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Infusions of neuropeptide Y into the lateral septum reduce anxiety-related behaviors in the rat

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ABSTRACT

Neuropeptide Y (NPY) is one of the most abundant peptides in mammalian brain and NPY-like-immunoreactivity is highly expressed in the lateral septum, an area extensively involved in anxiety regulation. NPY counteracts the neurochemical and behavioral responses to acute threat in animal models, and intracerebroventricular (i.c.v.) administration of NPY at low doses is anxiolytic. Less is known about the specific contributions of the lateral septum to NPY-mediated anxiety regulation. In Experiment 1, the effects of infusions of NPY (1.5 μg) into the lateral septum were investigated in three animal models of anxiety: the elevated plus-maze, novelty-induced suppression of feeding, and shock-probe burying tests. Experiment 2 examined the role of the NPY Y1 receptor in these models by co-infusing the Y1 antagonist BIBO 3304 (0.15 μg, 0.30 μg) with NPY into the lateral septum. In the elevated plus-maze, there were no changes in rats' open arm exploration, the index of anxiety reduction in this test. In the novelty-induced suppression of feeding test, rats infused with NPY showed decreases in the latency to consume a palatable snack in a novel (but not familiar) environment, suggesting a reduction in anxiety independent of increases in appetite. This anxiolysis was attenuated by co-infusion with BIBO 3304 (0.30 μg) in Experiment 2. Lastly, rats infused with NPY showed decreases in the duration of burying behavior in the shock-probe burying test, also indicative of anxiety reduction. However, unlike in the feeding test, BIBO 3304 did not attenuate the NPY-induced anxiolysis in the shock-probe test. It is concluded that NPY produces anxiolytic-like actions in the lateral septum in two animal models of anxiety: the novelty-induced suppression of feeding, and shock-probe burying tests, and that this anxiolysis is dependent on Y1 receptor activation in the feeding test.

1. Introduction

Neuropeptide Y (NPY) is a 36-amino acid peptide that is distributed in mammalian brain in higher concentrations than any other peptide studied to date (Gray and Morley, 1986). NPY has many physiological and behavioral functions, including the regulation of stress and anxiety responses and may act endogenously through opposing the behavioral responses of anxiety under acutely stressful conditions (for review: Heilig, 2004). Interestingly, NPY-transgenic rats behave like wild type animals in the elevated plus-maze under normal conditions, but do not display the expected anxiogenic response observed in normal rats following acute restraint stress (Thorsell et al., 2000). On the other hand, intracerebroventricular (i.c.v.) administration of NPY decreases anxiety-like behaviors in many animal models, including the plus-maze, open field, and conflict tests (Britton et al., 2000; Heilig and Murison, 1987; Heilig et al., 1989). Expectedly, NPY-like-immunoreactivity (NPY-IR) is highly expressed in many structures that also regulate these behaviors, including the amygdala, hippocampus, hypothalamus, and lateral septum (Allen et al., 1983; Chronwall et al., 1985; Heilig, 2004; Kask et al., 2002).

The lateral septum regulates anxiety as part of a circuit that includes multiple interconnections with the structures mentioned above (Risold and Swanson, 1997; Sheehan et al., 2004). Both lesions and pharmacological perturbations of the lateral septum reduce anxiety-like behaviors; i.e., by increasing rats' open arm exploration in the elevated plus-maze and reducing defensive burying in the shock-probe burying test (e.g., Bondi et al., 2007; Menard and Treit, 1996; Pesold and Treit, 1992, 1996; Trent and Menard, 2010). Given that the lateral septum has a high density of NPY binding sites (Allen et al., 1983; Larsen et al., 1993; Martel et al., 1986) it seems likely that NPY actions at that site may mediate rats' behavioral responses in anxiety-related paradigms. Thus, infusions of NPY into the lateral septum decreased anxiety in the social interaction and plus-maze tests and antagonized the anxiogenic effects of corticotrophin releasing hormone (Kask et al., 2001; Molina-Hernandez et al., 2010). In the current study, we were interested in

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investigating lateral septal NPY in a wider range of paradigms because not all defensive behaviors share the same neural circuitry (e.g., Pesold and Treit, 1994; Treit and Menard, 1997; Treit et al., 1993). In addition, although the lateral septum has been implicated in both open-arm avoidance and shock-probe burying these responses are nonetheless differentially regulated by distinct receptor types within that structure. For example, lateral septal infusions of either the 5-HT1A receptor agonist, 8-OH-DPAT or the NMDA receptor antagonist, AP-5 suppressed burying while leaving rats' normal levels of open-arm avoidance intact (Menard and Treit, 1998, 2000). By contrast, chronic infusions of the vasopression V1/V2 receptor antagonist, d(CH2)5-D-Tyr(Et)VAMP into the lateral septum increased open-arm avoidance while leaving the burying response intact (Everts and Koolhaas, 1999).

The purpose of the current study was two-fold: to investigate the effects of infusions of NPY into the lateral septum across a range of anxiety-related behaviors (Experiment 1) and to examine the role of the Y1 receptor by pre-treating rats with the Y1 antagonist BIBO 3304 prior to the NPY infusions (Experiment 2). The development of specific NPY receptor antagonists has allowed for a detailed investigation into the role of each receptor subtype. The actions of NPY are mediated through at least four G-protein linked receptors: NPY Y1, Y2, Y4, and Y5 (Dumont et al., 1998; Eaton et al., 2007; Harro, 2006). The Y1 receptor has been the most associated with mediating the anxiolytic actions of NPY (for review: Kask et al., 2002) and is located mostly postsynaptically (King et al., 1999). The lateral septum has a high expression of Y1 receptors (Martel et al., 1986; Dumont et al., 1996, 1998), which may play a putative role in anxiety regulation at this site. In a prior study, co-infusion of the Y1 antagonist BIBO 3304 with NPY in the lateral septum attenuated lateral septal NPY-induced anxiety reduction in the social interaction test (Kask et al., 2001). Infusion of BIBO 3304 into the lateral septum did not alter anxiety on its own, implying that NPY-mediated anxiety regulation may be phasic at this site (Kask et al., 2001). Infusions of Y1 antagonists alone usually have no effect on anxiety, except when infused into the periaqueductal gray or dentate gyrus, where infusions have been found to either increase or decrease anxiety, respectively (Kask et al., 1998a,c; Smialowska et al., 2007).

We tested rats in three paradigms: the elevated plus-maze test, novelty-induced suppression of feeding test, and the shock-probe burying test. In the plus-maze test, a reduction in anxiety suppresses the normal tendency of rodents to avoid open areas, measured as an increase in the proportion of entries or time spent on the open arms of the maze (Carobrez and Bertoglio, 2005; Pellow et al., 1985). In the novelty-induced suppression of feeding test, anxiety reduction is indexed by a decrease in the latency to initiate consumption of a palatable snack in a novel environment without changing latency to snack consumption in the home-cage (Merali et al., 2003). One advantage of this test is that it allows us to examine potential NPY-related changes in appetite. This is important, because NPY is known to increase appetite (e.g., Hanson and Dallman, 1995; Polidori et al., 2000) and exploration-based animal models of anxiety are sensitive to changes in appetitive motivation (Genn et al., 2003a,b; Inoue et al., 2004). For example, chronic food restriction has been shown to selectively increase rats' open-arm exploration in the plus-maze without altering their behavioral responses in the social interaction test (Genn et al., 2003b). Lastly, we also tested rats in the shock-probe burying test, in which anxiety reduction is indexed by a decrease in burying behavior, that is, the natural tendency of rats to bury an electrified probe by using their forepaws to push bedding material towards and over the probe (Treit et al., 1981). Inclusion of this test allowed us to examine the effects of lateral septal NPY on rats' defensive responses to a noxious, localizable threat source. To the best of our knowledge, there have been no prior investigations into the potential anxiolytic effects of lateral septal NPY on either novelty-induced suppression of feeding or shock-probe burying. Given the established role of the lateral septum in anxiety regulation, we hypothesized that infusions of NPY into the lateral septum would reduce rats' anxiety-like behavior in all three animal models: the elevated plus-maze, novelty-induced suppression of feeding and shock-probe burying tests (Experiment 1) and further that this NPY-induced anxiolysis could be attenuated by the Y1 receptor antagonist BIBO 3304 (Experiment 2).

2. Materials and methods

2.1. Subjects

Subjects were naïve, male Long Evans rats from Charles River, Quebec weighing 300–400 g at the time of surgery. Rats were given at least 1 week to acclimatize to the colony conditions before undergoing surgery. Prior to surgery, rats were double housed in polycarbonate cages, given ad libitum food and water, and maintained on a 12:12 light/dark cycle (lights on at 0700 h). The temperature of the colony room was maintained at approximately 21 °C. Following surgery, rats were individually housed under the same conditions as before surgery. The treatment of all animals was in compliance with the guidelines of the Canadian Council on Animal Care, and was approved by Queen's University Animal Care Committee.

2.2. Drugs

Human, rat NPY_{1-36} was obtained from Polypeptide Laboratories in San Diego, CA and BIBO 3304 trifluoroacetate was obtained from Tochris Bioscience in Strasbourg, France. Owing to poor solubility, NPY and BIBO 3304 were dissolved in sterile water rather than physiological saline.

2.3. Surgery

Rats were anesthetized with isoflurane (1.5%–4.5%) in oxygen at a rate of 1.5–2 L/min. Buprenorphine (0.04 mg/kg s.c.) was administered preoperatively to reduce pain. The rats' heads were shaved and they were injected subdermally with the analgesic Marcaine (2 mg/kg). Rats were then placed in a Kopf stereotaxic apparatus. The scalp was thoroughly sterilized and an incision was made to expose the skull. Stereotaxic procedures were used to drill burr holes through the skull, bilaterally, over the right and left lateral septum and two 23-gage stainless-steel guide cannula were implanted, according to flat skull coordinates from Paxinos and Watson (1998) (0.5 mm AP, ±1.2 mm ML, and 3.2 mm DV to bregma at 7° angled medially). Guide cannulae secured by cementing 4 small jeweler's screws to the skull using dental acrylic. At the end of surgery a pin was inserted into each cannula to keep the tract clear of debris. Immediate post-operative care included: analgesic treatment using ketoprofen (5 mg/kg s.c.), rehydration with injection of lactated ringer solution (5 ml s.c.), and maintenance of body temperature by placing the rat under a heat lamp. After animals recovered from anesthesia they were transferred from the surgery room to a recovery room (separate from the home colony) where they remained for a minimum of 3 recovery days. On each recovery day rats were given a morning injection of buprenorphine (0.04 mg/kg s.c.) and afternoon injections of both buprenorphine (0.04 mg/kg s.c.) and ketoprofen (5 mg/kg s.c.). The recovery room temperature was set to approximately 25 °C, which was slightly higher than the regular colony conditions. Once recovery was complete, the animals were returned to the regular colony where they were left undisturbed (except for regular maintenance) for at least 4–6 days prior to behavioral testing.

2.4. Infusions

Following post surgical recovery, rats were randomly assigned to one of the following infusion conditions: Experiment 1: (a) physiological