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Abstract

The novel human differentiating factor peptide fragment HLDF6 (Thr-Gly-Glu-Asn-His-Arg) was synthesized and purified. HLDF6 (0.1 mg/kg i.p. but not 1 mg/kg i.p.) improved not only long-term (24 h) memory in adult rats in the water maze behavioural paradigm but also performance in the delayed matching-to-position (DMTP) task (0.3 and 1.0 but not 0.1 mg/kg i.p.). Hence, HLDF6 not only enhanced allocentric spatial learning and reference memory (water maze) but also improved temporal, spatial and working memory processes in the DMTP behavioural paradigm. Immunoreactivity blotting analysis of HLDF (the protein precursor of HLDF6) was performed and the following rank order of visual intensities from brain structures was noted: hippocampus > cerebral cortex > cerebellum > hypothalamus > striatum. Subsequently, we found that the highest absolute levels of HLDF were expressed in the

hippocampus and cerebral cortex as detected by ELISA. We also demonstrated that HLDF6 enhanced [³H]-thymidine and [¹⁴C]-leucine incorporation into whole brain and hippocampal homogenates (maxima occurring within the range 10⁻¹²–10⁻⁶ M) suggesting that this hexapeptide promoted de novo DNA and protein biosynthesis. We discuss this data in terms of their implications for links with other integrative metabolic pathways involving immediate early gene activation which may underpin a potential application for HLDF6 in limiting memory impairments associated with neurodegenerative diseases.

Keywords

learning, memory, human leukaemia differentiating factor HLDF6, DNA, protein biosynthesis

Introduction

During the course of retinoic acid-induced differentiation of human promyelocytic HL-60 cells, an 8.2-kDa protein factor (human leukaemia differentiation factor – HLDF) was isolated and the primary structure determined by parallel sequencing of the protein (N-terminal region) and corresponding cDNA. HLDF is glycosylated and consists of 54 amino acid residues (Kostanyan *et*

al., 1995). Secretion of HLDF was accompanied by the co-production of cytokines such as tumour necrosis factor (TNF) (Marmenout *et al.*, 1985, Smolewski *et al.*, 2002) and various interleukins (Kohase *et al.*, 1986) all of which were able to induce HL-60 cell differentiation into granulocytes. Later, it was discovered that some active peptide fragments corresponded to sequences within the HLDF structure. One of the isolated fragments was identified as the hexapeptide Thr-Gly-Glu-Asn-His-Arg (₄₁TGENHR₄₆) and

has been termed HLDF6 (Kostanyan *et al.*, 2000). The HLDF6 peptide retains the ability of its precursor factor HLDF to induce differentiation, but in contrast, it arrests the proliferation of HL-60 cells. Moreover, HLDF6 is capable *in vivo* and *in vitro* of protecting neuronal cells from neurotoxic insults induced by sodium azide and cold stress (Goncharenko *et al.*, 2002; Zhokov *et al.*, 2004). Furthermore, HL-60 cells do not carry specific surface receptors to HLDF6. However, the peptide increases the fluidity of the cell membrane and modifies cell surface binding of the cell proliferation cytokine interleukin 1 β (IL-1 β) (Kostanyan *et al.*, 2000) which has been shown to modulate learning and memory processes (Pugh *et al.*, 2001). More recently, we have demonstrated that HLDF6 restores long-term habituation of the acoustic startle reaction after chemically induced hypoxia (Zhokov *et al.*, 2004). The cerebellum and hippocampus are implicated in this type of defence behaviour (Lopiano *et al.*, 1990; Daenen *et al.*, 2003; Fujisaki *et al.*, 2004) and because the latter neuroanatomical structure has been widely designated a major learning and memory centre (Sutherland and Rudy, 1989; Jarrand, 1993; Squire, 1992; Cayre *et al.*, 2002; Dash *et al.*, 2004) our initial aim in the present work was to demonstrate whether the parent peptide (HLDF) was present in the CNS. Our preliminary data revealed HLDF immunoreactivity in rat brain, particularly in the hippocampus. Since our recent behavioural study was performed on memory which had been impaired under pathological conditions (Zhokov *et al.*, 2004), the subsequent goal was to determine whether the peptide fragment HLDF6 modified learning and memory processes (water maze and delayed matching to position (DMTP) tasks) in the non pathological state and if there were any concurrent changes in brain protein and DNA biosynthesis.

Materials and methods

Animals

Male Wistar rats with a starting weight of 220 g were used throughout all behavioural and biochemical experiments. They were allowed food and water *ad libitum* and housed in groups of three or four in standard laboratory cages under 11 h–12 h light-dark conditions (lights on 0800) with an ambient room temperature of 21–23 °C. In the water maze paradigm, animals ($n = 10$ per group) were allowed food and water *ad libitum*. Animals ($n = 7$ per group) employed for DMTP were kept at approximately 85–90% of their free-feeding weight by restricting access to food for 1 h per day for 5 days of the week. Food was available *ad libitum* during all other days and water was always freely available except during testing. All animal test protocols conformed with European Community and Russian guidelines for the use of animals and were approved by the local ethical committees for Cardiff University and the P. K. Anokhin Institute of Normal Physiology.

Drugs and chemicals

HLDF6 was prepared as described below and dissolved in normal apyrogenic saline for behavioural experiments. ^3H -Thymidine (740 Bq/mmol, 5 Ci/mmol), ^{14}C -Leucine (185 Bq/mmol,

50 $\mu\text{Ci}/\text{mmol}$) and the Blot-protein detection kit as well as the immunoreagents for ELISA were obtained from Amersham (UK).

Synthesis and purification of peptide HLDF and HLDF6 were synthesized by the solid phase technique using the Boc/Bzl methodology. Polystyrene resin Boc-Arg(Tos)-PAM RESIN containing 250 μmol of the starting amino acid per analogue was used. Peptidyl polymers were synthesized in a flow-through reactor of variable volume using swellographic monitoring to determine the duration of operation cycles (Rodionov *et al.*, 1992). The resulting peptides were purified by gel filtration on Sephadex G-50 SF (column 26 \times 90 mm) in 1 M ethanol followed by medium-pressure liquid chromatography on a PepRPC/TM column (FPLC-System; Pharmacia, Sweden). The purity of preparations was determined using amino acid analysis and MALDI-TOF mass spectrometry. Mass-spectrometric analysis was carried out on a Vision 2000 instrument (Thermo Bioanalysis, UK).

Dot-blotting of HLDF immunoreactivity in homogenates from rat brain structures

HLDF antibody production Outbred rabbit immunization with HLDF and purification of the IgG fraction were performed according to standard procedures (Weir, 1986). Final purification of the antibodies was carried out chromatographically on HLDF-Sepharose 6B. Working titres of antibodies were determined by ELISA at an optimum of 1:1000 (Dranitsina *et al.*, 2000).

Dot-immunoblotting procedure Dissected rat brain structures (hippocampus, cortex, cerebellum, hypothalamus, striatum) ($n = 8$ per group) were homogenized using a glass mortar and teflon pestle in PBS (pH 7.4) and centrifuged at 3500 rpm. The precipitate was removed and brain structure supernatants and control HLDF samples (1.0–100 ng/ μl for calibration) were transferred to and developed on Hybond C nitrocellulose paper. Non-specific binding sites were displaced by paper immersion in 5% solution of reconstituted dried skimmed milk in TBS containing 0.5% Tween (1 h at 21 °C). Membranes were triple washed and incubated firstly, with primary antibodies to HLDF at a dilution of 1:1000 (1 h at 37 °C), secondly, with anti-rabbit biotinylated antibodies (dilution 1:250 for 1 h, at 37 °C), then, with streptavidin-biotinylated alkaline phosphatase complex (1:1000, 4 min at 37 °C). Finally, nitrocellulose membranes were developed with a substrate mixture of bromochloroindolylphosphate (0.38 M)/nitroblue tetrazolium (0.4 M) suspended in 100 mM Tris buffered saline (pH 9.5) with 10 mM MgCl_2 . HLDF immunoreactivity was visualized and compared with controls.

Determination of HLDF by ELISA

In immobilized ligand experiments, microtitre plate wells (Costar, USA) were coated with previously described purified rat IgG to HLDF (1:400 in 0.2 M PBS, pH 7.4, containing 0.05% sodium azide) and incubated (16 h, 4 °C). After washing, plates were covered with homogenates of rat brain structures (hippocampus, cerebral cortex, cerebellum, hypothalamus, striatum and blood

sera, $n = 8$ per group) or control HLDF samples (0.5 ng – 25 mcg of protein in PBS pH 7.4 for the calibration curve) then incubated (2 h at 37°C). After a second wash, plates were covered and incubated with rabbit immunoglobulins to HLDF (1:4000, 2 h at 37°C). Binding was quantified after a third wash by using goat secondary anti rabbit IgG conjugated with horseradish peroxidase and the signal – developed with orthophenylenediamine (Sigma, USA) in sodium acetate buffer at pH 6.0. The reaction was allowed to proceed for 30 min and stopped by adding 0.5 N H₂SO₄. Data were subsequently quantified via a microtitre plate reader (Flow Lab., USA) and expressed in ng/mg tissue or sera protein.

[³H]-thymidine incorporation in de novo synthesized DNA

Cellular DNA biosynthesis was assayed in whole rat brain and hippocampus ($n = 8$ per group). The tissue was homogenized in Solutio Hanksi in the presence of MgSO₄ (50 mg/l), KH₂PO₄ (50 mg/l), glucose (1 g/l) and centrifuged at 3500 rpm for 20 min at 8°C (Sorval 5B, Du Pont, USA). The precipitate was removed. Either HLDF6 dissolved in aqueous solution at concentrations of 10⁻¹²–10⁻⁶ M, or saline as control, along with ³H-thymidine (3.7 μCi/μM) was added to 0.5 ml of supernatant and incubated for 30 min at 37°C. Subsequently, incubates were admixed with 1% protamine sulphate solution (Serva, Sweden) and passed through Millipore filters (0.45 μm) then washed three times with Tris-HCl buffer (pH 7.5) plus 0.3 M NaCl. The filters were dried and transferred to glass vials followed by addition of 10 ml of ReadySolvEP scintillation fluid (Beckman, USA). After standing for 24 h (22°C), radioactivity was counted using a Rac-Beta 5 counter (LKB/Wallac). Data were calculated and expressed as cpm/mg tissue protein.

[¹⁴C]-leucine incorporation in de novo synthesized soluble proteins

Cellular soluble protein biosynthesis was assayed in whole rat brain and hippocampus ($n = 8$ per group). The tissue was homogenized in Solutio Hanksi in the presence of MgSO₄ (50 mg/l), KH₂PO₄ (50 mg/l), glucose (1 g/l) and centrifuged at 3500 rpm for 20 min at 8°C (Sorval 5B, Du Pont, USA). The precipitate was removed. Either HLDF6 dissolved in aqueous solution at concentrations of 10⁻¹²–10⁻⁶ M, or saline as control, along with ¹⁴C-leucine (2.5 μCi/μM) was added to 0.5 ml of supernatant and incubated for 30 min at 37°C. Insoluble proteins were precipitated by addition of 7% TCA and centrifuged at 3500 rpm. The soluble protein fraction was retained by 0.45 μm Millipore filters and washed three times with 7% TCA then triple washed with Tris-HCl buffer (pH 7.5). The filters were dried and transferred to glass vials followed by addition of 10 ml of ReadySolvEP scintillation fluid. After standing for 24 h (22°C), radioactivity was counted and data were calculated and expressed as cpm/mg tissue protein.

Protein concentration

All brain structure and blood sera samples were assayed for the protein content according to the method of Bradford (1976).

Water maze paradigm

Apparatus Experiments were performed in a test room in which many external cues including a window covered by blinds, tables and boxes were present (this would be an allocentric memory task). A circular aluminium swimming tank (diameter 1.4 m, wall height 60 cm) was filled to a depth of 40 cm with luke warm water (25°C) rendered opaque by the addition of a small quantity of powdered milk according to Nakamura *et al.* (2001). The pool was divided into four quadrants (north, south, east and west). A hidden square clear plexiglas platform (10 cm²) was placed in one quadrant near the tank wall and submerged to a depth of 5 mm where it would not be visible to an animal on the water surface. A Video-monitoring system (TSE, Germany) was used for recording the main parameters (time to reach the platform and length) of training in water maze paradigm.

Behavioural training Individual animals ($n = 10$ per group) were trained by exposure to six trials daily for the first day then tested for six trials on the second day. Each trial was commenced by carefully placing each animal into the water facing the wall of the pool at one of three random start positions avoiding the quadrant including the platform. In each trial, the time taken to escape on to the hidden platform (swimming latency, sec) was recorded. A cut-off time of 120 s for non-escapers was imposed. Such subjects were then placed on the platform and allowed to remain there for 30 s. At the end of each trial, animals were immediately returned to their home cage. The intertrial interval was ~1 min. Group performance in each trial was assessed by mean swimming latency to reach the platform (sec ± SEM).

Drug testing HLDF6 (0.1 mg/kg and 1.0 mg/kg) or vehicle was injected intraperitoneally once on the first day 1 h before initial training.

Delayed matching to position paradigm

Behavioural training and testing apparatus Animals were trained and tested in operant chambers (Campden Instruments, UK) modified to accommodate an additional non-retractable lever mounted on the wall opposite to the two standard retractable levers sited either side of the food collection panel. Each box was equipped with four functioning lights: the ceiling mounted house-light, two sample lights mounted above each of the two retractable levers and one back light mounted above the back wall non-retractable lever. Software controlling the boxes was written by D. M. Pache based on the programming language SPIDER (Paul Fray, UK).

Behavioural training Following standard autoshaping procedures, animals were trained to lever press on a schedule of continuous reinforcement in order to obtain a small food reward (45 mg precision food pellet; Campden Instruments, UK). Once lever responding was established, subjects were subsequently trained to perform a delayed matching to position schedule. Training for this schedule began with the simultaneous insertion of a pseudorandomly chosen sample lever into the operant chamber and the

illumination of the light situated above it. A response on this lever caused its retraction, the light above to be switched off and the rear lever light switched on. The animal was then required to respond on the rear lever below in order to acquire the small food reward. Once appropriate responding was achieved a matching to position schedule was started. This consisted of an additional choice stage to the procedure described above. A response on the back lever caused the light above it to be switched off, and the simultaneous insertion of both levers situated either side of the food dispenser along with the illumination of their respective lights. The animal was then required to choose a lever. A response on the original sample lever was rewarded with food and the lights switched off for the duration of an intertrial interval before the next trial began. A response on the alternative lever caused both lever lights to be switched off without the delivery of any food and initiated the intertrial interval. Once asymptotic performance was attained, delays were introduced between the sample phase and the choice phase; this caused the animal to respond on the rear lever for the duration of the delay. The first rear lever press after the expiry of the delay initiated the choice phase. Intertrial intervals were initially 8 s with delays set at 1 s. They were then gradually incremented to 1, 4, 8 and 16 s with an intertrial interval of 32 s. Once asymptotic responding was reached, experiments were commenced. During experiments only delays were extended to 1, 8, 16 and 24 s with a 48 s intertrial interval. Maintenance training was conducted twice a week on Mondays and Thursdays, with Tuesdays and Fridays reserved for experiments. All other days were rest days.

Drug testing During testing, each agent or vehicle was administered intraperitoneally as a 10 min pretreatment and the % correct lever selection (response accuracy) determined.

Data analysis Data are presented as means \pm standard errors of mean (SEM). $p < 0.05$ was considered to represent significant differences. Analysis of variance (ANOVA) was used in biochemical data.

Data for the water maze experiments was analysed using one-way ANOVA with Tukey's post hoc test for identifying significance between individual treatment groups. In order to determine the generic effect of HLDF6 on response accuracy in delayed matching to position, data from the two shortest delays (1 s and 8 s) were combined and a statistical comparison conducted between treatment doses using one-way ANOVA followed by Tukey's post hoc test. Data from the two longest delays (16 s and 24 s) were treated similarly.

Results

Dot-blotting immunoreactivity with HLDF antibodies

Using the immunoblotting technique with polyclonal monospecific rabbit antibodies against HLDF, we were able to visually identify HLDF-like qualitative immunoreactivity in all brain structures studied. The immunoreactivity in anatomical areas was detected with the following rank order of visual intensities: hippocampus > cerebral cortex > cerebellum > hypothalamus > striatum.

Consequently we used the ELISA technique to measure the specific quantitative HLDF immunoreactivity levels.

Determination HLDF by ELISA

The mean HLDF immunoreactivity content of some adult rat brain areas is shown in Table 1 and ranged from 12.1 ng/mg tissue protein in hippocampus, and 5.9 ng/mg in cortex down to less than 1.0 ng/mg tissue protein in the striatum. By comparison, this blood-derived factor was predictably detected in the blood sera of the same rats at a level of 1.1 ng/mg sera protein, which was 11-fold lower than the richest brain region (hippocampus). In the brain, HLDF is the precursor polypeptide for HLDF6, a fragment which we have previously shown to express neuroprotective activity (Zhokov *et al.*, 2004) inevitably implicating de novo synthesis of proteins (Okuda and Ogita, 2002; Roth, 2004). We therefore proceeded to examine HLDF6 activity on markers of de novo DNA/protein synthesis since these molecular mechanisms are also involved in memory processes (D'Agata and Cavallaro, 2002; Hinoi *et al.*, 2002).

Activity of HLDF6 on de novo DNA synthesis ($[^3\text{H}]$ -thymidine incorporation) in adult rat brain tissue

HLDF6 was investigated with respect to $[^3\text{H}]$ -thymidine incorporation into adult rat brain homogenates as a marker of de novo DNA synthesis. Concentrations of 10^{-12} up to 10^{-6} M HLDF6 produced a biphasic concentration-incorporation relationship with significant increases occurring at 10^{-11} M (37.1%; $p < 0.05$) up to a peak at 10^{-9} M (57.7%; $p < 0.01$) then decreasing to 19.5% at 10^{-6} M ($p < 0.05$) versus controls (Fig. 1). We also measured incorporation of $[^3\text{H}]$ -thymidine in hippocampal homogenates and found that over the concentration range 10^{-12} – 10^{-6} M HLDF6, the peak utilization (mean \pm SEM) of the radiolabelled marker ($49, 850 \pm 3100$ cpm/mg protein) also occurred at 10^{-9} M HLDF6 ($p < 0.05$).

Activity of HLDF6 on de novo protein synthesis ($[^{14}\text{C}]$ -leucine incorporation) in adult rat brain tissue

The activity of HLDF6 was studied at concentrations ranging from 10^{-12} up to 10^{-6} M on $[^{14}\text{C}]$ -leucine incorporation compared to saline control in rat whole brain homogenates as a reflection of de

Table 1 HLDF content in adult rat blood and brain structures

Tissues	HLDF (ng/mg of tissue or sera protein)
Hippocampus	12.1 \pm 3.3
Cortex cerebral	5.9 \pm 1.7
Cerebellum	3.2 \pm 0.9
Hypothalamus	1.0 \pm 0.2
Striatum	<1.0
Blood sera	1.1 \pm 0.3

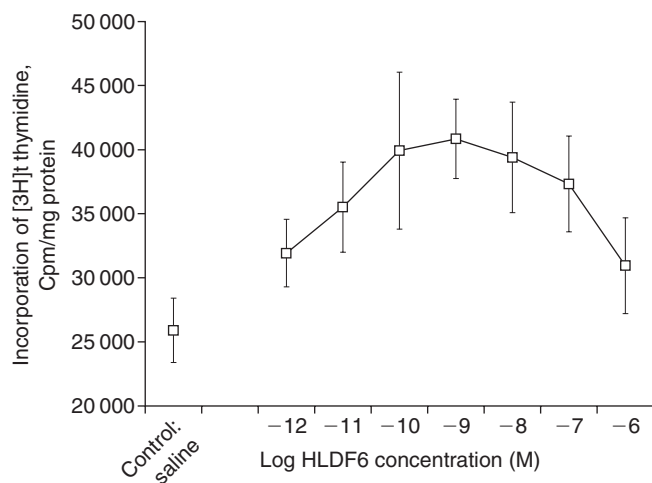


Figure 1 Influence of HLDF6 on incorporation of [^3H]-thymidine in DNA synthesized de novo in adult rat brain homogenates

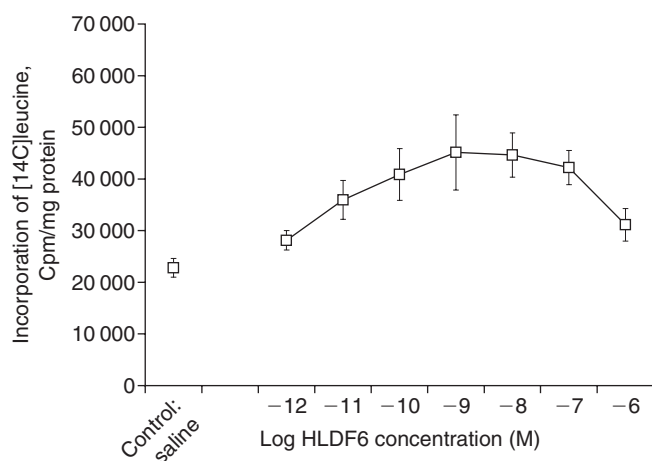


Figure 2 Influence of HLDF6 on incorporation of [^{14}C]-leucine in protein synthesized de novo in adult rat brain homogenates

novo protein synthesis. It was demonstrated that the peptide also produced a biphasic concentration-incorporation relationship increasing up to an optimum rate almost twice that of the control (98%; $p < 0.05$) occurring at 10^{-9}M . This degree of leucine incorporation subsequently declined over concentrations up to 10^{-6}M (36.6%; Fig. 2). In homogenates of hippocampus, maximal incorporation (mean \pm SEM) of [^{14}C]-leucine (59350 ± 3965 cpm/mg protein) also occurred at a concentration of 10^{-9}M HLDF6 ($p < 0.05$).

Activity of HLDF6 on learning in the Morris water maze

Administration of HLDF6 (0.1 mg/kg but not 1.0 mg/kg i.p.) significantly enhanced ($p < 0.05$) the spatial reference memory

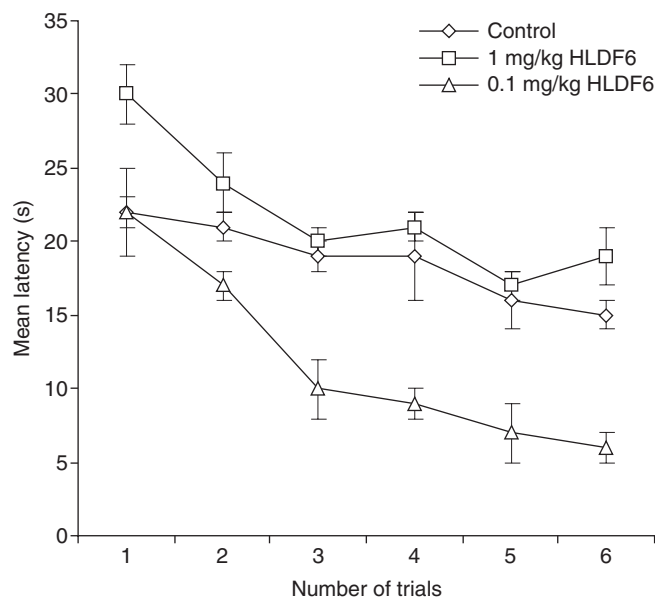


Figure 3 Influence of HLDF6 on the spatial memory of adult rats trained in the Morris water maze

(Nakamura *et al.*, 2001) of rats trained in the Morris water maze (Fig. 3). Thus, in Fig. 3, there was a significant reduction (-19.0% , $p < 0.05$) compared to control (21.0 ± 1.0 s) in the mean time taken to reach the platform during the second trial and this reduction was maintained up to the sixth trial where it attained a magnitude of -60.3% . At the higher dose (1.0 mg/kg, i.p.) there was no significant difference between control and test at any of the trials sessions.

Activity of HLDF6 on the delayed matching to position task

Using the putative short-term memory task, delayed matching to position (DMTP), HLDF6 at 0.3 mg/kg ($p < 0.05$) and 1.0 mg/kg ($p < 0.01$) but not 0.1 mg/kg, enhanced performance at long (16 s and 24 s), but not short (1 s and 8 s) delays (Fig. 4). No effect was observed on trial completion or panel press activity at any dose level.

Discussion

In the present study, HLDF6 (Thr-Gly-Glu-Asn-His-Arg), one of the isolated peptide fragments of human leukaemia differentiation factor was synthesized and purified. Initial qualitative dot-blotting immunoreactivity with HLDF demonstrated that this precursor protein was present in several adult brain structures (hippocampus, cerebral cortex, cerebellum, hypothalamus and striatum). Subsequent study with the ELISA technique confirmed the presence of quantities of HLDF which correlated with the rank order of

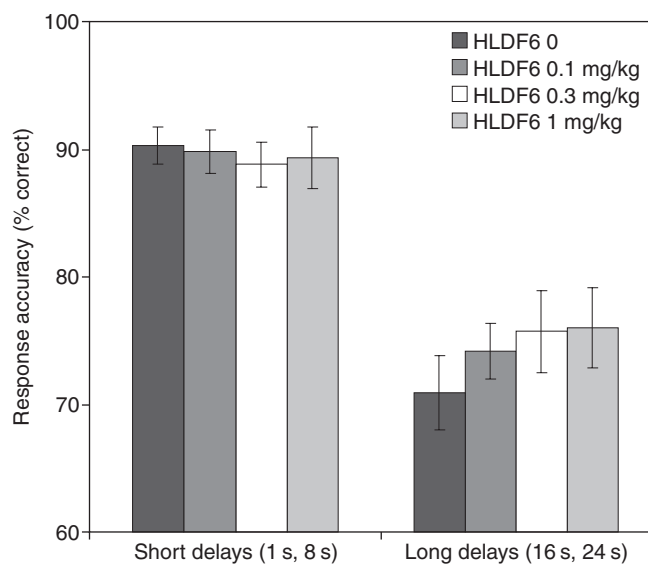


Figure 4 Influence of HLDF6 on the memory of adult rats in the delayed matching-to-position task

visual intensities from brain structures using the dot-blotting data. In particular, we detected high HLDF levels in the hippocampus and cerebellum which is the first occasion that such a finding has been reported.

It is well known that the hippocampus in particular, is implicated in memory processes (Sutherland *et al.*, 1989). It is tempting therefore, in view of the hippocampal localization of the HLDF6 precursor, to suggest that there might be a link between expression of HLDF in the brain, its limited proteolysis to intermediate active cleavage peptide fragments and an involvement of these peptides in molecular mechanisms of learning and memory.

The behavioural tasks employed in this investigation were chosen to reflect a spectrum of elements associated with learning and memory processes thereby providing a profile of activity for HLDF6. Hence, learning in the Morris water maze is thought to test permanent allocentric spatial learning capacity and reference memory (Nakamura *et al.*, 2001). The delayed matching-to-position task (Christoffersen *et al.*, 1998) by comparison, invokes temporal as well as spatial working memory processes. In the Morris water maze paradigm, HLDF6 at a low dose (0.1 mg/kg) significantly enhanced platform seeking performance time from the second trial onwards to the end of testing. Hence, HLDF6 enhanced 24h (long-term) spatial learning in the Morris water maze. Interestingly, in the delayed matching to position task, the lowest dose of HLDF6 (0.1 mg/kg) failed to modify task performance. However, at the higher doses (0.3 and 1.0 mg/kg) a significant improvement was observed. Positive nootropic effects in DMTP paradigms are rarely reported and as such, though there is only a small improvement in task performance against vehicle, it represents an interesting, if somewhat uncommon finding. Due to the short delay time frame of the task, the improvement in DMTP

response accuracy is unlikely to be a reflection of increased DNA or protein synthesis. These events clearly require more time than that available during even the longest delay stage (24s) of a delayed matching task. Conversely, the ability of HLDF6 to improve water maze performance where re-testing was conducted 24h after training, can be more readily attributable to such biosynthetic mechanisms.

The enhancement of cognitive function observed to HLDF6 in the two behavioural tasks led us to investigate possible underlying molecular mechanism(s). In this context, it is accepted that long-term memory formation requires *de novo* DNA, RNA and protein biosynthesis (Clements and Rose, 1997; Bourthouladze *et al.*, 1998; Tiunova *et al.*, 1998; Luo *et al.*, 2001; Anokhin *et al.*, 2002). This has been corroborated in experiments using protein synthesis inhibitors for example cycloheximide (Litvin and Anokhin, 2000).

Following incubation of HLDF6 with rat brain homogenates, protein synthesis *in vitro* was doubled and this fact inevitably stemmed from a *de novo* process and was also reflected by the fact that we found biosynthetic activity located in the hippocampus. HLDF6 also stimulated DNA synthesis as marked by *in vitro* [³H]-thymidine incorporation in whole brain as well as hippocampal tissue.

Luo *et al.* (2001), have identified more than 28 genes associated with maze-learning. Some of them are involved with Ca²⁺ signalling, Ras activation and kinase cascades which may regulate neural transmission, synaptic plasticity and neurogenesis. It might be hypothesized that the currently reported HLDF6 outcome on DNA may affect *in vivo* immediate early genes such as c-fos and c-jun which can be regarded as mediators by which brief stimuli trigger long-term changes in synaptic connections thus encoding spatial memory (Clements and Rose, 1997; He *et al.*, 2002).

Immediate early genes may function as a switch between short- and long-term memory by triggering late, structural genes. These late genes are responsible for the *de novo* synthesis of structural glycoproteins e.g., the cell adhesion molecules NCAM and L1, which facilitate synaptic remodelling and the formation of long-term memory in learning tasks (Clements and Rose, 1997).

Moreover, such a modulatory mechanism involving HLDF and the HLDF6 fragment may be closely linked with other cellular metabolic pathways. For instance, we demonstrated that HLDF6 interacts with cell membrane lipids to alter fluidity (Kostanyan *et al.*, 2000), which can influence the expression and specific receptor binding of IL-1 β in the HL-60 cell line (Kostanyan *et al.*, 2000). More recently, behavioural evidence has been presented that IL-1 β impairs the consolidation of hippocampal-dependent memory and this is blocked by antagonizing the actions of IL-1 β (Pugh *et al.*, 2001).

In summary, it can be concluded that the novel human leukaemia differentiation factor peptide fragment HLDF6 may improve memory processes probably by changing brain DNA and protein biosynthesis. Finally, the effects of HLDF6 on cognitive function may well underpin a potential application for HLDF6 in limiting memory impairments associated with neurodegenerative diseases.

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