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Effect of Humanin Analogues on Experimentally Induced Impairment of Spatial Memory in Rats

GABRIELA KREJCOVA,[a]*JIRI PATOCKA[a] and JIRINA SLANINOVA[b]

[a] Department of Toxicology, Purkyně Military Medical Academy, Třebešská 1575, 500 01 Hradec Králové, Czech Republic
[b] Institute of Organic Chemistry and Biochemistry AVČR, Flemingovo sq. 2, 16610 Prague, Czech Republic

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Abstract: Humanin and its analogues have been shown to protect cells against death induced by various Alzheimer's disease genes and amyloid-β-peptides *in vitro;* the analogue [Gly^{14}]-humanin has also been shown to be potent in reversing learning and memory impairment induced by scopolamine in mice *in vivo*. It is important to validate these results by using other behavioral methods. In this study, the effect of [Gly^{14}]-humanin and des-Leu-PAGA, another analogue (0.2 μmol kg^{-1}, i.p.) on the 3-quinuclidinyl benzilate-induced (2 mg kg^{-1}, i.p.) impairment of spatial memory in the multiple T-maze in rats has been evaluated. Both peptides reversed the impairment of spatial memory. These results indicate the potential of humanin analogues in modulation of the cholinergic system. Copyright © 2004 European Peptide Society and John Wiley & Sons, Ltd.

Keywords: humanin; [Gly^{14}]-humanin; humanin analogue; memory; T-maze test; Alzheimer's disease; 3-quinuclidinyl benzilate

INTRODUCTION

Humanin (Met-Ala-Pro-Arg-Gly-Phe-Ser-Cys-Leu-Leu-Leu-Leu-Thr-Ser-Glu-Ile-Asp-Leu-Pro-Val-Lys-Arg-Arg-Ala; MW = 2686.3; $C_{119}H_{203}N_{34}O_{32}S_2$) and its analogues have been shown to protect neuronal cells against death induced by various Alzheimer's disease (AD) genes and amyloid-β-peptides *in vitro* [1–5]. However, testing their *in vivo* effect is desirable. Direct suppression of neuronal cell death would be promising for the development of anti-AD therapeutics [2]. For the evaluation of the effect of compounds on memory functions, i.e. memory formation, consolidation, impairment, on long-term and short-term memory etc, there are several behavioral tests [6]. Only one study of the effect of the humanin analogue [Gly^{14}]-humanin *in vivo* in the Y-maze test after i.c.v. administration in mice has appeared [7]: it showed the analogue to be potent in reversing the impairment of spontaneous alternation behaviour induced by scopolamine in the Y-maze, an index of short-term memory in mice. It is important to validate these results by using other behavioral methods. In this study, the effect was evaluated of [Gly^{14}]-humanin and the humanin analogue des-Leu-PAGA on 3-quinuclidinyl benzilate (QNB)-induced impairment of spatial orientation and memory in the multiple T-maze in rats. QNB is an anticholinergic drug which affects both the peripheral and central nervous system. It is a potent competitive inhibitor of acetylcholine at postganglionic muscarinic synaptic sites, although in high doses it can also affect nicotinic sites [8]. Pharmacologically, it acts on the central and peripheral nervous systems like scopolamine. It is able to cross the blood–brain barrier. This drug is commonly

*Correspondence to: Gabriela Krejcova, Department of Toxicology, Purkyně Military Medical Academy, Třebešská 1575, 500 01 Hradec Králové, Czech Republic; e-mail: krejcova@pmfhk.cz
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used for experimental modeling of memory deficits in animals [9–11].

MATERIALS AND METHODS

Peptides

[Gly^{14}]-Humanin from Clonestar Biotech (Brno, Czech Republic) was re-purified on semipreparative HPLC and gave a single peak on analytical HPLC; MS confirmed the molecular weight. Des-Leu-PAGA (Pro-Ala-Gly-Ala-Ser-Cys-Leu-Leu-Leu-Thr-D-Ser-Glu-Ile-Asp-Leu-Pro) was obtained from PolyPeptide Laboratories and showed also a single peak on analytical HPLC; MS confirmed the molecular weight. PAGA is a shorter active core (3–19) analogue of humanin (the name comes from the first four amino acids in the sequence, Pro-Ala-Gly-Ala) [12]. Both peptides were dissolved in physiological saline to give 0.2 mM solutions. QNB was synthesized at Purkyně Military Medical Academy Laboratory.

Animals

Male albino Wistar rats weighing 180–200g were obtained from VÚFB Konárovice (Czech Republic). They were kept in an air-conditioned room and maintained on a 20 h food deprivation schedule with food available only in the maze and for 4 h after the daily trial. Tap water was available *ad libitum*. Handling of the experimental animals was done under supervision of the Ethics Committee of the Medical Faculty of Charles University and the Purkyně Military Medical Academy in Hradec Králové (Czech Republic).

Multiple T-maze test

The T-maze [13,14] consisted of five segments 12 cm wide, 20 cm long and 11 cm high. One of the arms (the goal compartment) contained a reward (several food pellets), the length of the correct track from start to the goal compartment was 185 cm. The rats were trained to pass the maze in less than 3 min without entering the wrong arm once a day for 20 days. Only rats that for four successive days had not made any mistake were used in the experiment. The rats were divided into ten groups of seven animals. QNB (2 mg kg^{-1}) was injected 15 min after the peptide injection (0.2 μmol/kg). All substances were administered intraperitoneally. Cognitive functions were tested before drug application and then 15 min, 24 h and 7 days following the QNB injection. Controls were tested at the same time intervals after physiological saline or peptide administration alone. The number of entries into the wrong arms, and passage times through the maze were recorded. Statistical analysis was performed on a PC with Statistica software '98 Edition. Analysis of variance (ANOVA) and the Scheffé method of contrasts were used for the determination of significant differences between the control and experimental groups [15]. The differences were considered significant when $p < 0.05$.

RESULTS

Our study confirmed that the performance in the T-maze of rats injected i.p. with QNB alone was changed in comparison to that before the injection or to that of control animals (rats injected with physiological saline). The rats showed a high number of entries into wrong arms of the maze, and prolonged passage times through the maze. Aggravation of the T-maze performance was observed 15 min and 24 h after drug application (Figure 1). Both peptides significantly attenuated the impairment of the performance, i.e. the spatial memory and orientation when injected 15 min before QNB: there was a reduction of passage time through the maze (Figure 1) in comparison with QNB alone. Although 15 min following QNB injection, the number of wrong entries by the group of [Gly^{14}]humanin- and QNB-treated rats was similar to that of the group which received QNB alone, the peptide-treated animals were able speedily to remedy their mistakes. 24 h after treatment, there was a statistically significant reduction in the number of wrong entries (Table 1) and passage times (Figure 1) if the rats were pretreated with either peptide.

DISCUSSION

This study examined the effect of [Gly^{14}]humanin and des-Leu-PAGA-humanin on spatial memory and orientation impairment induced by QNB. This drug is used for experimental induction of central anticholinergic syndrome [16] with a negative impact on memory and learning [17,18]. Cholinergic neuronal systems play an important role in the cognitive

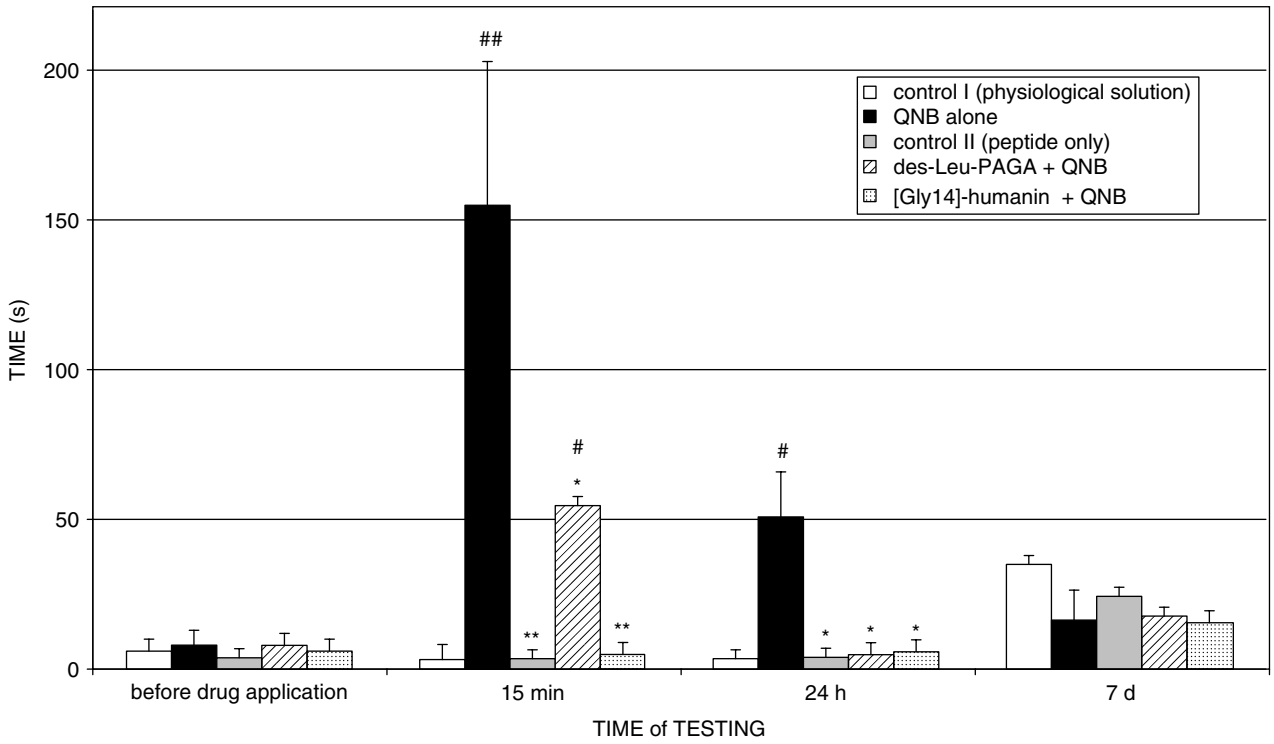

Figure 1 Performance of rats in the T-maze — passage times of the variously treated groups of rats before and 15 min, 24 h and 7 days after drug injection (statistical significance: # and ## vs control I; $p < 0.05$ and $p < 0.01$, respectively; * and ** vs QNB alone, $p < 0.05$ and $p < 0.01$, respectively).

Table 1 Performance of Rats in the T-maze — Number of Wrong Entries of Variously Treated Rats with Statistical Differences (statistical significance [a] vs control I, $p < 0.05$; [b] vs QNB alone, $p < 0.05$)

Group of rats	Number of error entries							
	Before drug application		After drug application					
			15 min		24 h		7 d	
	Ø	±s	Ø	±s	Ø	±s	Ø	±s
Control I ($n = 7$)	0	0	0	0	0	0	0.29	0.49
QNB alone ($n = 7$)	0	0	2.57[a]	2.76	0.57[a]	0.13	0.14	0.38
Control II ($n = 7$)	0	0	0	0	0[b]	0	0.29	0.49
des-Leu-PAGA + QNB ($n = 7$)	0	0	1.29[a]	1.61	0[b]	0	0.43	0.54
[Gly^{14}]-humanin + QNB ($n = 7$)	0	0	3.00[a]	2.77	0[b]	0	0.14	0.38

deficits associated with AD and other neurodegenerative diseases [19]. The study of spatial recognition and memory is a useful method to evaluate potentially antiamnesic drugs [13]. In our study, all drugs were injected i.p., and were found to be active. The fact that the peptides were active after i.p. administration is very significant for drug development. No evidence that humanin analogues cross the blood–brain barrier has so far been published, and the finding of a central effect of humanin analogues following administration in our experiment is therefore fundamental. Peptides are generally rapidly degraded by gastrointestinal enzymes, and thus injection is the preferred route of administration.

This is valid also for humanin [20]. Both peptides used had a significant antiamnesic effect — they reversed memory impairment induced by QNB in rats. Our results confirm the antiamnesic potential of [Gly^{14}]-humanin and demonstrate the neuroprotective effect of des-Leu-PAGA. This humanin analogue has a shorter chain length than humanin (16 versus 24 residues) and lacks one leucine in the middle sequence of the molecule (it has three successive leucines instead of the four present in humanin). This feature simplifies the synthesis (unpublished observation). These results indicate the potential of humanin analogues for the modulation of the cholinergic system and shows that they are able to improve both short-term and long-term memory in rodents.

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