Behavioural Brain Research 209 (2010) 274–280

Contents lists available at ScienceDirect

Behavioural Brain Research

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

Research report

Environmental factors during early developmental period influence psychobehavioral abnormalities in adult PACAP-deficient mice

Toshihiro Ishihama[a,1], Yukio Ago[a,1], Norihito Shintani[b], Hitoshi Hashimoto[b,c,d], Akemichi Baba[b], Kazuhiro Takuma[a], Toshio Matsuda[a,c,d,*]

[a] Laboratory of Medicinal Pharmacology, Graduate School of Pharmaceutical Sciences, Osaka University, 1-6 Yamada-oka, Suita, Osaka 565-0871, Japan
[b] Laboratory of Molecular Neuropharmacology, Graduate School of Pharmaceutical Sciences, Osaka University, 1-6 Yamada-oka, Suita, Osaka 565-0871, Japan
[c] Department of Experimental Disease Model, The Osaka–Hamamatsu Joint Research Center For Child Mental Development, Graduate School of Medicine, Osaka University, 2-2 Yamada-oka, Suita Osaka, 565-0871, Japan
[d] United Graduate School of Child Development, Osaka University, Kanazawa University and Hamamatsu University School of Medicine, Osaka University, 2-2 Yamada-oka, Suita Osaka, 565-0871, Japan

ARTICLE INFO

Article history:
Received 16 December 2009
Received in revised form 30 January 2010
Accepted 1 February 2010
Available online 6 February 2010

Keywords:
PACAP
Gene-environment interaction
Social isolation
Enriched environment
Psychomotor abnormalities
Age-dependency

ABSTRACT

Mice lacking the neuropeptide pituitary adenylate cyclase-activating polypeptide (PACAP) (PACAP$^{-/-}$) display behavioral abnormalities, and genetic variants of the genes encoding PACAP are associated with schizophrenia. Clinical studies show that environmental factors, besides genetic factors, play a key role in etiology of many psychiatric disorders. This study examined the effects of environmental factors such as short-term social isolation and an enriched environment on behavioral abnormalities of PACAP$^{-/-}$ mice. Rearing in isolation for 2-weeks from 4-weeks old induced hyperlocomotion and aggressive behaviors in the PACAP$^{-/-}$ mice without affecting the behavioral performance of the wild-type controls. Adult PACAP$^{-/-}$ mice showed not only hyperactivity, jumping behavior, and depression-like behavior, but also decreased social interaction. These abnormal behaviors were improved by rearing for 4-weeks in an early enriched environment (from 4-weeks old), although the deficits of prepulse inhibition (PPI) were not influenced by the enriched condition. In contrast, rearing for 4-weeks in late enriched environment (from 8-weeks old) did not affect the hyperactivity and jumping behaviors in the PACAP$^{-/-}$ mice. These results suggest that abnormal behaviors except PPI deficits in PACAP$^{-/-}$ mice depend on the environmental factors during the early stages of development.

1. Introduction

Pituitary adenylate cyclase-activating polypeptide (PACAP) belongs to the vasoactive intestinal peptide (VIP)/secretin/glucagon superfamily and widely distributed in the brain as well as peripheral organs [41]. In addition, the PACAP-specific receptor (PAC1) is predominantly expressed in the brain, especially in the neocortex, the limbic system and brain stem [13]. Thus, PACAP is thought to exert a wide range of biological effects as a neurotransmitter, neuromodulator and/or neurotrophic factors [41]. Mice lacking the *Adcyap1* gene encoding the PACAP (PACAP$^{-/-}$) display remarkable behavioral abnormalities, including hyperlocomotion and jumping behavior in a novel environment, increased novelty-seeking behavior, deficits in prepulse inhibition (PPI) and depression-like behavior, and these abnormal behaviors are ameliorated by antipsychotic drugs [11,12,14,37]. Furthermore, a recent genetic analysis revealed that genetic variants of the genes encoding PACAP and its selective receptor, PAC1, are associated with schizophrenia [14]. These findings suggest that alterations in PACAP signaling might contribute to the pathogenesis of schizophrenia and its related disorders.

Besides genetic factors, the contribution of environmental conditions experienced early in life to the onset and course of psychiatric disorders are increasingly recognized. Especially, experiences during a critical period of brain development are considered to alter maturation of the brain [16,30], and then influence behavior for a lifetime, which could be attributable to the etiology of psychiatric disorders [6,10]. In this line, rodent studies have demonstrated the influence of the environment in early life on psychological behaviors in adulthood. Environmental stress, such as maternal separation or social isolation during development, can induce a variety of behavioral abnormalities including

* Corresponding author at: Laboratory of Medicinal Pharmacology, Graduate School of Pharmaceutical Sciences, Osaka University, 1-6 Yamada-oka, Suita, Osaka 565-0871, Japan. Tel.: +81 6 6879 8161; fax: +81 6 6879 8159.
E-mail address: matsuda@phs.osaka-u.ac.jp (T. Matsuda).
[1] These authors contributed equally to this work.

0166-4328/$ – see front matter © 2010 Elsevier B.V. All rights reserved.
doi:10.1016/j.bbr.2010.02.009

increased aggressiveness, anxiety-related behaviors and hyperlocomotion [6,42–44]. Furthermore, rearing animals in larger and more complex environment, "enriched environment", influences brain structure and function, and improves or delays behavioral impairments in animal models of CNS disorders such as Huntington's disease, Alzheimer's disease, Parkinson's disease, and amyotrophic lateral sclerosis [21,27,35]. Gene-environment interactions may play a key role in the pathogenesis of CNS disorders. However, it is not known whether environmental factors may modify or interact with the expression of behavioral abnormalities of PACAP$^{-/-}$ mice.

The present study was designed to investigate the role of early environmental factors in the expression of abnormal behaviors of genetically engineered PACAP$^{-/-}$ mice. First, we examined whether the expression of abnormal behaviors of PACAP$^{-/-}$ mice is age-dependent, and then the effects of two developmental environmental conditions (short-term social isolation and enriched environment) in early life on their behaviors. The effect of an enriched environment on adolescent mice was also compared with that on adult mice to clarify any age-dependency of the effect.

2. Materials and methods

2.1. Animals

Homozygous PACAP-null mutant mice were generated and characterized as described previously [12]. The null mutation mice were backcrossed for more than 10 generations onto CD1 (ICR) mice, and then male PACAP$^{+/+}$ (wild-type) and PACAP$^{-/-}$ mice used were obtained from the intercross of the heterozygous animals. Genotypes of all mice were identified by PCR analysis of DNA isolated from tail biopsies. The wild-type and mutant mice were housed separately under a standard 12-h light/dark cycle (lights on at 8:00 a.m.) at a constant temperature of 22 ± 1 °C with free access to food and water throughout the experiments. All mouse studies were approved by the Animal Care and Use Committee of Graduate School of Pharmaceutical Sciences, Osaka University, the Guiding Principles for the Care and follow the United States National Institutes of Health Guide for the Care and Use of Laboratory Animals. In this study, there was no significant difference in body weight between the wild-type and PACAP$^{-/-}$ mice [4-weeks old, 23.4 ± 0.8 (wild-type) and 21.7 ± 0.5 g (PACAP$^{-/-}$); 6-weeks old, 33.9 ± 0.6 (wild-type) and 33.3 ± 0.4 g (PACAP$^{-/-}$); 8-weeks old, 37.0 ± 1.0 (wild-type) and 34.4 ± 0.6 g (PACAP$^{-/-}$); 12-weeks old, 40.0 ± 0.6 (wild-type) and 38.2 ± 1.1 g (PACAP$^{-/-}$): expressed as means ± SEM (n = 6–9), Student's t-test].

2.2. Rearing conditions

For social isolation experiments, mice at 4-weeks old were divided into two groups. The mice for the isolation group were individually housed for 2-weeks in wire-topped opaque polypropylene cages (28 cm × 17 cm × 12 cm), while the control group continued to be housed under normal group-housed conditions (five to six per cage) in wire-topped clear plastic cages (28 cm × 17 cm × 12 cm). For the environmental enrichment experiments, mice at 4 or 8-weeks old were divided into two groups. The mice for the enriched environment group were housed for 4-weeks in large sized acrylic boxes (65 cm × 35 cm × 30 cm) (five to six per cage), containing two running wheels (15.2 cm in diameter), two nesting materials (Shepherd Shack®; Shepherd Specialty Papers, Watertown, TN) and a variety of objects, including toys, tunnels, and hiding places [3,29] (Supplementary Fig. 1). The arrangement of these objects was changed twice per week, taking care to make the environment novel each time for the mice. The control mice were housed for the same period under group-housed conditions. Unless otherwise specified, behavioral and biochemical tests were carried out immediately after rearing under the conditions indicated.

2.3. Open-field test

The open-field test was carried out according to the method previously reported [37]. Each mouse was placed in the center of an open cubic transparent acrylic box with a black Plexiglas floor (45 cm × 45 cm × 30 cm), and allowed to freely explore the environment for 60 min under an illumination of 100 lx of white light. During this time, the ambulation of the mice was monitored using the Panlab Infrared (IR) Actimeter System (LE8815) with acquisition software Acti-Track® 2.65 for Windows (Panlab, Barcelona, Spain). Paths taken by each mouse were stored permanently as x–y coordinate sequences, and the total travelling distance was assessed as locomotor activity. Vertical activity over 16 cm was scored as jumping, and the number of times that the animals entered into inner area (25 cm × 25 cm) from outer area was also recorded. The open-field was wiped clean with 70% ethanol before each mouse was placed in it.

2.4. Forced swim test

Forced swim test was conducted by placing the mice into an individual acrylic cylinder (25 cm height × 19 cm diameter) containing 25 ± 1 °C water at a depth of 13 cm, so that the mice could not support themselves by touching the bottom with their paws. The performance of the mice for 6 min in the swimming session was videotaped using a digital camera for subsequent analysis. After the session, the mice were removed from the cylinders, dried with paper towels and placed under a warming lamp until completely dry, and then returned to their home cages. The total time of immobility was measured during 4 min (range of 2–6 min) in the swimming session by an observer blind to the treatment conditions [1].

2.5. Aggressive behaviors

Aggressive behaviors were assessed according to the method previously reported [24] with minor modification. 4-weeks old male mice were housed in isolation for 2-weeks and used for an aggressive behavior experiment. A 5-weeks old male ddY mouse, a different strain *albino* mouse, was used as an intruder. One isolated 6-weeks old mouse and one intruder were simultaneously introduced into a novel clear polycarbonate cage (28 cm × 17 cm × 12 cm). The aggressive behaviors of the isolated mouse were recorded for 20 min using a digital camera. The aggressive behaviors were defined by four agonistic behaviors: biting, pushing under, sideways posturing, and aggressive grooming. Total time of aggressive behaviors during the session and latency to first attack were measured by an experienced observer blind to the treatment conditions. Latency to the first attack was recorded as 20 min if no attack against the intruder occurred during the session.

2.6. PPI analysis

Acoustic startle responses were measured in a startle chamber (SR-LAB®; San Diego Instruments, San Diego, CA) as described previously [20,45]. Animals were placed in the chamber under moderately bright light conditions (240 lx), and then allowed to acclimate for 10 min in the presence of 65 dB background white noise. The test session consisted of startle trials (40 ms burst of 120 dB white noise), no-stimulus trials (only the background noise) and PPI trials [a prepulse (20 ms burst of white noise at 68, 71 or 74 dB intensity) preceded the 120 dB startle pulse (40 ms) by 100 ms]. Trials were pseudo-randomly presented with an inter-trial interval between 7 and 23 s (average: 15 s), ensuring that each trial was presented 20 times. Each animal received total 100 trials and the session lasted approximately 30 min. Spontaneous activity during the no-stimulus trials was 10–20 (arbitrary unit). The startle response was recorded for 100 ms (measuring the response every 1 ms) after onset of a startle stimulus. The maximum startle amplitude recorded during the 100 ms sampling window was used as the dependent variable. PPI was calculated as a percentage score for each prepulse trial type: PPI (%) = (1 − [(startle response for pulse with prepulse)/(startle response for pulse alone)]) × 100.

2.7. Social interaction test

A social interaction test was carried out as described previously [39] with minor modifications. Mice were placed individually as a resident in an observation cage (38 cm × 22 cm × 20 cm) under bright light conditions (350 lx) for 60 min. Thereafter, a male ICR mouse of the same age that had been housed in an independent cage, was introduced into the same cage. The interaction between the two mice was recorded for 20 min using a digital camera. An interaction was defined by body contact including inspection and anogenital sniffing. The duration and total number of body contacts were scored by an experienced observer blind to the treatment conditions.

2.8. Statistics

The experimental data were statistically analyzed using StatView® 5.0 for Windows (SAS Institute, Cary, NC). The significance of differences was determined by ANOVA, followed by the Tukey-Kramer *post hoc* test. The criterion for statistical significance was $P < 0.05$.

3. Results

3.1. Age-dependent expression of abnormal behaviors in PACAP$^{-/-}$ mice

Previous studies show that the expression of PPI deficits depends on age in PACAP$^{-/-}$ mice [37]. But, it is not known whether other phenotypes of the mice also depend on age. In this study, we examined age-dependency of jumping behavior, hyperactivity and depression-like behavior of PACAP$^{-/-}$ mice. PACAP$^{-/-}$ mice at 4-weeks old had already displayed a significant increase in jumping, compared with age-matched wild-type controls (Fig. 1A). This