baseline ACh level (=100%). Alterations in the dialysate ACh levels after PREP inhibitor treatment were expressed as a percentage of this value. After the baseline collection, either KYP-2047 or JTP-4819 (15 or 50 μmol/kg) or vehicle (5% Tween® 80 in saline) was administered intraperitoneally, and the dialysate was collected for 5 h in 20 min fractions. The dialysates were immediately frozen (−20 °C) and later stored at −80 °C until analyzed within two weeks of collection.

After the microdialysis experiments, randomly selected rats were deeply anaesthetized and then decapitated and the correct location of the probes in the hippocampus and cortex were verified as described earlier [10]. All examined probes were correctly located (data not shown).

2.4. Analytical and statistical methods

ACh levels in the microdialysates were measured with a highly sensitive liquid chromatography/tandem mass spectrometry (LC/MS/MS) method as described earlier [12].

A mixed model was used to assess the difference between groups in the ACh levels at different time points (time, group and their interaction as fixed effects and rat number as random effect) using SPSS for Windows 14.0.1 software (SPSS Inc., Chicago, USA) [10]. The differences with p-values <0.05 were considered statistically significant. All results are presented as the group means ± S.E.M.

3. Results

The mean baseline extracellular ACh levels were 9.0 ± 1.9 nM in hippocampus and 12.0 ± 1.1 nM in medial prefrontal cortex. In hippocampus, KYP-2047 had no significant effect on the ACh levels at the dose range of 15–50 μmol/kg (Figs. 1A and 2A), but a trend towards decreased levels was observed at the higher dose at 40–80 min after drug administration. JTP-4819 had no effect on the hippocampal ACh levels at the lower dose (15 μmol/kg) (Fig. 1A), but the higher dose (50 μmol/kg) significantly decreased ACh levels in hippocampus by about 25% ($p < 0.05$ at 200–260 min vs. control) (Fig. 2A).

In medial prefrontal cortex, both compounds decreased extracellular ACh levels. In the case of KYP-2047, the smaller dose (15 μmol/kg) decreased ACh levels maximally by 25% ($p < 0.05$ at 260 and 300 min) (Fig. 1B), and a similar (ns) effect was also