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Short communication

Amygdalar neuropeptide Y Y_1 receptors mediate the anxiolytic-like actions of neuropeptide Y in the social interaction test

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Abstract

The effects of intra-amygdalar neuropeptide Y infusions were assessed in rats using the social interaction test. Neuropeptide Y administered into the central nucleus of the amygdala did not alter behavior, while injections into the basolateral nucleus of the amygdala produced an increased social interaction time. Furthermore, the anxiolytic-like effect was antagonized by co-administration of the potent neuropeptide Y Y_1 receptor antagonist ((*R*)-*N*-[[4-(aminocarbonylaminomethyl)-phenyl]methyl]-*N*2-(diphenylacetyl)-argininamide trifluoroacetate) 3304, but not with the inactive enantiomer ((*R*)-*N*-[[4-(aminocarbonylaminomethyl)-phenyl]methyl]-*N*2-(diphenylacetyl)-argininamide trifluoroacetate) 3457. Therefore, neuropeptide Y produces an anxiolytic-like effect in the social interaction test through neuropeptide Y Y_1 receptors located in the basolateral amygdala.

Keywords: Amygdala; Anxiety; Basolateral nucleus; Central nucleus; Neuropeptide Y_1 receptor antagonist; Social interaction

1. Introduction

Neuropeptide Y, a 36 amino acid neuropeptide, produces a number of central nervous system mediated effects. The best studied of these is a marked increase in food intake with intracerebroventricularly or intrahypothalamically administered neuropeptide Y (for review, see Gehlert, 1998). In addition, centrally administered neuropeptide Y has been reported to produce an 'anxiolytic' effect in rodent models of anxiety (for review see Heilig and Widerlöv, 1995). These results were observed using several experimental paradigms including the elevated plus maze and the conflict test. Utilizing microinjection techniques, Heilig et al. (1993) localized the actions of neuropeptide Y to the amygdala.

At the cellular level, neuropeptide Y produces its effects through an interaction with a portfolio of G-protein coupled receptors. At the present time, four receptor subtypes have been cloned in the rat. These receptors include the Y_1, Y_2, Y_4, and Y_5 receptors (for review see Gehlert, 1998). The neuropeptide Y Y_1 receptor has been implicated in the anxiolytic responses to neuropeptide Y infused into the amygdala (Heilig and Widerlöv, 1995). In the present study, we assessed the effects of neuropeptide Y infused into the central nucleus and basolateral nuclei of the amygdala using the social interaction test, a validated test for assessing anxiety-like behavior in rodents (File, 1980). In addition, we evaluated the effects of the specific neuropeptide Y Y_1 receptor antagonist, ((*R*)-*N*-[[4-(aminocarbonylaminomethyl)-phenyl]methyl] -*N*2- (diphenylacetyl)-argininamide trifluoroacetate) 3304 (Weiland et al., 1998),on the effects produced by neuropeptide Y.

2. Materials and methods

2.1. Animals

All experiments were conducted using male Wistar rats (300–350 g) from Harlan Laboratories (Indianapolis, IN). Animals were housed individually in a temperature controlled room (72°F) and maintained on a 12-h light–dark cycle. Food and water were given ad libitum.

2.2. Surgical implantation of guide cannulae

Rats were anesthetized using pentobarbital (50 mg/kg i.p.) with atropine (1 mg/kg i.p.). Utilizing a stereotaxic instrument with incisor bar set at −3.3 mm, bilateral guide

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cannulae (26-gauge, 10 mm in length; Plastic Products, Roanoke, VA) were implanted into the central nucleus (A, −2.1; L, 4.0; V, −8.0 relative to bregma) and the basolateral amygdala (A, −2.1; L, 5.0;V, −8.5 relative to bregma). Coordinates for placement of cannulae were determined using the atlas of Paxinos and Watson (1986). The guide cannulae were secured in place using cranioplastic cement and three 2.4-mm stainless steel screws anchored to the skull. Dummy cannulae were then inserted to seal the guide cannulae. Animals were allowed three days to recover before tests were conducted.

2.3. Intracranial (i.c.) injections

Two 33-gauge microinjection cannulae (Plastic Products, Roanoke, VA) which extended 1 mm beyond guide cannulae, were used to bilaterally administer the peptide and the ((*R*)-*N*-[[4-(aminocarbonylaminomethyl)-phenyl] methyl]-*N*2-(diphenylacetyl)-argininamide trifluoroacetate) compounds. Both neuropeptide Y (Peninsula Laboratories, England) and ((*R*)-*N*-[[4-(aminocarbonylaminomethyl)-phenyl]methyl]-*N*2-(diphenylacetyl)-argininamide trifluoroacetate) compounds (generously provided by Drs. W. Engel and Klaus Rudolf, Boehringer Ingelheim Pharma, Germany) were delivered in 100 nl of 1% bovine serum albumen. The dose of neuropeptide Y used for injections into the amygdala was determined from a dose response study. Once the effective dose of NPY was determined, a twenty fold higher dose of the neuropeptide Y Y_1 receptor antagonist ((*R*)-*N*-[[4-(aminocarbonylaminomethyl)-phenyl]methyl]-*N*2-(diphenylacetyl)-argininamide trifluoroacetate) 3304 was used to reverse the effects of NPY. The dose ratio of antagonist to agonist was similar to that utilized by Weiland et al. (1998) for microinjections into the paraventricular nucleus. Polyethylene (PE-50) tubing (Fisher Scientific, Pittsburgh, PA) connected the injection cannulae to a 10 μl Hamilton syringe. The Hamilton syringe was placed on an infusion pump (Harvard Apparatus, Holliston, MA, model PHD 2000) and set to automatically deliver 100 nl over 30 s per side. Following the injection, the cannulae remained in place for an additional minute before being removed. Precise flow of the solutions was verified before and after each injection to ensure compound delivery.

2.4. Behavioral measurement (social interaction)

Experimental anxiety was assessed using the social interaction test (File, 1980). All behavioral testing was conducted under low light familiar conditions and recorded via a video camera situated directly above the social interaction box. The 'experimental' rat and the 'partner' rat were simultaneously placed in an open topped 36 in. L × 36 in. W × 12 in. H box. The 'partner' rat was another rat of similar sex and weight that was housed under identical conditions as the 'experimental' rat, yet had no previous contact with the 'experimental' rat. Social interaction times were determined as previously described (Sajdyk and Shekhar, 1997).

2.5. Experimental protocol

Rats were allowed 72 h to recover from surgery. social interaction testing days were carried out under low light/familiar conditions and separated by 48 h. Two groups of animals were assessed, one with cannulae placement in the central nuclei and one with cannulae in the basolateral nuclei of the amygdala. On experimental day 1, animals in both groups were placed in the social interaction box with a partner rat and assessed for their social interaction time. On experimental day 2, rats were administered neuropeptide Y(10 pmol/side) then evaluated using the social interaction test 30 min after the injection. On experimental day 3, both groups of rats were further divided in half. The first half received the neuropeptide Y Y_1 receptor antagonist ((*R*)-*N*-[[4-(aminocarbonylaminomethyl)-phenyl]methyl]-*N*2-(diphenylacetyl)-argininamide trifluoroacetate) 3304 (100 pmol/side) and the second half received ((*R*)-*N*-[[4-(aminocarbonylaminomethyl)-phenyl] methyl]-*N*2-(diphenylacetyl)-argininamide trifluoroacetate) 3304 (200 pmol/side). On experimental day 4, the same procedure was repeated with the animals receiving the other treatment. On experimental day 5, only rats in the group with cannulae in the basolateral nuclei were injected with the combination of neuropeptide Y (10 pmol/side) and ((*R*)-*N*-[[4-(aminocarbonylaminomethyl)-phenyl] methyl]-*N*2-(diphenylacetyl)-argininamide trifluoroacetate) 3304 (200 pmol/side). Thirty minutes following treatment, animals were behaviorally assessed in the social interaction test. The same experimental procedure was followed on day 7, except the inactive enantiomer ((*R*)-*N*-[[4-(aminocarbonylaminomethyl)-phenyl]methyl]-*N*2-(diphenylacetyl)-argininamide trifluoroacetate) 3457 (200 pmol/side) was administered with neuropeptide Y (10 pmol/side) in place of ((*R*)-*N*-[[4-(aminocarbonylaminomethyl)-phenyl]methyl]-N2-(diphenylacetyl)-argininamide trifluoroacetate) 3304.

2.6. Histology

Upon completion of the study, all animals were killed with carbon dioxide. The brains were rapidly removed and rinsed in 0.9% saline. They were then wrapped in parafilm and foil and placed in a −70° freezer. Within approximately one week, the brains were sliced into (40 μm) sections and mounted onto microscope slides. Subsequently, the sections were stained with Cresyl violet to verify the location of the cannulae. Only data from the animals that had successful bilateral implants into the central nucleus or basolateral nucleus of the amygdala were utilized in the statistical analyses.

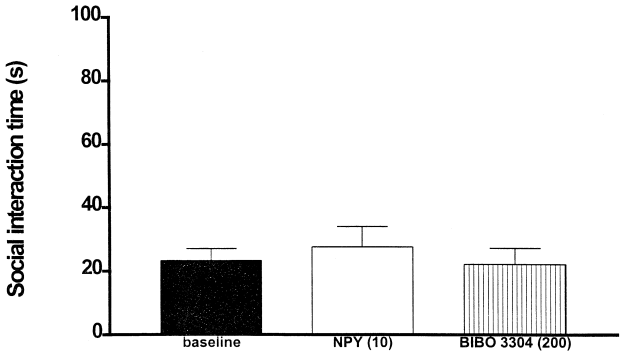

Fig. 1. Social interaction times at baseline and following administration of neuropeptide Y (10 pmol/side), and ((*R*)-*N*-[[4-(aminocarbonylaminomethyl)-phenyl]methyl]-*N*2-(diphenylacetyl)-argininamide trifluoroacetate) BIBO 3304 (200 pmol/side). All injections were bilateral in 100 nl of 1% bovine serum albumin solution delivered into the central nucleus ($n = 8$).

2.7. Statistical analysis

Social interaction times are given as the total time the 'experimental' rat initiated interaction with the 'partner' rat during the 5-min test interval. A repeated measures ANOVA with a Newman–Keuls post-hoc test was utilized for all data analysis. The significance level for all analysis was set at $\alpha = 0.05$.

3. Results

To precisely localize the effects of neuropeptide Y, guide cannulae were placed into the central and basolateral nuclei of the amygdala and small (100 nl) injection volumes were used to reduce diffusion of the administered peptides. The animals behavior was monitored using the social interaction test and the changes in social interaction times resulting from treatment were compared to the animals' baseline behavior, which was determined at the beginning of the study. When neuropeptide Y (10 pmol) was administered into the central nucleus, no changes in social interaction times were noted (Fig. 1). Likewise, there was no effect on social interaction times when the neuropeptide Y Y_1 receptor antagonist ((*R*)-*N*-[[4-(aminocarbonylaminomethyl)-phenyl]methyl]- *N*2- (diphenylacetyl)argininamide trifluoroacetate) 3304 was administered into the central nucleus (200 pmol/side). No statistically significant effect was observed at a lower (100 pmol) dose of ((*R*)-*N*-[[4-(aminocarbonylaminomethyl) -phenyl] methyl] -*N*2-(diphenylacetyl)-argininamide trifluoroacetate) 3304 (data not shown). Contrary to animals with cannulae place ment in the central nucleus, animals which received 10 pmol of neuropeptide Y into the basolateral amygdala showed statistically significant increases in social interaction times (Fig. 2). Subsequent dose–response

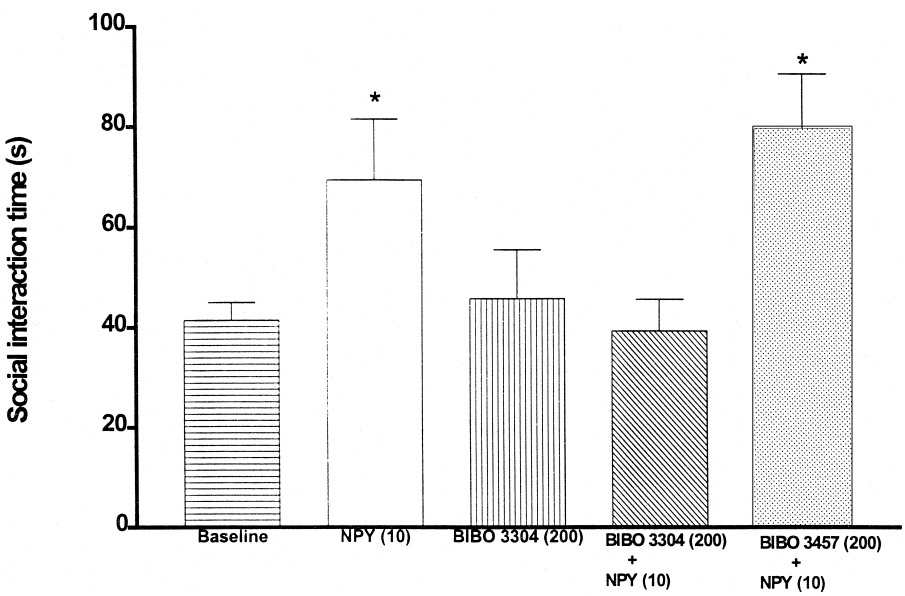

Fig. 2. Social interaction times at baseline and following administration of neuropeptide Y (10 pmol/side), ((*R*)-*N*-[[4-(aminocarbonylaminomethyl)-phenyl]methyl]-*N*2-(diphenylacetyl)-argininamide trifluoroacetate) BIBO 3304 (200 pmol/side), ((*R*)-*N*-[[4-(aminocarbonylaminomethyl)-phenyl]methyl]-*N*2-(diphenylacetyl)-argininamide trifluoroacetate) BIBO 3304 (200 pmol/side) + neuropeptide Y (10 pmol/side), and ((*R*)-*N*-[[4-(aminocarbonylaminomethyl)-phenyl]methyl]-*N*2-(diphenylacetyl)-argininamide trifluoroacetate) BIBO 3457 (200 pmol/side) + neuropeptide Y (10 pmol/side). All injections were bilateral in 100 nl of a 1% BSA solution delivered into the basolateral amygdala. * Significantly different from baseline, ((*R*)-*N*-[[4-(aminocarbonylaminomethyl)-phenyl]methyl]-*N*2-(diphenylacetyl)-argininamide trifluoroacetate) BIBO 3304 (200 pmol/side) and ((*R*)-*N*-[[4-(aminocarbonylaminomethyl)-phenyl]methyl]-*N*2-(diphenylacetyl)-argininamide trifluoroacetate) BIBO 3457 + neuropeptide Y (10 pmol/side), $P < 0.05$ ($n = 6$).

studies showed doses of 10 pmol of neuropeptide Y produced a statistically significant increase in social interaction times [$F(3,18) = 8.724$, $P = 0.0014$], while doses of 1 and 3 pmol did not (data not shown). In subsequent experiments, the specific neuropeptide Y Y_1 receptor antagonist, ((*R*)-*N*-[[4-(aminocarbonylaminomethyl)-phenyl]methyl]-*N*2-(diphenylacetyl)-argininamide trifluoroacetate) 3304,was administered into the basolateral amygdala and animals were assessed in the social interaction test. When administered alone at doses of 100 pmol (data not shown) and 200 pmol, ((*R*)-*N*-[[4-(aminocarbonylaminomethyl)-phenyl]methyl]-*N*2-(diphenylacetyl)argininamide trifluoroacetate) 3304 did not significantly alter behavior from baseline levels. Microinjections of ((*R*)-*N*-[[4(aminocarbonylaminomethyl)-phenyl]methyl]-*N*2-(diphenylacetyl)-argininamide trifluoroacetate) 3304 (200pmol/side) with 10 pmol/side neuropeptide Y produced a complete inhibition of the previously seen increase in social interaction time with neuropeptide Y alone. To confirm the specificity of the response, neuropeptide Y (10 pmol/side) was infused with the inactive enantiomer ((*R*)-*N*-[[4-(aminocarbonylaminomethyl)-phenyl]methyl]-*N*2-(diphenylacetyl)-argininamide trifluoroacetate) 3304 (200 pmol) and the response was similar to neuropeptide Y infusion alone (Fig. 2). An overall comparison of treatments within the basolateral nucleus showed a significant treatment effect [$F(5,30) = 7.412$; $P = 0.0002$] with a post-hoc analysis revealing a significant difference between neuropeptide Y (10 pmol/side) given alone and neuropeptide Y (10 pmol/side) +((*R*)-*N*-[[4-(aminoarbonylaminomethyl)-phenyl]methyl]-*N*2-(diphenylacetyl) rgininamide trifluoroacetate) 3457 (200 pmol/side) given together from all other treatments and baseline.

4. Discussion

In the present study, we examined the effects of neuropeptide Y administered into the rat amygdala on the behavior of the rat in the social interaction test. Infusion of neuropeptide Y into the central nucleus of the amygdala did not produce any alterations in the animals social interaction time. On the other hand, neuropeptide Y produced a significant increase in social interaction time as compared to the animals' baselines when injected into the basolateral nucleus. In order to determine the neuropeptide Y receptor mediating the anxiolytic behavior in the social interaction test, the selective neuropeptide Y Y1 receptor antagonist, ((*R*)-*N*-[[4-(aminocarbonylaminomethyl)phenyl]methyl]-*N*2-(diphenylacetyl)-argininamide trifluoroacetate) 3304 was utilized. ((*R*)-*N*-[[4-(aminocarbonylaminomethyl)-phenyl]methyl]-*N*2-(diphenylacetyl)-argininamide trifluoroacetate) 3304 is a nonpeptide antagonist with subnanomolar affinity for the rat neuropeptide Y Y_1 receptor (IC_{50} values 0.72 ± 0.42 nM), while the inactive enantiomer ((R)*N*-[[4-(aminocarbonylaminomethyl)-phenyl]-methyl]-*N*2(diphenylacetyl)-argininamide trifluoroacetate) 3457 has a very low affinity for the rat neuropeptide Y Y_1 receptor (IC_{50} values > 1000 nM; Weiland et al., 1998). The anxiolytic-like effect was inhibited by the neuropeptide Y Y_1 receptor antagonist ((*R*)-*N*-[[4-(aminocarbonylaminomethyl)-phenyl]methyl]-*N*2-(diphenylacetyl)-argininamide trifluoroacetate) 3304 and not by the inactive enantiomer. Therefore, the anxiolytic effect of neuropeptide Y in the social interaction test appears to be mediated through neuropeptide Y Y_1 receptors in the basolateral amygdala.

Prior studies of the anxiolytic effects of neuropeptide Y utilized either the punished responding test or the elevated plus maze (see Heilig and Widerlöv, 1995). In these studies, the anxiolytic effect was attributed to receptors localized in the amygdala (Heilig et al., 1993). In the present study, we provide evidence that neuropeptide Y administered into the amygdala produces an anxiolytic-like action in the social interaction test. Using small injection volumes we have been able to further localize the effect to the basolateral amygdala. Infusions of neuropeptide Y into the adjacent central nucleus resulted in no significant alterations in social interaction times.

In previous experiments, neuropeptide Y analogs were used to help define the receptor subtypes that mediated the anxiolytic-like behaviors seen in the conflict test (Heilig et al., 1993). In this study, the Y_1,Y_4, Y_5, selective agonist neuropeptide Y-[Leu^{31}-Pro^{34}] produced anxiolysis in the conflict test at lower doses than the neuropeptide Y Y_2 selective peptide, neuropeptide Y-(13-36). Subsequently, antisense oligonucleotides were administered intraventricularly. to reduce the levels of neuropeptide Y Y_1 receptors in the rat brain (Wahlestedt et al., 1983; Heilig and Widerlöv, 1995). These animals exhibited a reduced response to neuropeptide Y in the elevated plus maze. Using the social interaction test, we found the specific neuropeptide Y Y_1 receptor antagonist, ((*R*)-*N*-[[4-(aminocarbonylaminomethyl)-phenyl]methyl]-*N*2-(diphenylacetyl)-argininamide trifluoroacetate) 3304, blocked the anxiolysis produced by neuropeptide Y. Therefore, these results are consistent with previous studies implicating the neuropeptide Y Y_1 receptor. However, administration of the antisense oligonucleotides appeared to produce an anxiogenic-like effect in the elevated plus maze. In the social interaction test, infusions of ((*R*)-*N*-[[4-(aminocarbonylaminomethyl)-phenyl]methyl]-*N*2-(diphenylacetyl)-argininamide trifluoroacetate) 3304 into the basolateral or central nuclei did not significantly affect social interaction times. This would suggest that neuropeptide Y Y_1 receptor activation within the amygdala is not an important component of the social interaction test under these conditions.

In conclusion, we have shown for the first time that neuropeptide Y produces an anxiolytic-like effect in the social interaction test when injected into the basolateral nucleus of the amygdala and have demonstrated the importance of neuropeptide Y Y_1 receptors in mediating this

response. These results add to the growing body of evidence that neuropeptide Y acting through neuropeptide Y Y_1 receptors can be an important component to experimental anxiety.

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