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The action of neuropeptide AF on passive avoidance learning. Involvement of neurotransmitters

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ABSTRACT

Neuropeptide AF (NPAF) is an amidated octadecapeptide, which is member of the RFamide peptide family. NPAF is encoded by the farp-1 gene and acts through the G protein coupled NPFF-1 and NPFF-2 receptors. NPAF is involved in several physiological functions of the central nervous system, however we have little evidence about the involvement of NPAF in learning and memory. Therefore, the aim of the present study was to investigate the action of NPAF on consolidation of memory in a passive avoidance learning paradigm in mice. We have also investigated the underlying neurotransmissions and the action of NPAF on β-amyloid-induced memory impairment. Accordingly, mice were pretreated with a nonselective muscarinic acetylcholine receptor antagonist, atropine, a non-selective 5-HT2 serotonergic receptor antagonist, cyproheptadine, a mixed 5-HT1/5-HT2 serotonergic receptor antagonist, methysergide, a D2, D3, D4 dopamine receptor antagonist, haloperidol, a non-selective opioid receptor antagonist, naloxone, a nitric oxide synthase inhibitor, nitro-L-arginine, a $\alpha_1/\alpha_{2\beta}$-adrenergic receptor antagonist, prazosin, a nonselective β-adrenergic receptor antagonist, propranolol or β-amyloid 25–35 in combination with NPAF administration. Our results demonstrate for the first time that NPAF improves the consolidation of passive avoidance learning. This effect is mediated through muscarinic cholinergic, 5HT1- and 5HT2-serotoninergic, dopaminergic, nitrergic and α- and β-adrenergic neurotransmissions, but not by opioid transmission, since atropine, cyproheptadine, methysergide, haloperidol, nitro-L-arginine, prazosin and propranolol reversed the action of NPAF, whereas naloxone was ineffective. The present study also shows that NPAF reverses the β-amyloid 25–35-induced memory impairment.

1. Introduction

Neuropeptide AF (NPAF, A18F amide, AGEGLSSPFWSLAAPQRFamide) is an amidated octadecapeptide, which was isolated together with neuropeptide FF (NPFF, F8F amide, FLFQPQRFamide) from bovine brain (Yang, Fratta, Majane, & Costa, 1985). Subsequently, another RFamide peptide, the neuropeptide SF (NPSF, SLAAPQRFamide), was isolated from rodent spinal cord (Bonnard et al., 2001). NPAF, NPFF and NPSF signal through two $G_{i/o}$-protein coupled receptors (GPCRs), known as NPFF-2 (GPR74, NPGPR, HLWAR77) and NPFF-1 (GPR147, OT7T022) (Fukusumi, Fujii, & Hinuma, 2006). These peptides play role in several physiological functions, including the regulation of nociception (Kavaliers, 1990; Yang et al., 1985; Yudin, Tamarova, & Krishtal, 2006), insulin and somatostatin release (Fehmann et al., 1990), food and water intake (Newmyer & Cline, 2009; Newmyer, Siegel, & Cline, 2010), adipocyte metabolism (van Harmelen et al., 2010), motility of colon (Raffa & Jacoby, 1989), body temperature (Desprat & Zajac, 1997), blood pressure (Roth, Disimone, Majane, & Yang, 1987), locomotion and releases of CRF, ACTH and corticosterone (Jaszberenyi, Bagosi, Csabafi, Palotai, & Telegdy, 2014; Jaszberenyi et al., 2009), anxiety and depression (Palotai, Telegdy, Tanaka, Bagosi, & Jaszberenyi, 2014). There is a growing base of evidence revealing the involvement of RFamide peptides in learning and memory as well. In 1993, Kavaliers and Colwell (1993) were the first who reported that a lower dose (1 μg) of NPFF improves, whereas a higher dose (10 μg) impairs long-term spatial memory acquisition in Morris water maze test (Kavaliers & Colwell, 1993). In 2010, Betourne et al. demonstrated that the non-selective NPFF receptor agonist 1DMe (D-Tyr1(NMe)Phe3]NPFF) impairs both the short-term retention in object location task and the long-term spatial memory in Morris water maze test (Betourne et al., 2010).

The expression and distribution of RFamide peptides and their receptors have been described particularly in the central nervous

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system (CNS). NPAF and NPFF are proteolytic products of their neuropeptide precursor encoded by the farp-1 gene, whereas NPFF-2 and NPFF-1 receptors are encoded by the frf-3 gene (Fukusumi et al., 2006). NPAF and NPFF are expressed in the hypothalamus and in the nucleus of the solitary tract. Immunoreactive fibers and terminals were found in several brain regions, such as the lateral septum, amygdala, hypothalamus, neurohypophysis, thalamus, periaqueductal gray, and several medullary nuclei (Kivipelto, Majane, Yang, & Panula, 1989). Expression of NPFF-1 and NPFF-2 receptors have been identified in brain sites, which directly or indirectly influences behavior, cognition and memory. In particular, NPFF receptors were identified in the septal nucleus, bed nucleus of stria terminalis (BNST), posteromedial cortical amygdaloid nucleus, parafascicular thalamic nucleus, medial mammillary nucleus, CA3 region of the ventral hippocampus (Bonini et al., 2000) and in distinct cortical areas (Gouarderes, Puget, & Zajac, 2004). Taking into consideration all the behavioral and autoradiographic data, we can assume that the RFamide peptides can be involved in learning and memory processes. Although the effect of NPFF on memory and the distribution of NPFF receptors have been investigated particularly, the action of NPAF on learning and memory remains to be elucidated.

Most neuropeptides are co-expressed with at least one classic neurotransmitter in the CNS. Generally, neuropeptides behave as neuromodulators exerting multiple actions on physiological brain functions and, consequently, on behavior. Their effects involve changes in membrane excitability, gene transcription, receptor affinity and in modulation of neurotransmitter release (Ogren, Kuteeva, Elvander-Tottie, & Hokfelt, 2010). It has been demonstrated that NPFF controls the release of serotonin, glutamate and GABA in the medial prefrontal cortex (mPFC) (Chen, Li, Liang, & Huang, 1999), whereas NPAF stimulates the release of dopamine in the amygdala and the striatum (Jaszberenyi et al., 2009). Another study also revealed that activation of NPFF receptors interferes with dopaminergic, serotoninergic and opioid transmissions (Huang, Li, Wong, Tan, & Chen, 2002). However, the involvement of distinct neurotransmissions in the action of NPAF on memory formation remains to be clarified.

β-amyloid plays a key role in the pathology of Alzheimer's disease, which is the most common form of dementia (Kanekiyo & Bu, 2014). Accordingly, behavioral investigations, using rodent models, revealed the inhibitory action of β-amyloid on memory consolidation (Chen, Wright, & Barnes, 1996; Telegdy, Tanaka, & Schally, 2009). The β-amyloid-induced neurotoxicity has been associated with oxidative stress, apoptosis, impaired mitochondrial function and receptor mediated effects (Bossy-Wetzel, Schwarzenbacher, & Lipton, 2004). A recently published study suggested that kisspeptin – which is also a member of the RFamide peptide family – can prevent the β-amyloid-induced neurotoxicity (Milton, Chilumuri, Rocha-Ferreira, Nercessian, & Ashioti, 2012). However, there is no published data about the action of NPAF on β-amyloid-induced memory impairment.

The aim of the present study was to clarify the involvement of NPAF in learning and memory. Therefore, we investigated the action of NPAF on memory consolidation in a passive avoidance learning paradigm in mice. We also investigated the underlying neurotransmissions and the action of NPAF on the β-amyloid-induced memory impairment. Accordingly, mice were pretreated with a nonselective muscarinic acetylcholine receptor antagonist, atropine, a non-selective 5-HT2 serotonergic receptor antagonist, cyproheptadine, a mixed 5-HT1/5-HT2 serotonergic receptor antagonist, methysergide, a D2, D3, D4 dopamine receptor antagonist, haloperidol, a non-selective opioid receptor antagonist, naloxone, a nitric oxide synthase inhibitor, nitro-L-arginine, a selective α_1-adrenergic receptor antagonist, prazosin, a nonselective β-adrenergic receptor antagonist, propranolol or β-amyloid 25–35 in combination with NPAF administration.

2. Methods and materials

2.1. Experimental animals and ethics statement

Male CFLP mice (Mus musculus, Bioplan Isaszeg, Hungary), weighing 25–28 g were used. The animals were maintained and treated during the experiments in accordance with the instructions of the Ethical Committee for the Protection of Animals in Research of the University of Szeged (Szeged, Hungary), which specifically approved this study. The mice were kept in their home cages at a constant temperature (23 °C) on a standard illumination schedule with 12-h light and 12-h dark periods (lights on from 6:00 AM). Commercial food and tap water were available ad libitum. To minimize the effects of nonspecific stress, the mice were handled daily. All surgery was performed under anesthesia, and all efforts were made to minimize suffering.

2.2. Surgery

For intracerebroventricular (i.c.v.) administration, the mice were implanted with a 10 mm long stainless steel Luer canulla (prepared from a hypodermic Luer needle of 20 G × 1.5 in., Henke-Sass Wolf, Tuttlingen, Germany) aimed at the right lateral cerebral ventricle under sodium pentobarbital (Nembutal, 35 mg/kg, intraperitoneally, i.p.) anesthesia. The stereotaxic coordinates were 0.2 mm posterior; 0.2 mm lateral to the bregma; 2.0 mm deep from the dural surface. Cannulas were secured to the skull with dental cement and acrylate. The i.c.v. treatment was applied via the cannula in 2 μl bolus injection. Post-operative care was provided in order to ensure the full recovery of the animals. To monitor anesthetic recovery, someone was always present with the animals recovering from anesthesia until the mice were ambulatory. Nursing support was also provided including quiet, darkened resting place, timely wound maintenance, increased ambient warmth, a soft resting surface, rehydration with oral fluids, and a return to normal feeding through the use of highly palatable foods. The mice were used after a recovery period of 5 days.

After the experiments, methylene blue was injected into the lateral ventricle, then the animals were decapitated and the brains were dissected to verify the permeability of the cannulas. Only animals with correctly located cannulas were used for statistical evaluation.

2.3. Treatments

NPAF (purchased from Bachem Inc., Switzerland) was applied via the i.c.v. cannula in a dose of 0.5 or 1.0 or 2.0 μg/animal. For combined treatment, only 1.0 μg NPAF was used. NPAF was administered after the learning trial.

Receptor blockers were applied immediately after the learning trial, followed 30 min later by NPAF administration. The following receptor blockers were used: atropine sulfate (EGYS, Budapest, Hungary), 2 mg/kg i.p.; cyproheptadine hydrochloride (Tocris, Bristol, UK), 5 mg/kg i.p.; methysergide hydrogen maleate (Sandoz, Cologne, Germany), 5 mg/kg i.p.; haloperidol (G. Richter, Budapest, Hungary) 10 μg/kg i.p.; naloxone hydrochloride (Endo Labs, Wilmington, USA), 0.3 mg/kg i.p.; nitro-L-arginine methylester hydrochloride (Sigma–Aldrich Inc., St. Louis, USA), 10 μg/2 μl i.c.v.; prazosin hydrochloride (Tocris, Köln, Germany), 62.5 μg/kg i.p. and propranolol hydrochloride (ICI Ltd., Macclesfield, UK), 5 mg/kg i.p. The doses of the receptor blockers were selected on the basis