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Behavioural phenotypic characterization of CD-1 mice lacking the neuropeptide S receptor

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ABSTRACT

Neuropeptide S (NPS) is the endogenous ligand of a previously orphan receptor now named NPSR. In the brain NPS regulates several biological functions including anxiety, arousal, locomotion, food intake, learning and memory, pain and drug abuse. Mice lacking the NPSR gene (NPSR(−/−)) represent an useful tool to investigate the neurobiology of the NPS/NPSR system. NPSR(−/−) mice have been generated in a 129S6/SvEv genetic background. In the present study we generated CD-1 congenic NPSR(+/+) and NPSR(−/−) mice and investigated their phenotype and sensitivity to NPS in various behavioural assays. The phenotype analysis revealed no locomotor differences between NPSR(+/+) and NPSR(−/−) mice. The behaviour of NPSR(+/+) and NPSR(−/−) mice in the righting reflex test was superimposable. No differences were recorded between the two genotypes in the elevated plus maze, open field and stress-induced hyperthermia tests, with the exception of rearing behaviour that was reduced in knockout animals. Moreover the behaviour of NPSR(+/+) and NPSR(−/−) mice in the forced swimming, novel object recognition and formalin assays was similar. The stimulatory effects of NPS in the locomotor activity test and its anxiolytic-like actions in the elevated plus maze and open field assays were evident in NPSR(+/+) but not NPSR(−/−) animals. In conclusion, the present study indicates that the NPS/NPSR system does not tonically control locomotion, sensitivity to diazepam, anxiety, depressive-like behaviours, memory and pain transmission in mice. Furthermore our results clearly show that the product of the NPSR gene represents the mandatory protein for all the NPS biological effects so far described.

1. Introduction

Neuropeptide S (NPS, human sequence SFRNGVGTGMKK TSFQRAKS) selectively binds and activates a previously orphan G protein coupled receptor now named NPSR (Xu et al., 2004). At cellular level NPS produces excitatory effects promoting calcium mobilization and increasing cAMP levels (Reinscheid et al., 2005). In the brain the ppNPS mRNA displays a very limited distribution: in fact, most of the NPS expressing neurons were located exclusively in the principle sensory trigeminal nucleus, the lateral parabrachial nucleus, and the peri locus coeruleus area (Xu et al., 2007, 2004). On the contrary the NPSR is widely distributed in the brain with high levels of mRNA expression found in the cortex, olfactory nuclei, thalamus, hypothalamus, amygdala, and subiculum (Xu et al., 2007, 2004). This profile of receptor expression suggests the involvement of the NPS/NPSR system in the regulation of multiple central functions. This has been confirmed by a series of behavioural studies demonstrating that the supraspinal injection of NPS in rodents modulates wakefulness, locomotor activity, stress and anxiety, food intake and gastrointestinal functions, memory processes, and drug abuse (for a recent review see (Guerrini et al., 2010)). However, little information is so far available about the physiological functions modulated by the endogenous NPSergic pathways. NPSR knockout (NPSR(−/−)) mice represent an useful and powerful tool to shed light on the biological roles played by this new peptidergic system. In addition NPSR(−/−) mice are instrumental to investigate the involvement of the NPSR receptor in the effects elicited by NPS. NPSR(−/−) mice were generated on a 129S6/SvEv genetic background as described in Allen et al. (2006). However the 129S6/SvEv strain provides an unfavourable genetic background, due to the low locomotor activity, high anxiety levels,

Abbreviation: EPM, elevated plus maze; FS, forced swimming; LA, locomotor activity; NOR, novel object recognition; NPS, neuropeptide s; NPSR, NPS receptor; NPSR(+/+), NPSR wild type; NPSR(−/−), NPSR knock out; OF, open field; RR, righting reflex; SIH, stress-induced hyperthermia.

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depressive-like behaviours and poor reproductive performance displayed by these mice (Bolivar et al., 2000; Bouwknecht and Paylor, 2002; Crawley et al., 1997; Ducottet and Belzung, 2005). These extreme traits could produce ceiling or floor effects that mask the outcome of the mutation. This consideration prompted us to change the genetic background of NPSR(−/−) mice from 129S6/SvEv to CD-1. This has been achieved by performing 8 cycles of backcross to the CD-1 strain.

Thus the present study investigated the behaviour of CD-1 congenic NPSR(+/+) and NPSR(−/−) mice in assays related to the NPS stimulant and arousal promoting effects (locomotor activity (LA) and recovery of righting reflex (RR)), and anxiolytic-like action (elevated plus maze (EPM), open field (OF), and stress-induced hyperthermia (SIH)). Furthermore, to understand if the NPS/NPSR system plays a role in controlling depressive-like behaviours the mice were subjected to the forced swimming (FS) test. Finally NPS has been reported to facilitate learning and memory (Han et al., 2009; Lukas and Neumann, 2012; Okamura et al., 2011) and to elicit antinociceptive effects (Li et al., 2009; Peng et al., 2010); therefore, the phenotype of NPSR(−/−) mice in the novel object recognition (NOR) task and in the formalin test was also investigated.

2. Materials and methods

2.1. Animals

All experimental procedures for in vivo studies complied with the standards of the European Communities Council directives (86/609/EEC), U.K. Animals (Scientific Procedures) Act, 1986 and associated guidelines, and National regulations (DL 116/92). All efforts were made to minimize animal suffering and to reduce the number of animals used. 129/SvEv NPSR(+/+) and NPSR(−/−) mice, generated as described in Allen et al. (2006), were obtained from Taconic Farms (Germantown, NY, USA). These mice were backcrossed for 8 generations to CD-1 mice (Harlan, Italy), to obtain NPSR(+/+) and NPSR(−/−) CD-1 congenic colonies. All mice were genotyped by polymerase chain reaction, using the protocol reported in Camarda et al. (2009). Experiments were conducted using adult male NPSR(+/+) and NPSR(−/−) mice 8–10 weaks old (weighing 28–35 g). They were housed in group of 4–5 per cage, in 20 × 27 × 14 mm cages (Tecniplast, MN, Italy), under standard conditions (24 °C, 55% humidity, 12-h light-dark cycle, lights on 7:00 a.m.) with food (MIL, standard diet Morini RE, Italy) and water ad libitum. All experiments were performed between 9:00 a.m. and 1:00 p.m. Mice were moved to the test room at least one week before the experiments to acclimate. Little is known about the potential carry over effects of one behavioural test to another (Voikar et al., 2004). In particular, mice repeatedly exposed to the same test display altered responses when reassessed in that given animal model of anxiety (Holmes and Rodgers, 1998). Taking into account these considerations, our experiments were performed by subjecting two different groups of NPSR(+/+) and NPSR(−/−) mice to the procedure described in Table 1. NPS was given to the lateral cerebral ventricle (intracerebroventricularly; i.c.v.). I.c.v. injections were performed using a stainless-steel guide cannula (22 GA) (Plastic One, Roanoke, VA, USA) stereotaxically implanted. The mice implanted with the i.c.v. cannula were different from mice used for the phenotype studies and they were used only once. In all the experiments food was not available during testing.

2.2. Surgery

Mice were anesthetized with isofluorane and placed in a stereotaxic apparatus. A vertical incision was made in the skin to expose the skull. A stainless-steel guide cannula was implanted into the lateral ventricle and was fixed with 3 skull screws and dental cement. Coordinates towards the bregma were $L - 1.1$ mm, $A - 0.5$ mm, $V + 3$ mm. To prevent occlusion, a dummy cannula was inserted into the guide cannula. The dummy cannula did not protrude the guide cannula. After surgery, the animals were allowed to recover for at least 5 days, and during this period, mice were gently handled every day to minimize the stress associated with manipulation of the animals throughout the experiments. For the i.c.v. injection, awake mice were gently restrained by hand and the drug solution (2 μl/mouse) was injected slowly. After completion of testing, mice were i.c.v. injected with trypan blue dye (2 μl) that was allowed to diffuse for 10 min. Then mice were decapitated, and their brains were removed and rapidly frozen. Examination of microtome slices was used to verify the placement of the cannula. Only the data from those animals with dispersion of the dye throughout the ventricles (>95% of the animals) were used.

Table 1
Experimental design adopted in the present study.

Group 1	EPM	OF	LA	NOR	FS	RR
Group 2	OF	EPM	LA	NOR	SIH	FT
Week	I	II	III	IV	V	VI

Animal groups consisted of 5–6 mice per genotype and the whole procedure was repeated twice.

2.3. Locomotor activity

For LA experiments the ANY-maze video tracking system was used (Ugo Basile, application version 4.52c Beta). Mice were positioned in square plastic cages (40 × 40 cm), one mouse per cage. Four mice were monitored in parallel for 60 min. Mouse horizontal activity was monitored by a camera while vertical activity was measured by an infrared beam array. The parameters measured were cumulative distance travelled (total distance in m that the animal travelled during the test), total time immobile (the amount of seconds the animal stays immobile during the test; the animal is considered immobile when 90% of his image remains in the same place for at least 2.5 s), and the number of animal rearings (the number of beam breaks due to vertical movements). Habituated mice were acclimatised to the test cage for 60 min before starting the test. NPS (1 nmol, i.c.v.) or saline were given 15 min before recording LA.

2.4. Righting reflex

The RR assay was performed according to the procedures described by Marley et al. (1986). Mice were given an i.p. injection of diazepam 15 mg/kg .When the animals lost the RR, they were placed in a plastic cage and the time was recorded by an expert observer, who was blind the genotype. Animals were judged to have regained the RR response when they could right themselves three times within 30 s. Sleep time is defined as the amount of time between the loss and regaining of the RR; it was rounded to the nearest minute.

2.5. Elevated plus maze

The EPM assay was carried out essentially as previously described by Pellow et al. (1985). The EPM apparatus (Hamilton–Kinder, Poway, CA, USA) consists of two open arms (30 × 5 × 0.6 cm), which are facing two opposite wall-enclosed arms (30 × 5 × 20 cm) connected by a central platform (5 × 5 cm) elevated 50 cm from the floor. A red light was focused on the central platform (~100 lux). Animals were placed at the centre of the maze, with the head facing an open arm. The number of entries and the time spent in both closed and open arms and some ethological variables (rearing, head-dipping and stretch attend postures) were recorded during a 5 min period by an experienced observer. An entry was scored as such only when the animal placed all four limbs into any given arm. The ratio of 'time spent in the open arms divided by time spent in all (open and closed) arms' and 'number of entries into open arms divided by total entries into all arms' was calculated and multiplied by 100, to yield the percentage of time spent in and the frequency of entries into open arms, respectively. NPS (1 nmol, i.c.v.) or saline were given 15 min before the beginning of the test.

2.6. Open field

The OF test was performed using the ANY-maze video tracking system (Ugo Basile, application version 4.52c Beta). Briefly the mouse was placed in a square plastic cage (40 × 40 cm) and ambulatory behaviour was monitored for 10 min. The central zone of the open field was defined as the central 20 × 20 cm square. Horizontal activity was monitored by a camera. Four mice were monitored in parallel in each experiment. The parameters measured were the same considered in the LA assay. The number of entries in the central zone and the time spent by the animal in the central area of the field were also measured. An entry in the central zone occurred when the entire area of the animal was in the central square and the time in the central zone is defined as the amount of time in seconds that the animal spent in the central square. NPS (1 nmol, i.c.v.) or saline were given 15 min before the beginning of the test.

2.7. Stress-induced hyperthermia

The SIH test was performed according to the method previously reported by Olivier et al. (2003). Rectal temperatures were measured to the nearest 0.1 °C using an ELLAB instruments thermometer (Copenhagen, Denmark) using a lubricated thermistor probe designed for mice (2 mm diameter) inserted 20 mm into the rectum, while the mouse was handheld near the base of the tail. The probe was left in place until steady readings were obtained (approximately 10 s). Rectal temperatures were measured twice in each mouse, at $t = 0$ min (T_1) and $t = 10$ min (T_2). The first rectal body temperature measurement (T_1) induces a mild stress that causes an increase in the second value (T_2). The difference in temperature (T_2-T_1) was considered to reflect SIH.