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Implication of neuropeptide-Y Y2 receptors in the effects of immune stress on emotional, locomotor and social behavior of mice

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ABSTRACT

Neuropeptide Y (NPY) is involved in the regulation of emotional behavior, and there is indirect evidence for a role of NPY in the cerebral responses to peripheral immune challenge. Since the NPY receptors involved in these reactions are not known, we investigated the effect of *Escherichia coli* lipopolysaccharide (LPS) on emotional, locomotor and social behavior, body temperature and circulating corticosterone in female Y2 (Y2−/−) and Y4 (Y4−/−) receptor knockout mice. LPS (0.1 mg/kg injected IP 2.5 h before testing) increased rectal temperature in control and Y4−/− mice to a larger degree than in Y2−/− animals. Both Y2−/− and Y4−/− mice exhibited reduced anxiety-related and depression-like behavior in the open field, elevated plus-maze and tail suspension test, respectively. While depression-like behavior was not changed by LPS, anxiety-related behavior was enhanced by LPS in Y2−/−, but not control and Y4−/− animals. Y2−/− mice were also particularly susceptible to the effect of LPS to attenuate locomotor behavior and social interaction with another mouse. The corticosterone response to LPS was blunted in Y2−/− mice which presented elevated levels of circulating corticosterone following vehicle treatment. These data show that Y2−/− mice are particularly sensitive to the effects of LPS-evoked immune stress to attenuate locomotion and social interaction and to increase anxiety-like behavior, while the LPS-induced rise of temperature and circulating corticosterone is suppressed by Y2 receptor knockout. Our observations attest to an important role of endogenous NPY acting via Y2 receptors in the cerebral response to peripheral immune challenge.

1. Introduction

Neuropeptide Y (NPY) is a messenger widely distributed in the peripheral and central nervous system. Its many functional implications include the control of sympathetic nervous system activity and immune function and the central regulation of energy balance, cognition, mood, anxiety and stress sensitivity (Kask et al., 2002; Heilig, 2004; Lin et al., 2004; Karl and Herzog, 2007; Bedoui et al., 2007). The physiological actions of NPY are mediated by several classes of NPY receptors, five of which (Y1, Y2, Y4, Y5 and Y6) have been elucidated at the gene and protein level (Michel et al., 1998; Redrobe et al., 2004). Coupled to $G_{i/o}$ signaling pathways, these Y receptors mediate the biologic actions of NPY.

Gene knockout studies have revealed that endogenous NPY acting via Y2 and Y4 receptors is involved in the regulation of anxiety, stress coping and energy homeostasis. Thus, anxiety- and depression-like behavior is significantly reduced in Y2 receptor knockout (Y2−/−) mice (Redrobe et al., 2003; Tschenett et al., 2003), and a similar anxiolytic and antidepressant phenotype has been observed in Y4 receptor knockout (Y4−/−) mice (Painsipp et al., in press).

There is indirect evidence that NPY-expressing neurons in the arcuate and paraventricular nuclei of the hypothalamus participate in the behavioral responses to immune stress and infection (McCarthy et al., 1995; Sonti et al., 1996; McMahon et al., 1999; Konsman and Dantzer, 2001; Romanovsky et al., 2005). These reactions are embodied in the term "sickness response" which is mediated by proinflammatory cytokines such as interleukin-1β and tumor necrosis-factor-α, which can excite vagal afferent neurons but also directly gain access to the brain (Goehler et al., 2000; Konsman et al., 2002; Romanovsky et al., 2005). As a result, fever, anorexia, a decrease in locomotor activity and social interaction and other pathophysiological changes (e.g., release of adrenal corticosteroids, altered brain monoamine activity and sleep disturbances) are brought about as typical features of the sickness response. This reaction can be reproduced by intraperitoneal (IP) injection of bacterial lipopolysaccharide (LPS; endotoxin), which causes the generation of proinflammatory cytokines. The behavioral responses

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to peripheral immune challenge involve changes in the central monoamine systems and in the hypothalamic–pituitary–adrenal (HPA) axis (Lacosta et al., 1999; Turnbull and Rivier, 1999; Dunn et al., 2005).

Although NPY has been implicated in the sickness response (Konsman and Dantzer, 2001; Romanovsky et al., 2005), the NPY receptors mediating the link between peripheral immune system and brain have not yet been characterized. Therefore, the overall aim of this study was to investigate whether some behavioral effects of LPS are altered by deletion of the Y2 or Y4 receptor gene. Our study addressed five specific issues in control, Y2−/− and Y4−/− mice: the influence of LPS on (i) fever, (ii) locomotor and anxiety-related behavior, (iii) social interaction, (iv) depression-like behavior, and (v) circulating corticosterone levels post-stress.

2. Methods

2.1. Experimental animals

This study was carried out with age-matched adult female mice, which were housed under controlled temperature (21 °C), relative air humidity (50 ± 15%) and light conditions (lights on at 7:00 h, lights off at 19:00 h, maximal intensity 150 lux). The experimental procedures and number of animals used in this study were approved by an ethical committee at the Federal Ministry of Science and Research of the Republic of Austria and conducted according to the Directive of the European Communities Council of 24 November 1986 (86/609/EEC). The experiments were designed in such a way that the number of animals used and their suffering was minimized. For this reason, only female mice were studied because in a preceding study only female control, Y2−/− and Y4−/−mice had been phenotyped with regard to their emotional–affective behavior (Painsipp et al., in press). The limited number of animals approved for the study made it necessary to inject LPS repeatedly to the animals in order to examine its influence on the behavioral parameters under study.

The germline Y2−/− and Y4−/− mice and non-induced conditional Y2 and Y4 receptor knockout (FY2 and FY4) mice were bred in the Department of Pharmacology of the Medical University of Innsbruck (Innsbruck, Austria), while all experiments were carried out at the Medical University of Graz. The genetic design of these animals has been described previously (Sainsbury et al., 2002a,c). Germline Y2−/− and Y4−/− mice were generated from the same founders on the same mixed C57BL/6:129/SvJ (50%:50%) background as the conditional FY2 and FY4 knockout mice. Germline Y2−/− and Y4−/− mice were obtained by crossing chimeric mice carrying a Y2 floxed gene ($Y2^{lox/lox}$) or a Y4 floxed gene ($Y4^{lox/lox}$), respectively, with oocyte-specific Cre recombinase-expressing C57BL/6 mice (Sainsbury et al., 2002a,c). Non-induced conditional FY2 and FY4 knockout mice were used as controls in all experiments and termed control mice throughout the paper. As demonstrated before, these non-induced conditional $Y2^{lox/lox}$ and $Y4^{lox/lox}$ mice do not differ from wild-type mice, as the level of expression of Y2 and Y4 receptors is not influenced by the introduction of the loxP sites (Sainsbury et al., 2002a,c). The deletion or presence of Y2 and Y4 receptors in the germline and non-induced conditional knockout mice was verified by receptor autoradiography using [^{125}I]PYY_{3-36} and [^{125}I]PP, respectively, in situ hybridization (data not shown) as well as by polymerase chain reaction using oligonucleotide primers recognizing DNA sequences adjacent to the loxP sites flanking the deleted or residing Y2 and Y4 receptor gene (Sainsbury et al., 2002a,c).

For the social interaction test, adult female mice of the outbred strain Him:OF1 (Division of Laboratory Animal Science and Genetics, Department of Biomedical Research, Medical University of Vienna, Himberg, Austria) were used as the partners, which the test mice could interact with.

2.2. Experimental protocols

Three different cohorts of animals of each genotype were used to address the questions under study. The first cohort of animals was used to examine the ability of LPS (0.1 mg/kg administered IP 2.5 h before the behavioral tests), relative to vehicle, to modify (i) locomotor and anxiety-related behavior in the open field (OF) and social interaction with another mouse of the same age and gender but different genotype, elevated plus-maze (EPM) and stress-induced hyperthermia (SIH) tests, (ii) rectal temperature, (iii) depression-like behavior in the tail suspension test (TST), and (iv) circulating corticosterone levels following exposure to the TST. In order to avoid tolerance to endotoxin (Beishuizen and Thijs, 2003), LPS was administered IP to the mice at intervals of at least 2 weeks, each administration being followed by a different test. The series of tests was started with the EPM test, continued with the SIH test, followed by the OF test combined with the social interaction test, and completed with the TST test combined with determination of circulating corticosterone 45 min post-TST.

This series of tests under the influence of LPS was replicated with a second cohort of animals. Since the results of the two test series were very similar, the data were pooled and are presented as one data set. A third cohort of animals was used to examine the effect of a high dose of LPS (0.83 mg/kg administered IP 2.5 h before the test) on OF behavior in naïve control and Y2−/− mice, i.e., in animals that had not been exposed to LPS before.

Throughout the experiments the animals were housed in groups of 3–4 animals per cage. After completion of each test, the animals were immediately returned to their cage mates in the home cage. Care was taken not to change the cage mates during the experiments.

2.3. Administration of lipopolysaccharide

LPS extracted from *E. coli* 0127:B8 (Sigma, Vienna, Austria) was dissolved in pyrogen-free sterile saline (0.9% NaCl) at a concentration of 1 mg/ml. This stock solution was diluted with pyrogen-free sterile saline to yield injection solutions of 0.01 and 0.083 mg/ml LPS, which were injected IP at a volume of 0.01 ml/g, equivalent to doses of 0.1 and 0.83 mg/kg LPS, respectively. Pyrogen-free sterile saline injected at the same volume was used as vehicle control. LPS was administered 2.5 h before the tests in question were carried out. This interval was chosen because the effect of LPS to depress social interaction has previously been found to become maximal 2–3 h post-LPS (Fishkin and Winslow, 1997; Konsman et al., 2000).

2.4. Behavioral tests

2.4.1. General precautions

Prior to all behavioral tests, the mice were allowed to adapt to the test room (22 ± 1 °C, 50 ± 15% relative air humidity, lights on at 7:00 h, lights off at 19:00 h, maximal light intensity 100 lux) for at least 2 days. The EPM, OF and social interaction test and TST were performed 2.5 h after the IP injection of LPS during the period of 10:30–13:30 h. The SIH test was carried out between 13:00 h and 13:30 h.

2.4.2. Elevated plus-maze test

The animals were placed in the center of a maze with four arms arranged in the shape of a plus (Belzung and Griebel, 2002). The maze consisted of a central quadrangle (5 × 5 cm), two opposing open arms (30 cm long, 5 cm wide) and two opposing closed arms of the same size but equipped with 15 cm high walls at their sides and the far end. The device was made of opaque gray plastic and elevated 70 cm above the floor. The light intensity at the central quadrangle was 70 lux, on the open arms 80 lux and in the closed arms 40 lux.

At the beginning of each trial, the animals were placed on the central quadrangle facing an open arm. The movements of the animals during a 5 min test period were tracked by a video camera above the center of the maze and recorded with the software VideoMot2 (TSE Systems, Bad Homburg, Germany). This software was used to evaluate the animal tracks and to determine the number of their entries into the open arms, the time spent on the open arms, the total distance traveled on the EPM and the total number of entries into any arm. Entry into an arm was defined as the instance when the mouse placed its four paws on that arm.

2.4.3. Open field and social interaction test

The OF consisted of a box (50 × 50 × 30 cm) that was made of opaque gray plastic and illuminated by 80 lux at floor level. The ground area of the box was divided into a 36 × 36 cm central area and the surrounding border zone. Mice were individually placed in a corner of the OF, and their behavior during a 5 min test period was tracked by a video camera positioned above the center of the OF and recorded with the software VideoMot2 (TSE Systems, Bad Homburg, Germany). This software was used to evaluate the time spent in the central area, the number of entries into the central area and the total distance traveled in the OF.

In the experiments, the mice were placed in the OF arena for two consecutive 5 min periods. During the first period, the locomotor behavior of the test mice was recorded in the absence of another mouse. During the second period, the behavior of the test mice was evaluated in the presence of a female partner mouse, which the test mice could interact with. The time spent in the central area, the number of entries into the central area, the total distance traveled and the number of social contacts, which the test mouse initiated with the partner mouse, were counted. Social contacts were defined as direct body-to-body contacts.

2.4.4. Stress-induced hyperthermia test

Measurement of the basal temperature in mice with a rectal probe represents a stressor that causes an increase in the temperature by about 1–1.5 °C within 15 min (Olivier et al., 2003). Measurement of the basal temperature (T_1) was followed by a second measurement of the temperature (T_2) 13 min later. This time interval had been found in pilot experiments to best portray the maximal increase in temperature, which returned to baseline levels within the following hour. Rectal temperature was determined with a digital thermometer (BAT-12, Physitemp Instruments, Clifton, New Jersey, USA) equipped with a rectal probe for mice. The stress-induced rise of temperature was expressed as the difference $\Delta T = T_2 - T_1$. Since SIH depends both on the time of the day and the light conditions (Peloso et al., 2002), the SIH test was carried out at 13:00–13:30 h when the stress-induced rise of temperature is maximal.