Neurobiology of Learning and Memory 140 (2017) 145–153

Contents lists available at ScienceDirect

Neurobiology of Learning and Memory

journal homepage: www.elsevier.com/locate/ynlme

The novel dehydroepiandrosterone (DHEA) derivative BNN27 counteracts delay-dependent and scopolamine-induced recognition memory deficits in rats

Nikolaos Pitsikas [a,*], Achille Gravanis [b]

[a] Department of Pharmacology, Faculty of Medicine, School of Health Sciences, University of Thessaly, Larissa, Greece
[b] Department of Pharmacology, School of Medicine, University of Crete, and Institute of Molecular Biology & Biotechnology, Foundation of Research & Technology-Hellas, Heraklion, Greece

ARTICLE INFO

Article history:
Received 28 October 2016
Revised 28 February 2017
Accepted 3 March 2017
Available online 6 March 2017

Keywords:
BNN27
Scopolamine
Recognition memory
Rat

ABSTRACT

Experimental evidence indicates that the neurosteroids dehydroepiandrosterone (DHEA) and dehydroepiandrosterone sulphate (DHEAS) are involved in cognition. BNN27 is a novel 17C spiroepoxy-DHEA derivative, which devoid of steroidogenic activity. The neuroprotective effects of BNN27 have been recently reported. The present study was designed to investigate the effects of BNN27 on recognition memory in rats. For this purpose, the novel object task (NOT), a procedure assessing non-spatial recognition memory and the novel location task (NLT), a procedure evaluating spatial recognition memory were used. Intraperitoneal (i.p.) administration of BNN27 (3 and 10 mg/kg) antagonized delay-dependent deficits in the NOT in the normal rat, suggesting that this DHEA derivative affected acquisition, storage and retrieval of information. In addition, BNN27 (3 and 10 mg/kg, i.p.) counteracted the scopolamine [0.2 mg/kg, subcutaneously (s.c.)]-induced non-spatial and spatial recognition memory deficits. These findings suggest that BNN27 may modulate different aspects of recognition memory, potentially interacting with the cholinergic system, relevant to cognition.

1. Introduction

Steroid hormones exert important functions in the control of growth, maturation and differentiation of the central and peripheral nervous system. Dehydroepiandrosterone (DHEA) and dehydroepiandrosterone sulphate (DHEAS) were the first steroids identified to be produced in large concentrations in the rat brain (Robel et al., 1987). DHEA and DHEAS were both synthesized by both neurons and glia, accumulated in the nervous system (Guazzo, Kirpatrick, Goodyer, Shiers, & Herbert, 1996).

It is now well established that DHEA and DHEAS play interesting roles in the central nervous system (Paul & Pardy, 1992). Specifically, these neurosteroids were found to increase the effects of the excitatory neurotransmitter glutamate (Bergeron, deMontigny, & Debonnel, 1996), to decrease the effects the inhibitory neurotransmitter γ-aminobutyric acid (GABA) (Majewska, 1992) and to stimulate acetylcholine (Ach) release in hippocampus (Rhodes, Li, Flood, & Johnson, 1996). DHEA seems to exert a strong neuroprotective effect via its binding and activation to both tyrosine kinase (Trk), and to pan-neurotrophin p75 ($p75^{NTR}$) (Charalampopoulos et al., 2004; Lazaridis et al., 2011; Pediaditakis et al., 2015). Nerve growth factor (NGF) was described as one of the first neurotrophins and its implication in neural plasticity and cognition are pivotal (Bothwell, 2014). Interestingly, the involvement of DHEA and DHEAS in learning and memory processes in rodents has been revealed in different behavioural paradigms. In particular, the beneficial effects of DHEA and DHEAS on rodents' memory abilities were observed in the T-maze paradigm (Flood, Smith, & Roberts, 1988; Roberts, Bologa, Flood, & Smith, 1987; Flood & Roberts, 1988); in the Morris water maze and the

Abbreviations: Ach, acetylcholine; ANOVA, analysis of variance; BBB, blood brain barrier; BNN27, [(R)-3β,21-dihydroxy-17R,20-epoxy-5-pregnene]; D, discrimination index; DHEA, dehydroepiandrosterone; DHEAS, dehydroepiandrosterone sulphate; F, familiar; FL, familiar location; GABA, γ-aminobutyric acid; i.p., intraperitoneally; ITI, intertrial interval; LTP, long-term potentiation; N, novel; NGF, nerve growth factor; NL, novel location; NLT, novel location task; NOT, novel object task; n.s., not significant $p75^{NTR}$ pan-neurotrophin p75; s.c., subcutaneously; T1, sample trial; T2, choice trial; Trk, tyrosine kinase membrane receptor.

* Corresponding author at: Department of Pharmacology, Faculty of Medicine, School of Health Sciences, University of Thessaly, Panepistimiou 3 (Biopolis), 41500 Larissa, Greece.

E-mail address: npitsikas@med.uth.gr (N. Pitsikas).

http://dx.doi.org/10.1016/j.nlm.2017.03.004
1074-7427/

Y-maze tasks (Frye & Sturgis, 1995; Maurice, Junien, & Privat, 1997) and in the passive avoidance test (Maurice et al., 1997).

BNN27 has recently been shown to specifically interact and activate only the TrkA receptor of NGF, in contrast to DHEA which binds to all vertebrate and invertebrate Trk receptors (Pediaditakis et al., 2015, 2016). Unlike to DHEA, BNN27 does not possess steroidogenic activity (Calogeropoulou et al., 2009). BNN27, is a small highly lipophilic molecule and it can cross the blood-brain-barrier (BBB) **(**Bennett, O'Brien, & Brohawn, 2016**).** BNN27 appears to be non toxic, and displays significant affinity and differential signalling on NGF TrkA receptors, exerting strong antiapopototic and neuroprotective effects (Calogeropoulou et al., 2009; Pediaditakis et al., 2016).

Recognition memory stems from a series of neural processes by which a subject becomes aware that a stimulus has been previously experienced, with recognition as the behavioural outcome of these processes. This type of memory requires that the perceived characteristics of the events are discriminated, identified, and compared with the memory of the characteristics of previously experienced events (Steckler, Drinkenburg, Sahgal, & Aggleton, 1998). Importantly, recognition memory is a type of memory that is impaired in Alzheimer's disease patients (Small, Molby, Laukka, Jones, & Backman, 2003). At the moment, it is not clear if and how BNN27 affects different stages of recognition memory formation (acquisition, storage and retrieval of information).

The integrity of the cholinergic system is critical for memory processing (Everitt & Robbins, 1997) and this system is known to degenerate during aging (Bartus, Dear, Beer, & Lippa, 1982). There are several lines of evidence suggesting that DHEA effects on memory are modulated by the cholinergic system. Specifically, in studies performed in rodents, DHEA enhanced, in a dose-dependent manner, Ach hippocampal release (Rhodes et al., 1996) and reversed memory deficits produced by the muscarinic cholinergic receptor antagonist scopolamine (Flood et al., 1988; Urani, Privat, & Maurice, 1998). In this context, the detrimental effect exerted by scopolamine on rats' recognition memory has been observed (Pitsikas et al., 2001).

Taken the above evidences into account, the first aim of our study was to examine the effects exerted by BNN27 on the aforementioned different mnemonic components (acquisition, storage and retrieval of information). Subsequently, the ability of this novel DHEA analogue to counteract disruption of recognition memory induced by scopolamine was also tested. For these studies, the novel object task (NOT) (Ennaceur & Delacour, 1988) and the novel location task (NLT) (Ennaceur, Neave, & Aggleton, 1997) were used. These behavioural procedures assess non-spatial and spatial recognition memory, respectively, in rodents.

2. Material and methods

2.1. Animals

Independent groups of naive male 3-month-old Wistar rats (Hellenic Pasteur Institute, Athens, Greece), weighing 250–300 g, were used in each of the described experiments. The animals were housed in Makrolon cages (47.5 cm length × 20.5 cm height × 27 cm width), three per cage, in a climate-regulated environment (21 ± 1 °C; 50–55% relative humidity) under a 12 h/12 h (lights on at 7:00 AM) light/dark cycle with free access to food and water.

The procedures that involved animals and their care were conducted in conformity with international guidelines and national and international laws and policies (EEC Council Directive 86/609, JL 358, 1, December 12, 1987; *Guide for Care and Use of Laboratory Animals*, NIH publication no. 85-23, 1985).

2.2. Novel object task (NOT)

The test apparatus consisted of a dark open box made of Plexiglas (80 cm length × 50 cm height × 60 cm width) that was illuminated by a 60 W light suspended 60 cm above the box. The light intensity was equal in the different parts of the apparatus. The objects to be discriminated (in triplicate) were made of glass, plastic, or metal, and had three different shapes: (i.e., metallic cubes, glass pyramids, and plastic cylinders, 7 cm high) and could not be moved by the rats.

The NOT was performed as described previously (Boultadakis & Pitsikas, 2010; Ennaceur & Delacour, 1988). Briefly, during the week before the test, the animals were handled twice per day for 3 consecutive days. Before testing, the rats were allowed to explore the apparatus for 2 min on 3 consecutive days. During testing, a session that consisted of two 2-min trials was conducted. During the "sample" trial (T1), two identical samples (objects) were placed in two opposite corners of the apparatus in a random fashion, 10 cm from the side walls. A rat was placed in the middle of the apparatus and allowed to explore the two identical objects. After T1, the rat was returned to its home cage, and an intertrial interval (ITI) followed. Subsequently, the "choice" trial (T2) was performed. During T2, a novel object replaced one of the objects that was presented during T1. Accordingly, the rats were re-exposed to two objects: a copy of the familiar (F) object and the novel (N) object. All combinations and locations of the objects were counterbalanced to reduce potential bias caused by preference for particular locations or objects. To avoid the presence of olfactory cues, the apparatus and objects were thoroughly cleaned with 20% ethanol after each trial and then wiped with dry paper.

Exploration was defined as the followings: directing the nose towards the object at a distance of 2 cm or less and/or touching the object with the nose. Turning around or sitting on the object was not considered exploratory behaviour. The time spent by the rats exploring each object during T1 and T2 was manually recorded with a stopwatch. Based on this measure, a series of variables was then calculated: the total time spent exploring the two identical objects in T1 and the time spent exploring the two different objects, F and N in T2. The discrimination between the F and N objects during T2 was measured by comparing the time spent exploring the familiar object with the time spent exploring the novel object. Because this time may be biased by differences in the overall level of exploration (Cavoy & Delacour, 1993), we used a discrimination index (D) to represent the preference for novel objects as opposed to familiar objects, calculated as D = (N − F/N + F) (Cavoy & Delacour, 1993).

2.3. Novel location task (NLT)

The test apparatus was the same apparatus as the one used in the NOT. The test arena was located in a large observation room with external cues (large and distinctive objects) that surrounded the experimental box to help rats complete the spatial memory task. These cues were kept in a constant location throughout the testing period. The objects were the same objects as in the object recognition task.

The NLT was performed as described elsewhere (Ennaceur et al., 1997; Pitsikas, 2007). Briefly, during the week before the test, the animals were handled twice daily for 3 consecutive days. Before testing, the rats were allowed to explore the apparatus for 2 min on 3 consecutive days. During testing, a session that consisted of two 2-min trials was conducted. During the "sample" trial (T1), two identical samples (objects) were placed in two opposite corners of the apparatus in a random fashion, 10 cm form the side wall. A rat was placed in the middle of the apparatus and allowed to explore these two identical objects. After T1, the rat was