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# Effects of FGF receptor peptide agonists on animal behavior under normal and pathological conditions

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ABSTRACT

Hexafins are recently identified low-molecular-weight peptide agonists of the fibroblast growth factor receptor (FGFR), derived from the β6–β7 loop region of various FGFs. Synthetic hexafin peptides have been shown to bind to and induce tyrosine phosphorylation of FGFR1, stimulate neurite outgrowth, and promote neuronal survival *in vitro*. Thus, the pronounced biological activities of hexafins *in vitro* make them attractive compounds for pharmacological studies *in vivo*. The present study investigated the effects of subcutaneous administration of hexafin1 and hexafin2 (peptides derived from FGF1 and FGF2, respectively) on social memory, exploratory activity, and anxiety-like behavior in adult rats. Treatment with hexafin1 and hexafin2 resulted in prolonged retention of social memory. Furthermore, rats treated with hexafin2 exhibited decreased anxiety-like behavior in the elevated plus maze. Employing an R6/2 mouse model of Huntington's disease (HD), we found that although hexafin2 did not affect the progression of motor symptoms, it alleviated deficits in activity related to social behavior, including sociability and social novelty. Thus, hexafin2 may have therapeutic potential for the treatment of HD.



## 1. Introduction

Fibroblast growth factors (FGFs) form a family of heparin-binding growth factors acting through distinct tyrosine kinase receptors and are involved in various physiological and pathological processes, including embryonic development, tissue repair, inflammation, angiogenesis, and tumor growth (for review, see Beenken and Mohammadi, 2009; Iwata and Hevner, 2009). Ten members of the FGF family have been shown to be widely distributed throughout the central nervous system and expressed in both neurons and glial cells (Gonzalez et al., 1995). Among the members of the FGF family in the brain, FGF1 (or acidic FGF) and FGF2 (or basic FGF) have been extensively studied and found to be abundant in neurons and astrocytes. Among the FGF receptors (FGFRs), FGFR1, FGFR2, and FGFR3 have been found to be expressed in the brain (for review, see Ford-Perriss et al., 2001).

FGF-mediated signaling has distinct functions at different stages of embryonic and early postnatal development and is required for multiple developmental processes, including determination of cell fate, promotion of cell survival, and axonal branching (for review, see Abe and Saito, 2001; Ford-Perriss et al., 2001; Iwata and Hevner, 2009; Ornitz, 2000; Reuss and von Bohlen und Halbach, 2003). In adult brain, FGFs play a critical role in the maintenance of normal neuronal function. Several members of the FGF family, particularly FGF2, are involved in neuroprotection and promote repair after traumatic brain injury, ischemia, and neurodegenerative disease (for review, see Alzheimer and Werner, 2002). After traumatic or ischemic injury to the brain, expression of FGF2 and its receptor increases in the tissue surrounding the lesions (Ganat et al., 2002; Grothe et al., 2001; Yoshimura et al., 2001; Yoshimura et al., 2003). The mechanisms by which FGF2 protects neurons against various oxidative, metabolic, and excitotoxic insults are not well understood. FGF2 has been suggested to promote the maintenance of $Ca^{2+}$ homeostasis, suppress the accumulation of reactive oxygen species and lipid peroxidation, stimulate antioxidant pathways, and increase neurogenesis (Arvidsson et al., 2002; Jin et al., 2003; Mudo et al., 2009; Wang et al., 2008; Xu et al., 2005).

Additionally, a strong line of experimental evidence has established a role for FGF2 in neuronal excitability, synaptic plasticity, and learning. These findings make the FGF system an important

*Abbreviations:* ANOVA, analysis of variance; BDNF, brain-derived neurotrophic factor; BSA, bovine serum albumin; CSF, cerebrospinal fluid; ELISA, enzyme-linked immunosorbent assay; EPM, elevated plus maze; FGF, fibroblast growth factor; FGFR, fibroblast growth factor receptor; HD, Huntington's disease; ITI, intertrial interval; NCAM, neural cell adhesion molecule; PCR, polymerase chain reaction; PD, Parkinson's disease; SRT, social recognition test; TMB, 3,3′,5,5′-tetramethylbenzidine.

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therapeutic target for modulating cognitive impairment and mood disorders in humans (Turner et al., 2006).

In various animal models, FGF2 has been shown to be a promising therapeutic compound for stroke (Ay et al., 1999; Ren and Finklestein, 2005) and Parkinson's disease (PD) (Grothe and Timmer, 2007; Jensen et al., 2008). A recent study showed that FGF2 improves neurological deficits and longevity in a transgenic mouse model of Huntington disease (HD) (Jin et al., 2005).

Despite the accumulating evidence of its neuroprotective potential, widespread use of FGF2 in the clinic is limited because of restricted passage of peptide hormones across the blood–brain barrier, poor pharmacokinetics, and severe adverse effects related to intravenous administration (Cheng et al., 2004). Therefore, the development of small peptidomimetics capable of activating FGFR signaling and promoting neurotrophic function is highly desirable.

Several synthetic peptide agonists of FGFR have recently been developed (for review, see Berezin and Bock, 2004), and a group of homologous peptides derived from various FGFs, termed hexafins, has been identified (Li et al., 2009). The hexafins bind to FGFR1 and FGFR2 and result in receptor phosphorylation. Hexafin1 and hexafin2 sequences are derived from FGF1 and FGF2, respectively. Hexafin2 (but not hexafin1) is able to promote neurite outgrowth from cerebellar granule neurons in an FGFR activation-dependent manner. Hexafin1 (but not hexafin2) has been shown to promote survival of primary neurons induced to undergo apoptosis (Li et al., 2009). Thus, profound biological activity of hexafins *in vitro* makes them attractive compounds for pharmacological studies *in vivo*.

The present study investigated the effects of hexafin1 and hexafin2 administration on adult rat behavior using tests for cognition, anxiety, and exploratory activity. Furthermore, we tested the potential efficacy of hexafin2 in ameliorating the motor and cognitive deficits in an R6/2 mouse model of HD.

## 2. Experimental procedures

### 2.1. Peptides and treatment

Hexafin1 (TGQYLAMDTDGLLYGS) and hexafin2 (ANRYLAMKEDGRLLAS) (Li et al., 2009) were purchased in dendrimeric form from Schafer-N (Copenhagen, Denmark). The peptides were synthesized using the Fmoc protection strategy on Tenta-Gel resin (Rapp Polymere, Tübingen, Germany) using Fmoc-protected amino acids (Calbiochem-Novabiochem, San Diego, CA, USA). The peptide dendrimers were composed of four monomers coupled to a lysine backbone. Biotinylation was achieved by substituting the *N*-terminal amino acid with the same amino acid labeled with biotin. All peptides were high-performance liquid chromatography-purified and dialyzed against sterile water.

Hexafin1 and hexafin2 were dissolved in sterile water (vehicle). Hexafin1 or hexafin2 (8 mg/kg) and vehicle (sterile water, 2.0 ml/kg) were administered subcutaneously.

### 2.2. Behavioral studies in normal rats

#### 2.2.1. Animals

Male Wistar rats were purchased from Charles River Laboratories (Sulzfeld, Germany). The animals weighed 200–250 g upon arrival and were housed in pairs in standard plastic cages (48 cm × 26 cm × 21 cm). Lighting was maintained on a 12 h/12 h light/dark cycle in a climate-controlled environment (21 °C, relative humidity 60%). The animals had *ad libitum* access to food and water throughout the experiment. All experiments were conducted in accordance with Danish legislation and with a license from the Danish Animal Experiments Inspectorate.

#### 2.2.2. Open field

Spontaneous locomotor activity and anxiety-like behavior were assessed in an open field chamber with a square arena (100 cm × 100 cm × 40 cm) equipped with hole-poke insert. Animals ($n$ = 12 per group) were placed individually into the center of the open field and left to explore the arena for 10 min. The time spent in the central and peripheral areas of the arena, total distance traveled, and number of rearings during the experiment were automatically recorded using the Ethovision video tracking system (Noldus Information Technology, Sterling, VA, USA). Head-dips and defecations were counted by the experimenters. The floor of the arena was washed with 10% ethanol solution to remove odors left by previous subjects. Hexafin1 or hexafin2 (8 mg/kg) and vehicle (sterile water, 2.0 ml/kg) were administered subcutaneously 24 h before the test.

#### 2.2.3. Elevated plus maze

The elevated plus maze (EPM) is used to assess anxiety-like behavior in rats and mice (Lister, 1987). The EPM apparatus consisted of two open arms (50 cm × 10 cm), two enclosed arms (50 cm × 10 cm) with 15 cm high opaque sides and end-walls, and a connecting central platform (15 cm × 15 cm). The maze was raised 100 cm above the floor. Animals ($n$ = 12 per group) were individually placed in the central platform facing an open arm and allowed to explore for 5 min. An Ethovision video tracking system (Noldus Information Technology, Sterling, VA, USA) was used to analyze the latency to the first open arm entry, percentage of time spent on the open arms, percentage of open arm entries, total number of entries. Hexafin1 or hexafin2 (8 mg/kg) and vehicle (sterile water, 2.0 ml/kg) were administered subcutaneously 24 h before the test.

#### 2.2.4. Social recognition test

The social recognition test (SRT) was performed as previously described (Thor et al., 1982). Twenty-four hours before the SRT, a habituation session was performed under conditions identical to the test session to adapt the animals to the experimental room, test cage, test duration, and contacts with juvenile rats (different juvenile animals were used for the habituation session and for the SRT).

On the test day, adult animals ($n$ = 11–13 per group) were individually housed 1 h before testing in the test cages (48 cm × 37 cm × 21 cm). Juveniles were isolated in individual cages 1 h prior to introduction to the test cage and between two successive sessions to prevent the formation of group odors.

The SRT consisted of two trials: the initial trial and the test trial. The duration of each trial was 4 min. The initial trial began by introducing a juvenile into the test cage of the adult rat for 4 min. At the end of the first exposure, the juvenile was removed and kept in an individual cage. After an intertrial interval (ITI) of 120 min, the test trial was performed. During the test trial, the experimental adult rats were exposed to the same juvenile or a novel juvenile. During each trial, the investigative behavior of the adult rat toward the juvenile (i.e., licking, sniffing, chewing the fur of the juvenile, and close following) was cumulatively measured. The time spent investigating the juvenile during the initial trial (T1) and the test trial (T2) was calculated. Social recognition is determined to be present during the test trial if the adult rat spends significantly less time investigating the previously introduced juvenile than during the initial trial. A lack of difference between the times spent investigating the juvenile during the first and second trials indicate that the adult failed to recognize the juvenile. Social memory retention was preliminarily tested in separate groups of animals at different ITIs (30, 60, 90, 120 min) under conditions identical to the experimental test. Animals tested with ITIs of 30 and 60 min could recognize the same juvenile. Because we tested whether the peptides could facilitate social memory retention, we used an ITI of 120 min. With