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Neurokinin$_3$-R agonism in aged rats has anxiolytic-, antidepressant-, and promnestic-like effects and stimulates ACh release in frontal cortex, amygdala and hippocampus

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Abstract

Neurokinin-3 receptors (NK_3-R) are localized in brain regions which have been implicated in processes governing learning and memory as well as emotionality. The effects of acute subcutaneous (s.c.) senktide (0.2 and 0.4 mg/kg), a NK_3-R agonist, were tested in aged (23–25 month old) Wistar rats: (a) in an episodic-like memory test, using an object discrimination task (this is the first study to test for deficits in episodic-like memory in aged rats, since appropriate tests have only recently became available); (b) on parameters of anxiety in an open field test, (c) on indices of depression in the forced swimming test and (d) on the activity of cholinergic neurons of the basal forebrain, using *in vivo* microdialysis and HPLC. Neither the saline-, nor senktide-treated aged animals, exhibited episodic-like memory. However, the senktide-, but not the vehicle-treated group, exhibited object memory for spatial displacement, a component of episodic memory. Senktide injection also had anxiolytic- and antidepressant-like effects. Furthermore, the active doses of senktide on behavior increased ACh levels in the frontal cortex, amygdala and hippocampus, suggesting a relationship between its cholinergic and behavioral actions. The results indicate cholinergic modulation by the NK_3-R in conjunction with a role in the processing of memory and emotional responses in the aged rat.

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1. Introduction

Neurokinins belong to the neuropeptide family of tachykinins, which include substance P (SP), neurokinin A (NKA) and neurokinin B (NKB) as the preferred endogenous ligands of three distinct neurokinin receptors: the NK_1-, NK_2- and NK_3-R. These receptors have been identified in the brain, with widespread distribution of NK_1- and NK_3-R, whereas the NK_2-R distribution is restricted to a few brain areas (Chen et al., 2001a,b; Saffroy et al., 2003).

Although relatively little is known about the behavioral and pharmacological mechanisms mediated by the NK_3-R, recent results suggest an involvement in processes underlying learning and memory (Siuciak et al., 2007; Zlomuzica et al., 2008) as well as emotionality (Ribeiro et al., 1999; Salome et al., 2006). For instance, promnestic effects of NK_3-R agonism were described in spatial memory tasks in mice (Kameyama et al., 1998; Ukai et al., 1998; Zlomuzica et al., 2008), and disruption of NK_3-R resulted in opposite effects in spatial memory and in avoidance tasks (Siuciak et al., 2007). Moreover, it was shown, that NK_3-R activation has reinforcing properties in a conditioned place preference paradigm (Ciccocioppo et al., 1998). On the other hand, with respect to its involvement in emotionality, current available studies provide contradictory results: In gerbils blockade of the NK_3-R had anxiolytic-like action (Salome et al., 2006), while in mice such an effect was found with NK_3-R agonism (Ribeiro et al., 1999). Similarly, antidepressant-like activity was found with NK_3-R agonists in mice (Panocka et al., 2001), but with NK_3-R antagonists in rats (Dableh et al., 2005).

With age, humans show a decline in memory functions, which can vary from mild impairment to debilitating cases of Alzheimer's disease. Individuals with mild cognitive impairment (MCI) are at risk of developing Alzheimer's disease (Arnaiz and Almkvist, 2003). The aged rat (18–24 months old rats) has shown to be a very useful model of the aged human (Ingram et al., 1994; Gallagher and Pelleymounter, 1988; Gallagher et al., 2003; LaSarge et al., 2007). In aged rats, degeneration of cholinergic cells of the basal forebrain has been associated with learning/memory impairments (Fischer et al., 1989; Hellweg et al., 1990; Martinez-Serrano et al., 1995). A similar relationship between cholinergic degeneration and cognitive impairment is also seen in patients affected with Alzheimer's disease (Patel and Tariot, 1991; Van den Berg et al., 2000).

Acute local application of NKB into the nucleus basalis magnocellulatis (NBM) prevented the decline in cortical ChAT activity associated with injection of NMDA into the NBM, attenuated a reference memory deficit in the radial maze produced by entorhinal cortex lesions (Wenk et al., 1997), and reversed effects of Aβ toxicity, which appears to be the main constituent of amyloid plaques, a hallmark of Alzheimer's disease (Mantha et al., 2006). These results support the hypothesis of potential beneficial function of the NK_3-R in the aging brain. Besides, NK_3-R are localized on basal forebrain cholinergic neurons (Chen et al., 2001a). Senktide, which was shown to be a highly potent NK_3-R agonist in rats, mice, gerbils and guinea pigs (Massi et al., 2000), increased striatal ACh when infused into the striatum (Steinberg et al., 1995), and hippocampal ACh when applied into the medial septum (Marco et al., 1998).

The present study aimed to assess the effects of the NK_3-R agonist senktide on learning and memory as well as emotionality and cholinergic neurotransmission in the aged rat. Since episodic memory loss is the main clinical characteristic of cognitive decline in MCI, dementia and Alzheimer's disease (Belleville et al., 2008; Sperling et al., 2010), aged rats were submitted to an episodic-like memory task, the first test of its kind in aged rats, since appropriate tasks have only recently been developed (Dere et al., 2007; Kart-Teke et al., 2006). The open-field and forced-swimming tests were used to ascertain effects on anxiety- and antidepressant-like behaviors, respectively. Changes in cholinergic neurotransmission upon s.c. senktide administration were assessed in the hippocampus, amygdala and frontal cortex – brain regions which are implicated in emotionality and processes of learning and memory – by *in vivo* microdialysis with HPLC-ECD.

2. Experimental procedure

2.1. Subjects

Experimentally naïve aged male Wistar rats at 23–25 month of age (weight 470–800 g) from the breeding colony of the University of Düsseldorf were used for all experiments. Animals were housed in translucent plastic cages (60.0×20.0×38.0 cm in height) under controlled laboratory conditions (temperature: 20±2 °C) with free access to food and water under an artificial reversed 12:12 light–dark cycle (light off at 07:00 a.m.). Experiments were performed during the animal's active period between 08.00 a.m. and 5.00 p.m. Thirty-nine animals were used for the open-field and episodic-like memory test, 46 animals for the forced swimming test, and 17 animals for the neurochemical study. They were housed in groups of two or three animals per cage. Animals for the neurochemical study were housed individually after surgery. The animals were allowed to adjust to the housing conditions for 2 weeks and were handled daily for 5 days preceding the experiments. All rats were weighed once a week and health status (food and drinking behavior, coat condition, and body's orifices) was controlled daily. All experiments were carried out according to the German Law of Animal Protection of 1998.

2.2. Drug administration

The NK_3-R agonist, senktide ([succinyl-Asp^6-Me-Phe^8]SP_{6-11}; Bachem, USA), was diluted with 5% dimethylsulphoxide in phosphate-buffered saline. The vehicle, 5% dimethylsulphoxide in phosphate-buffered saline, served as control. Animals were randomly assigned to either vehicle, 0.2 mg/kg or 0.4 mg/kg senktide (De Souza Silva et al., 2006; Jocham et al., 2007; Zlomuzica et al., 2008) and injected subcutaneously (s.c.) in the back of the neck 30 min prior to behavioral testing. The injection volume was 1 ml/kg of body weight.

2.3. Open field test

The open field used was a square arena (60×60 cm) with grey acrylic walls (30 cm high) and open roof, located in a sound-attenuating room with masking noise (60 dB). The arena was illuminated by four 40 W light bulbs that provided a light density of approximately 13 lx at the centre and 6 lx in the corners of the field. A video camera (SSC-M388CE; Sony), was mounted 1.6 m above the arena to record the experiment on video tapes for post-hoc analysis.

Animals were individually placed into the center of the open field and allowed to explore it for 15 min. The arena was cleaned with 60%