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Anxiolytic effect of the GPR103 receptor agonist peptide P550 (homolog of neuropeptide 26RFa) in mice. Involvement of neurotransmitters

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ABSTRACT

The GPR103 receptor is a G protein-coupled receptor, which plays a role in several physiological functions. However, the role of the GPR103 receptor in anxiety has not been clarified. The first aim of our study was to elucidate the involvement of the GPR103 receptor in anxious behavior. Mice were treated with peptide P550, which is the mouse homolog of neuropeptide 26RFa and has similar activity for the GPR103 receptor as neuropeptide 26RFa. The anxious behavior was investigated using an elevated plus-maze paradigm. The second aim of our study was to investigate the underlying neurotransmissions. Accordingly, mice were pretreated with a nonselective muscarinic acetylcholine receptor antagonist, atropine, a γ-aminobutyric acid subunit A ($GABA_A$) receptor antagonist, bicuculline, a non-selective 5-HT_2 serotonergic receptor antagonist, cyproheptadine, a mixed 5-HT_1/5-HT_2 serotonergic receptor antagonist, methysergide, a D_2, D_3, D_4 dopamine receptor antagonist, haloperidol, a nonselective α-adrenergic receptor antagonist, phenoxybenzamine and a nonselective β-adrenergic receptor antagonist, propranolol. Our results demonstrated that peptide P550 reduces anxious behavior in elevated plus maze test in mice. Our study shows also that $GABA_A$-ergic, α- and β-adrenergic transmissions are all involved in this action, whereas 5-HT_1 and 5-HT_2 serotonergic, muscarinic cholinergic and D_2, D_3, D_4 dopaminergic mechanisms may not be implicated.

1. Introduction

The RFamide peptides are neuropeptides with a Arg-Phe-NH_2 motif at their C-termini, which seem to act as neurotransmitters and neuromodulators [8,41]. The first RFamide peptide was identified in 1977 [29]. In the past 30 years, a broad diversity of RFamide peptides was discovered. Reverse pharmacology brought the breakthrough in the research of RFamide peptides by matching the "orphan" G protein-coupled receptors (GPCRs) to their natural ligands. These discoveries had a prominent impact on our understanding of neuromodulation and brain functions [6,8,41].

To date, the RFamide peptide family includes five peptide groups, namely the neuropeptide FF (NPFF) group, the prolactin-releasing peptide (PrRP) group, the gonadotropin-inhibitory hormone (GnIH) group, the kisspeptin group, and the 26RFa (also known as 26RFa/QRFP) group. The most recently discovered members of the RFamide peptide family are the 43RFa (also known as pyroglutamylated RFamide peptide (QRFP) or QRFP-43) and the 26RFa (also known as P518, QRFP or QRFP-26), which consist of 43 and 26 amino acids, respectively. (Previous studies referred to both peptides using the QRFP term [5,34]. To be more specific, we will use the 43RFa and the 26RFa terms.) Both peptides belong to the 26RFa group and have been identified as the endogenous ligands of the GPR103 (also known as AQ27 or SP9155 or QRFP) receptor [5,41–43].

The GPR103 is a "deorphanized" $G\alpha_{i/o}$ and $G\alpha_q$ protein-coupled receptor that has a single encoding gene in humans, whereas there are two isoforms, designated as $GPR103_A$ and $GPR103_B$ in rodents [5,8,41,42]. The 43RFa and the 26RFa bind to both isoforms with nearly similar affinity [5,41]. GPR103 expression was detected in several brain regions, including the paraventricular and magnocellular hypothalamic nuclei, striatum, bed nucleus of stria terminalis (BNST), lateral septum (LS), medial supramammillary nucleus, olfactory bulb as well as in the brainstem in rodents [3,34]. The GPR103/43RFa/26RFa system plays a role in

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the regulation of several physiological functions, including bone formation [2]; adipogenesis [23]; food intake and energy homeostasis [10,18,20,22,34]; aldosterone [9], gonadotropin [24,27], prolactin [44] and insulin secretion [12,22]; nociceptive transmission [46,47]; blood pressure and locomotor activity [34].

The first aim of our study was to clarify the involvement of the GPR103 receptor in mouse-specific anxious behavior, hereafter referred to as anxiety. Therefore, we treated mice with the synthetic GPR103 receptor agonist peptide P550 (also known as orphan GPCR SP9155 agonist peptide P550, which is the mouse homolog of 26RFa [16]) and the anxious behavior was investigated using an elevated plus-maze test. The second aim of this study was to investigate the underlying neurotransmissions. Accordingly, mice were pretreated with a nonselective muscarinic acetylcholine receptor antagonist, atropine, a γ-aminobutyric acid subunit A ($GABA_A$) receptor antagonist, bicuculline, a non-selective 5-HT_2 serotonergic receptor antagonist, cyproheptadine, a mixed 5-HT_1/5-HT_2 serotonergic receptor antagonist, methysergide, a D_2, D_3, D_4 dopamine receptor antagonist, haloperidol, a nonselective α-adrenergic receptor antagonist, phenoxybenzamine and a nonselective β-adrenergic receptor antagonist, propranolol.

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 is based on the EU Directive 2010/63/EU and 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 stainless steel Luer cannula 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. Cannulae were secured to the skull with dental cement and acrylate. The mice were used after a recovery period of 5 days.

2.3. Chemicals

The P550 was obtained from Bachem Inc. (Bubendorf, Switzerland); atropine sulfate, from EGIS Pharmaceutical Plc (Budapest, Hungary); bicuculline, from Sandoz (Basel, Switzerland); cyproheptadine hydrochloride, from Tocris Bioscience (Bristol, UK); methysergide hydrogen maleate, from Sandoz (Basel, Switzerland); haloperidol, from Gedeon Richter Plc (Budapest, Hungary); phenoxybenzamine hydrochloride, from Smith Kline & French (Herts, UK) and propranolol hydrochloride, from Imperial Chemical Industries Ltd. (Macclesfield, UK).

2.4. Treatments

All the experiments were performed in the morning. The P550 in a quantity of 10 μg per ampoule was lyophilized and stored at −20 °C. Immediately before the experiments, P550 was dissolved in sterile pyrogen-free 0.9% saline and administered i.c.v. in a volume of 2 μl via the cannula. The control animals received only saline (2 μl i.c.v.). First, different doses of P550 (0.25, 0.5, 1.0, 2.0 μg/2 μl) were used to establish the dose-response effect of P550 on time spent in open arm (open/total) (Fig. 1). Then only the most effective dose of P550 (1.0 μg) was used in combination with the receptor antagonists. The effective doses of the receptor antagonists were selected on the basis of previous experience where the minimal doses were effective (in other tests), but did not themselves influence the tests [36,39]. The receptor antagonists were dissolved in 0.9% saline and were injected i.p. 30 min before the i.c.v. administration of P550 or saline. The tests started 30 min following the i.c.v. administration.

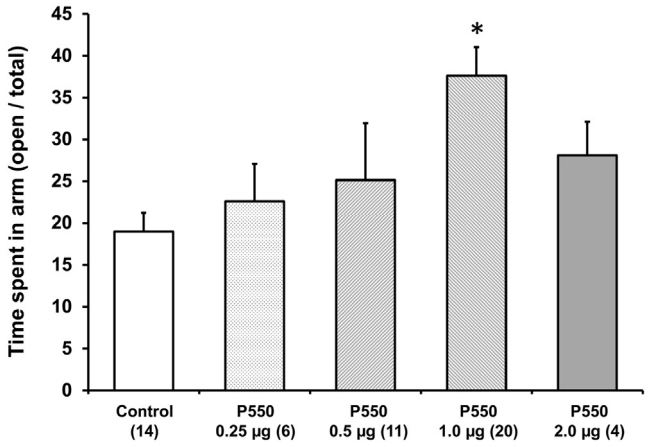

Fig. 1. The anxiolytic action of P550 in elevated plus maze. P550 (0.25 μg/2 μl, i.c.v.), P550 (0.5 μg/2 μl, i.c.v.), P550 (1.0 μg/2 μl, i.c.v.) *$p < 0.05$ vs. control, P550 (2.0 μg/2 μl, i.c.v.). Data are expressed as means ± S.E.M. Numbers in brackets denote the numbers of animals used.

2.5. Behavioral testing

2.5.1. Elevated plus maze test

The idea of the elevated plus maze (EPM) test [28] is that open arms are more fear provoking and that the ratio of the times spent in open versus closed arms or the ratio of the entries into open versus closed arms reflects the relative 'safety' of closed arms, as compared with the relative danger of open arms. The wooden maze consisted of two open (25 × 5 cm) and two closed (25 × 5 × 20 cm) arms, which were connected by a 5 × 5 cm central square and elevated to a height of 50 cm above the floor. Arms were angled at 90° to each other and the same types of arms were positioned opposite to each other. The animals were placed individually at the center of the maze, facing one of the closed 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 of the numbers of entries into the open and closed arms. Entry into an arm was defined as the entry of all four feet into that arm. These scores were converted into percentages (open/total). Total number of entries into arms provided a measure of overall activity. Each animal was tested only once in the plus maze apparatus.

2.6. Statistical analysis

Statistical analysis of the behavioral testing was performed by analysis of variance (ANOVA), which was followed by Tukey's post hoc comparison test. Only the mean percentages were plotted and the standard error of the mean (SEM) is given in the figure captions.