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Research report

The selective neuropeptide Y Y_5 agonist [cPP$^{1-7}$,NPY$^{19-23}$,Ala^{31},Aib^{32},Gln^{34}]hPP differently modulates emotional processes and body weight in the rat

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ABSTRACT

The neuropeptide Y (NPY) has been suggested to act as a major regulator of emotional processes and body weight. The full spectrum of biological effects of this peptide is mediated by at least four classes of receptors known as the Y_1, Y_2, Y_4, and Y_5 subtypes. However, the respective contribution of each of these receptor subtypes, especially the Y_5 subtype, in emotional processes is still mostly unknown. In the present study, we investigated the effect of long term administration of a selective Y_5 agonist [cPP$^{1-7}$,NPY$^{19-23}$,Ala^{31},Aib^{32},Gln^{34}]hPP on emotional processes and body weight using two rat models of emotional dysfunctions, the corticosterone (CORT)-induced anxiety model as well as the olfactory bulbectomized (OBX) model of depression and anxiety in Wistar and Sprague-Dawley rats, respectively. The sub-chronic administration of the Y_5 agonist reversed the high levels of locomotion, rearing and grooming in the open field test and the impaired social activity induced by OBX, while increased the percentage of entries and time in the open arm of the elevated plus maze in CORT-treated rats. Furthermore, this Y_5 agonist increased body weight in both strains of control rats. These data further demonstrate that Y_5 receptors are not only involved in the control of body weight but also mediate emotional processing under challenged conditions. Thus, the pharmacotherapeutic administration of a Y_5 agonist could be considered as a potentially novel strategy to alleviate some forms of anxiety and depression in humans.

1. Introduction

Anxiety comprises multiple disorders with an excessive social burden and a very high prevalence [1]. These disorders also present high comorbidity with other emotional and physical disabilities including depression [2,3]. Current treatments of choice include various benzodiazepines but their long term administration induces several side effects including cognitive deficits as well as the development of tolerance [4]. Thus, the search for novel approaches for the treatment of anxiety-related disorders is one of the foremost challenges in mental health research.

The neuropeptide Y (NPY), an abundant peptide expressed in various regions of the central nervous system (CNS), is known to play different roles in emotional processing ([5,6]). Cumulative evidence has shown that the NPY levels are decreased in animal models of depression-like behaviors [7–9] and in concordance, the exogenous administration of NPY exerts anxiolytic- and antidepressant-like behaviors in naïve rodents [10–12]. Furthermore, numerous studies have reported the presence of lower levels of NPY in depressive subjects [13–15] and a negative correlation has been shown between NPY gene expression and emotional processing in humans [13,16,17]. These data support the significance of the NPY system in emotionally dysfunctional conditions in humans.

The biological effects of this peptide are mediated by the activation of at least four classes of receptors known as the Y_1, Y_2, Y_4 and Y_5 subtypes [18]. Most of the actions of NPY have been linked to the activation of the largely expressed Y_1 and Y_2 subtypes in the CNS ([19,6]). Little information is rather available on the role of Y_5 receptor subtype in the brain and low levels of this receptor have been reported in discrete brain regions by Dumont et al. [20]. However, recent data suggest a broader distribution in areas such as the olfactory bulbs, hippocampus, amygdala and hypothalamus [21,22].

Early on, Cabrele et al. [23] reported that the Y_5 agonist, [cPP$^{1-7}$,NPY$^{19-23}$,Ala^{31},Aib^{32},Gln^{34}]hPP, had minimum three times higher affinity than the natural ligand (NPY) for the Y_5 receptor subtype and a significant selectivity for this receptor *versus* other NPY receptor subtypes *in vitro*. This compound had an IC50 of 0.24

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for Y_5 receptors compared to >500 for Y_1 and Y_2 receptors *in vitro* [23]. Lecklin et al. [24] also observed that this compound had a K_i value of 1.32 nmol for Y_5 receptors compared to 85 nmol for Y_1 receptors *in vitro* in guinea pigs. Acute ICV administration of this Y_5 agonist increased food intake in control rats. This agonist also showed a strong modulatory role in hippocampal excitatory transmission [25]. Thus, this modified hPP analog clearly represents a suitable tool to further investigate the role of Y_5 receptor *in vivo*.

Previous studies have suggested that the Y_5 receptors are involved in anxiety and sedation [26,27]. However, these earlier studies were performed using only an acute dose of peptides in control naïve animals and the selective agonist mentioned here has never been investigated in animal models of emotionally dysfunctional conditions. Hence, we studied the effects of a long term administration of this Y_5 agonist on anxiety- and depression-like behaviors in two well-studied rat models, namely the corticosterone (CORT)-induced anxiety model [28] and the olfactory bulbectomized (OBX) lesion model [29], since OBX induces a variety of emotional disturbances resembling symptoms of anxiety and depression in rat [30,31]. We also evaluated the effect of this modified peptide on the body weight of these animals.

2. Materials and methods

2.1. Animals

Male Wistar and Sprague Dawley rats weighing 150–170 g (Charles River Canada, Montréal, QC, Canada) at the beginning of the treatment were housed two per cage and maintained on a 12 h light/dark cycle (lights on at 8:00 AM) with *ad libitum* access to food (Purina Lab Chow) and water. Animals were weighed once a week until behavioral tests and constantly monitored to ensure their health.

All procedures were approved by the McGill Animal Care Committee and according to the guidelines of the Canadian Council on Animal Care and the National Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH Publication No. 8023). All efforts were made to minimize animal suffering and to reduce the number of animals used. Fig. 1 shows a flow chart of procedures.

2.2. Corticosterone administration

Wistar rats were administered with a single injection of 10 mg/mL/kg of CORT (subcutaneously, s.c., Sigma–Aldrich, Montréal, Canada) as previously described [28]. CORT was dissolved in peanut oil as vehicle on the day of injection. Control animals were administered with the same volume of vehicle. The dose of CORT injected resembles several hours of physiological stress [32]. The behavioral tests were performed 12 days after the injection of CORT or vehicle as previously reported [28].

2.3. OBX surgery

Bilateral olfactory ablation was performed in Sprague-Dawley rats as described earlier [30,33]. Briefly, 5% isoflurane (Baxter, Mississauga, ON, Canada) was used to induce anesthesia and subsequently maintained at 2.5% during the surgical procedure. A cranial window 5.2 mm anterior to the bregma was created in the frontal bone and the olfactory bulbs were cut and aspirated out. Sham operations were performed in the same manner but the bulbs were left intact. Prevention of blood loss from the cranial window was achieved by filling the open space with a haemostatic sponge (Gelfoam, Pfizer Canada Inc., Montréal, QC). Following surgery, rats were administered with carprofen (0.1 mL/100 g) (Pfizer Animal Health, Montréal, QC) and saline solution (0.9% NaCl) (Hospira Healthcare Corporation, Montréal, QC) and were placed in pairs in their respective cages to recover for two weeks. Only data from animals with complete removal of olfactory bulbs and no damage to the frontal cortex (determined by examination following brain removal) were included in the analysis.

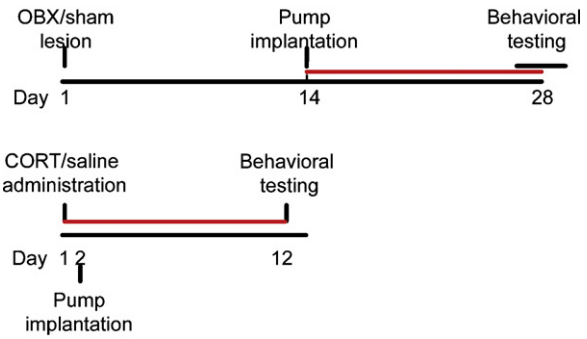

Fig. 1. Flow chart depicting the procedural order used in this study.

2.4. Osmotic minipump implantation

An osmotic minipump (Alzet, model 2002, Durect Cupertino, CA, USA) connected to an indwelling ICV cannula was implanted to deliver the Y_5 agonist, [cPP$^{1-7}$,NPY$^{19-23}$,Ala^{31},Aib^{32},Gln^{34}]hPP or vehicle (0.9% NaCl) at a flow rate of 0.5 μl/h for 12 days in CORT and 14 days in OBX rats [30,34]. The pumps were implanted one day after the administration of CORT or vehicle in the CORT-induced anxiety model and two weeks after the OBX or sham lesion. This treatment delivers 1 nmol/day in CORT-treated and OBX rats. Pumps were filled a day prior to surgery and incubated at 37 °C overnight in a sterile saline solution for priming. The peptide [cPP$^{1-7}$,NPY$^{19-23}$,Ala^{31},Aib^{32}, Gln^{34}]hPP was synthesized as described previously with some modifications. In brief, it was obtained by automated multiple-solid-phase peptide synthesis (SPPS) on a Syro II peptide synthesizer (MultiSynTech, Bochum, Germany) by Fmoc/tBu-strategy using Rink amide resin (resin loading = 0.045 mmol/g) to yield C-terminally amidated peptides [23,35]. Side chain protecting groups included tBu (Ser, Tyr, Asp, Glu), Trt (Asn, Gln, and His), Pbf (Arg) and Boc (Lys). The peptide was purified to homogeneity (>95%) by preparative RP-HPLC (Phenomenex Jupiter Proteo C-18 column, 22 mm × 250 mm, 4 μm/90 Å). The identity of the peptide was verified by MALDI-ToF mass spectrometry (Ultraflex III MALDI-ToF/ToF, Bruker Daltonics).

For osmotic pump implantation, rats were anesthetized with 5% isoflurane to induce anesthesia and subsequently maintained at 2.5% during the extent of the surgical procedure. In brief, animals were placed in a stereotaxic instrument (David Kopf Instruments, Tujunga, CA, USA) and a cannula was implanted into the left lateral ventricle (anterior–posterior: −0.8 mm from bregma, lateral: −1.3 mm from bregma, and vertical: −4.5 mm from dura) as previously reported [30]. The cannula was sealed with dental cement and connected to an osmotic pump by medical grade vinyl tubing. The pump was placed into a subcutaneous pocket in the dorsal region. Animals from the same treatment and condition group were left in pairs until behavioral testing. The lateral ventricles as well as the dentate gyrus were examined for any possible changes in those areas as a result of the long term infusion after behavioral tests were finished. No differences were seen between the ipsilateral and the contralateral ventricles or dentate gyri (data not shown).

2.5. Behavioral tests

Behavioral tests included the elevated plus maze (EPM) for the CORT-treated rats and the open field test (OFT), the forced swim test (FST) and the social interaction test (SIT) for OBX rats. The EPM was conducted on CORT treated rats and its respective controls, 12 days after the treatment as previously described [28]. In addition, a series of three behavioral tests was performed on three consecutive days in the OBX rat as reported earlier [30]. The OFT test was carried out on day 27, the FST was performed on day 28, whereas the SIT was done on day 29 post OBX or sham surgeries. All three behavioral tests were carried out during the light phase (9:00–13:00) of the light–dark cycle. Rats were kept in the same housing conditions throughout all tests. Ten to eighteen animals per group were assessed.

2.5.1. Elevated plus maze

The EPM apparatus consisted of a maze with two open arms (50 cm × 10 cm) opposite each other crossed by two walled (closed) arms (50 cm × 10 cm × 40 cm) raised 80 cm above ground with central area (10 cm × 10 cm) forming the intersection of the four arms. The rats were placed in the central area facing one of the open arms. The number of entries, the time, the percentage of entries and time in each of the arms were analyzed as well as the distance traveled in the closed arm for a 10 min period. All sessions were video recorded and analyzed off-line using the Videotrack system (Viewpoint Life Science, Montréal, Québec, Canada) *via* an automatic differential movement analysis. Behaviors were determined as anxiogenic when the number of entries, the percentage of entries and/or the percentage of time in the open arms is decreased compared to control animals [28,36]. The percentage of entries in open arms was evaluated as [# of entries in open arm/(# of entries in open arm + # of entries in closed arm)]. The percentage of time in open arms was evaluated as [time in open arm/(time in open arm + time in closed arm)]. The locomotion was also evaluated with the number of entries, distance