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Research report

Transmitter mediation of the anxiolytic action of apelin-13 in male mice

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HIGHLIGHTS

- Apelin-13 is anxiolytic in plus maze in mice.
- The action is mediated by α-β-adrenergic, dopaminergic, and serotonergic mediation.
- Cholinergic, opiate and GABA-A mediation is not involved in this action.

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ABSTRACT

The widespread distribution of apelin-13 and apelin receptors in the brain and periphery suggests an important function of this neuropeptide in regulatory processes in the organism. In previous work we found that apelin-13 facilitates the consolidation of passive avoidance learning in rats. In the present work we demonstrate that apelin-13 exerts anxiolytic action in an elevated plus maze in mice. In order to assess the possible involvement of transmitters in this action, the animals were pretreated with the following receptor blockers in doses which themselves did not influence the behavioral paradigm: atropine (a nonselective muscarinic acetylcholine receptor antagonist), haloperidol (a D2, D3, D4 dopamine receptor antagonist), phenoxybenzamine (a nonselective α1-adrenergic receptor antagonist), methysergide (a nonselective 5-HT2 serotonergic receptor antagonist), propranolol (a β-adrenergic receptor antagonist), naloxone (a nonselective opioid receptor antagonist) and bicuculline (a γ-aminobutyric acid subunit A receptor antagonist. Phenoxybenzamine, haloperidol, propranolol and methysergide prevented the action of apelin-13, whereas atropine, naloxone and bicuculline were ineffective. The data suggest that apelin-13 elicits its anxiolytic action via α-adrenergic, dopaminergic, β-adrenergic and 5-HT2 serotonergic mediation.

1. Introduction

The first apelin, apelin-36 was isolated from bovine stomach extracts as [1]. It is derived from a 77-amino acid precursor, preproapelin, identified in human and bovine tissues [1], which is processed to the molecular forms apelin-36, apelin-26, apelin-19, apelin-17, apelin-13 and apelin-12 in different tissues [2,3]. Apelin-36 is the endogenous ligand of an orphan G protein-coupled receptor, APJ, identified in a human gene by O'Dowd et al. [4].

The synthetic C-terminal 13 to 19-amino acid fragments of preproapelin, exhibit significantly higher activities at the receptors than that of apelin-36 [2,5]. The most extensively studied apelin-13 participates in the regulation of the pituitary–adrenal axis [6,7], fluid homeostasis [[7,8] and the cardiovascular function [8,9]. The apelin receptors and apelin are widely distributed in the central nervous system (CNS) [10–13], suggesting that apelin may be of importance in the regulation of certain CNS functions. We earlier demonstrated that apelin-13 increases the open-field activity, the plasma corticosterone level and the core temperature in male rats [6]. Its influence on learning and memory function is mediated by a number of transmitters [14]. The aim of the present work was to elucidate the action of apelin-13 administered i.c.v. on anxiety in mice. The possible involvement of neurotransmitters *in the anxiolytic action* was studied by pretreating the animals with certain neurotransmitter antagonists.

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2. Methods and materials

2.1. Animals

CFLP male mice weighing 25–28 g, were used. The animals were kept and handled during the experiments in accordance with the Regulation of the Albert Szent–Györgyi Medical University Ethical Committee for the Protection of Animals in Research. Five animals per cage were housed in a light – (lights on at 0600 h and off at 1800 h) and temperature – controlled room (23 °C) and had free access to food and water.

2.2. Surgery

The mice were anesthetized with sodium pentobarbital (Nembutal 35 mg/kg i.p.) and a cannula was introduced into the lateral cerebroventricle and fixed to the skull with dental cement and acrylic resin. The animals were allowed to recover for 5 days. The correct location of the cannula was checked by dissecting the brain following completion of the experiments. Only animals with the correct location of the cannula were used in the evaluation of the experiments. All experiments were performed in the morning period.

2.3. Materials

Different doses of apelin-13 (Bachem, Bubendorf, Switzerland) dissolved in saline, or saline alone (control animals), in a volume of 2 μl, were injected i.c.v. into conscious mice. Apelin-13 in a quantity of 10 μg per ampoule was lyophilized and stored at −20 °C. Immediately before the experiments apelin-13 was dissolved in sterile pyrogen-free 0.9% saline and administered i.c.v. in a volume of 2 μl via a cannula.

2.4. Treatments

For the administration of the apelin-13 (Bachem, Bubendorf, Switzerland) a stainless steel cannula with an external diameter of 0.7 mm was implanted stereotaxically into the right lateral brain ventricle. The peptides were injected i.c.v. via the cannula in a volume of 2 μl. in doses of 0.5, 1 and 2 μg. For the transmitter interaction the only effective dose of apelin-13 (0.5 μg) was selected.

The antagonists of neurotransmitters was given intraperitoneally 30 min before apelin-13 administration. Following receptor blockers were used: atropine sulphate from EGYS (Budapest, Hungary, haloperidol from G. Richter (Budapest, Hungary); phenoxybenzamine hydrochloride was obtained from Smith Kline & French (Herts, UK); propranolol hydrochloride from ICI Ltd., (Macclesfield, UK); naloxone hydrochloride (Endo Labs, Wilmington USA). methysergide hydrogenmaleate was from Sandoz (Basle, Switzerland); bicuculline methiodide from Sandoz (Basle, Switzerland). Experiments were carried out between 8 and 10 am.

2.5. Behaviural testing

2.5.1. Elevated plus-maze test

The elevated plus-maze test was carried out by the method of Pellow et al. [15]

Apelin-13 was administered i.c.v at doses of 0.5, 1, and 2 μg, 30 min before the behavioral testing started. The animals were placed at the center of the maze, facing one of the open arms. During a 5-min test period, the behavior of the animal was recorded by an observer sitting 1 m from the center of the maze. Recordings were made of the times spent in the open and the closed arms and the numbers of entries into the open and the closed arms. Entry into an arm was defined as the entry of all four feet into that arm. These scores were converted into percentage (open/open + closed) values. Total entries into arms provided a measure of the overall activity. Each animal was tested only once in the plus-maze apparatus.

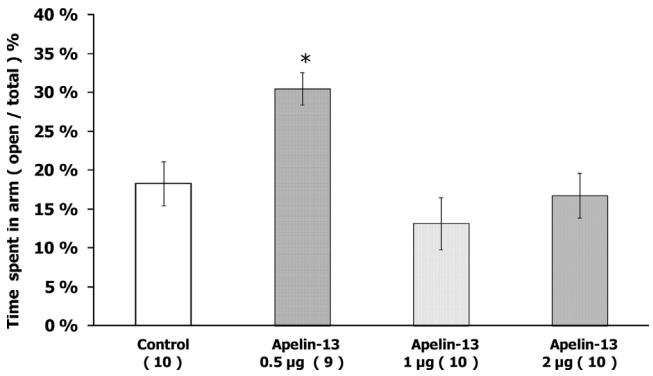

Fig. 1. The action of different doses of apelin-13 on anxiolytic action in elevated plus maze. Data are expressed as means ± S.E.M. Number in brackets are the numbers of animals used. Symbols: * $P < 0.05$ vs. control.

2.6. Statistical analysis

The two-way analysis of variance (ANOVA) test was followed by Tukey's test for multiple comparisons with unequal cell size. Probability values (P) of less than 0.05 are considered significant.

3. Results

In the figures, only the time of entries is presented because the numbers of entries led to the same conclusion. None of the treatments influenced the overall activity of the animals relative to the controls, therefore these results are not shown either.

Apelin-13 tested in different doses for anxiolytic action (0.5, 1.0 and 2.0 μg). 0.5 μg significantly increased the time spent in the open arms in plus maze [$F(3,35) = 6.61$]; $P < 0.001$. For combined testing with antagonist the only effective dose of apelin-13 (0.5 μg) was used (Fig. 1).

Apelin-13 in combination with atropine, a nonselective muscarinic acetylcholine receptor antagonist, the apelin-13 in a dose of 0.5 μg i.c.v. significantly increased the time spent in the open arms in the plus maze [$F(3,35) = 5.42$]; $P < 0.004$. Atropine (2 mg/kg i.p.) alone had no action on the test, but could not block the anxiolytic action of apelin-13 (Fig. 2).

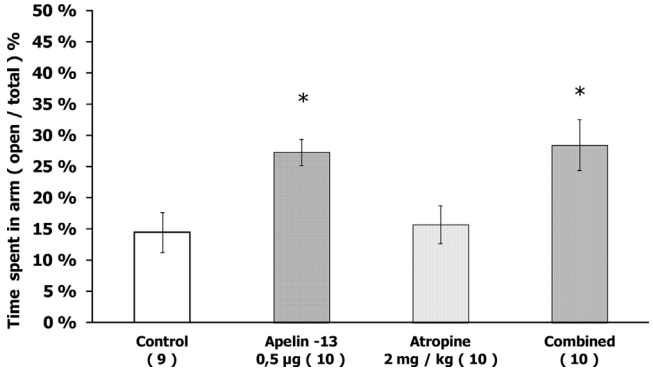

Fig. 2. The effect of a nonselective muscarinic acetylcholine receptor antagonist, atropine (2 mg/kg i.p.) on apelin-13-induced anxiolytic action in elevated plus maze. Data are expressed as means ± S.E.M. Number in brackets are the numbers of animals used. Symbols: * $P < 0.05$ vs. control.