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Research Article

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# Interaction of Cocaine- and Amphetamine-regulated Transcript and Neuropeptide Y on Behavior in the Central Nervous System

Aynur MÜDÜROĞLU KIRMIZIBEKMEZ[1], Murat MENGİ[2], Ertan YURDAKOŞ[2]

[1]Department of Electroneurophysiology, Nişantaşı University, İstanbul, Turkey
[2]Department of Physiology, İstanbul University Cerrahpaşa School of Medicine, İstanbul, Turkey

**ABSTRACT**

**Introduction:** In the central nervous system, cocaine- and amphetamine-regulated transcript (CART) 55–102 peptide is localized in areas, such as the ventral tegmental area, amygdala, hypothalamus, and hippocampus, where emotional activity is regulated. Studies on the effects of the intracerebroventricular (ICV) administration of CART peptide on behavior remain limited. The findings from these studies suggest that this neuropeptide has anxiogenic-like effects. In the central nervous system, neuropeptide Y (NPY) has similar localization as CART. Previous behavioral studies have demonstrated that the ICV administration of NPY has anxiolytic-like effects.

**Methods:** In our study, we established five experimental groups of male Wistar rats to study the competitive effects of NPY and CART peptide. These groups were sham (n=10), CART (n=10), NPY (n=10), CART-NPY (n=10), and NPY-CART (n=10). The open field test, elevated plus maze test, and Porsolt swim test were performed for behavioral analyses. Moreover, the rats were decapitated after the behavioral tests, and the amount of these two peptide in their brains was quantified.

**Results:** Our study revealed that the ICV administration of CART peptide is anxiogenic and inhibits animals undergoing learned helplessness in the Porsolt swim test. When we evaluated the results of our study with respect to NPY, we observed its anxiolytic-like effects; in the Porsolt swim test, although it reduced the duration of immobilization, it did not affect the period of struggle.

**Conclusion:** Our results revealed that during the competitive interaction of these two peptides, anxiogenic CART peptide suppressed the anxiolytic effects of NPY.

**Keywords:** Cocaine- and amphetamine-regulated transcript, neuropeptide Y, anxiety, rat, behavior

## INTRODUCTION

Neuropeptide Y (NPY) is abundant in the mammalian brain, and its effects on food intake and energy expenditure, hormone secretion and reproduction, circadian rhythms, seizures, and ethanol consumption have been studied (1,2), besides studies on behavior such as anxiety and aggression (3,4,5).

In recent years, the relevance of NPY for neuropsychiatric disorders and cognitive functions, such as learning and memory, has been studied (6,7,8). Studies revealed that NPY mainly exerts its antidepressant and anxiolytic-like properties via the Y1 receptor (9,10,11). Cocaine- and amphetamine-regulated transcript (CART) was discovered in 1981 in the hypothalamus of sheep and was first cloned by Douglass et al. in 1995 (12). There have been a wide range of studies regarding the effects of CART peptide on feeding, neuroendocrine response to conditions of stress, drug addiction, general behavior, and neurodegenerative and neuropsychiatric disorders (13,14,15,16,17,18,19).

The intracerebroventricular (ICV) administration of CART peptide in rodents induces anxiety-like behavior in elevated plus maze and social interaction tests (20). The ICV administration of CART peptide causes an increase in the expression of c-fos in the paraventricular nucleus where corticotropin-releasing hormone (CRH) is located; thus, it is involved in the release of CRH (21). Moreover, there are studies that suggest that CART peptide has anxiogenic effects (19,22,23). As drug addiction is associated with anxiety, the relationship between NPY and CART peptide and drug addiction has been investigated. Various studies regarding the relationship between NPY and addictive substances are available. NPY decreased the consumption of addictive substances when intracerebroventricularly administered (24,25). The administration of NPY reduces the effects of acute alcohol use (26,27). Although the impact of CART peptide on addiction is unclear, there are a few studies that demonstrate that CART peptide improves the deterioration in behavior related to addiction (28,29).

**Correspondence Address:** Aynur Müdüroğlu-Kırmızıbekmez, Nişantaşı Üniversitesi, Elektronörofizyoloji Bölümü, İstanbul, Türkiye
E-mail: aynur.muduroglu@gmail.com

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The half-life of NPY is approximately 20 min (30), while the half-life of CART peptide is between 40 and 60 min (31).

Receptors for CART peptide remain to be identified; therefore, it was not possible to set up a study of the interaction of NPY receptors and CART peptide receptor/receptors. In our study, we have consecutively intracerebroventricularly administered CART peptide and NPY and aimed to observe the behavioral consequences of the interaction of these peptide. Furthermore, we measured the amounts of NPY and CART peptide in rat brains.

## METHODS

### Subjects

In our study, we used adult male Wistar albino rats weighing 250–300 g, which were obtained from the University of the Istanbul Institute of Experimental Medical Research. The rats were housed in standard laboratory conditions in a 12-h dark/12-h light schedule, where the room temperature was 20–22°C. Four or five rats were placed in each cage. Tap water and pellet rat food were supplied ad libitum to each cage during the experiments. Before initiating the test procedure, the rats were habituated to bare hand contact by the researcher who implemented the behavior tests. This enabled the prevention of aversion of rats to hand contact during the test processes. All guidelines and requirements were according to the NIH Guide for Care and Use of Animals.

Fifty rats were randomly divided into five groups as follows: sham (n=10), CART (n=10) (0.1 µg/5 µL), NPY (n=10) (8 µg/5 µL), NPY-CART (n=10) (8 µg/5 µL NPY and 10 min later, 0.1 µg/5 µL CART), and CART-NPY (n=10) (0.1 µg/5 µL CART peptide and 10 min later, 8 µg/5 µL NPY). Injections were intracerebroventricularly administered to the groups, and 5 µL saline was administered to the sham group. In the CART-NPY group, first, CART peptide and 10 min later, NPY were injected. Similarly, NPY and CART peptide were administered to the NPY-CART group. Ten minutes after the last administration, behavioral tests were conducted in the groups.

### Anesthesia and Surgery Techniques

#### Anesthesia

Ketamine (50 mg/kg) (ketamine hydrochloride 50 mg/mL: Ketalar, Pfizer, İstanbul, Turkey) was intraperitoneally administered to animals. The depth of anesthesia was checked via the response to a painful stimulus, and extra anesthetic was administered if required.

#### Surgical Implantation of Chronic ICV Cannula

A chronic ICV cannula was implanted using the stereotaxic method. For this procedure, the cannula (Acute Guide Cannula C-313 GA) was mounted into an angled mounting holder stereotaxic apparatus (Stoelting Co. Stellar Cat. No. 51400). The cannula was inserted into the skull and located at a depth of 3.5-mm dorsoventral from the dura inside the left lateral ventricle in compliance with the instructions in the guide (32). To fix the cannula, two screws (Stainless Steel Mounting Screws, 0.80×3/32) were used, and they were molded with acrylic cement (denture material liquid, VERTEX+denture material powder, VERTEX). After the cement had dried, the initial incision in the skull was sutured with purse string (4.0 clear monofilament polyglyconate, MAXON) around the cement, enabling the skin to hug the cap.

Seven days after the completion of the chronic ICV cannula implantation, the rats were taken for experiments. The cannula cap was replaced with a cap without the internal part (Dust cap 303DC). The injections were intracerebroventricularly administered via the internal cannula (Single Internal Cannula C313I/SPC) that was attached in the tip of a Hamilton syringe in a regimen of 1 µL/15 s. After the injection was completed, we waited for 1.5 min to prevent regurgitation, and then, the internal cannula was removed and the cannula was capped.

### Behavior Tests Administered

#### Elevated Plus Maze

The elevated plus maze is a test used to screen the anxiolytic and anxiogenic effects of pharmacological agents. The height of the apparatus is 50 cm. It is composed of two open arms (50×10 cm), two closed arms (50×10×50 cm), and a central platform that connects the two together (10×10 cm) (33). For 5 min the rats were observed for the time spent on and the number of entries they made into the open arms. The time that the test group animals spent on the open arms compared with the sham group, a decline in the number of entries into the open arms, and a decrease in the time spent on the open arms were associated with anxiety (34,35). The maze was cleaned with 70% ethanol between trials.

#### Open Field Test

The open field test apparatus comprises a 90×90 $cm^2$ arena with a 30-cm wall that is marked in 64 equal squares. In this test, the animals were screened for immobilization, ambulation (number of squares crossed), and rearing for 6 min. The squares that were crossed were considered as an indication of explorative behavior. The number of squares that the animals crossed was counted throughout the procedure. A smaller number of squares crossed, longer time of immobilization, and less rearing of the test groups in comparison with the sham group were considered as indicators of anxiety (36). The field was cleaned with 70% ethanol between subjects.

#### Porsolt Swim Test

This test is used to evaluate behavioral despair by means of a learned helplessness paradigm comprising a stressful swim test (37). It is implemented using a cylindrical acrylic glass with a diameter of 30 cm and a height of 50 cm. The glass is filled with tap water at 25°C with a depth of 15 cm. The animals were forced to swim in the cylinder twice for 10 min at an interval of 24 h. The immobility time, which was scored as floating and treading water just enough to keep the nose above water, and struggle time, i.e., escape behaviors, including diving, vigorous paddling with all four legs, circling the tank, and clambering at the walls, were measured for the first 5 min on the second day of the experiment (PST II). All these behavior tests were videotaped and watched using a double-blind technique.

#### Enzyme Immunoassay (EIA)

On the day of the study, brain tissues were removed from -80°C and homogenized in ice. Before homogenization, the tissues were weighed, and a homogenization solution was prepared. The tissues were covered with vortex, and the process was continued to achieve complete homogenization. After the homogenization process, the tissues were centrifuged at 15,000 rpm for 20 min. The upper layer of the fluid was removed because of its cytoplasmic protein content.

When preparing the homogenization solution, 4 µL aprotinin as a protease inhibitor in 1 mL phosphate-buffered saline was used per gram of

tissue. After the homogenization phase, the amounts of CART peptide and NPY in the brain tissue were determined in the samples that were obtained from the supernatants using the RayBio rat CART EIA kit and RayBio rat NPY EIA kit, according to the manufacturer's procedure.

### Statistical Analysis

For intergroup comparisons of the behavior tests and amounts of NPY and CART peptide in the brain tissue, one way ANOVA, followed by Tukey's HSD test as a post hoc test were performed. A p value of <0.05 was considered to be statistically significant.

## RESULTS

### Open Field Test Assessments

The statistical analyses of the differences among the groups in terms of immobilization time and the scores for ambulation and rearing in the open field test were evaluated.

No significant difference was found among the groups in terms of immobilization time (Table 1, Figure 1).

When the differences in ambulation were analyzed, the ambulation scores of the NPY group compared with the NPY-CART ($p<0.05$) and CART-NPY ($p<0.01$) groups were found to be significantly higher (Table 1, Figure 2).

When the differences in the rearing scores were analyzed, the rearing score of the sham group was found to be significantly higher compared with the NPY-CART ($p<0.01$) and CART-NPY ($p<0.05$) groups. The same score displayed a significant increase in the NPY group compared with the CART ($p<0.05$), CART-NPY ($p<0.05$), and NPY-CART ($p<0.01$) groups (Table 1, Figure 3).

### Elevated Plus Maze Assessments

The statistical analyses of the differences among the groups in terms of entry into the open arms and time spent in the open arms in the elevated plus maze test were evaluated.

The analysis of the differences in entry into the open arms revealed that the NPY group had significantly more entries into the open arms when compared with the sham ($p<0.05$), CART ($p<0.01$), NPY-CART ($p<0.001$), and CART-NPY ($p<0.05$) groups (Table 2, Figure 4).

When the times that the groups spent in the open arms were analyzed, it was observed that the NPY group spent significantly more time in the open arms than the sham ($p<0.001$), CART ($p<0.001$), NPY-CART ($p<0.001$), and CART-NPY ($p<0.001$) groups (Table 2, Figure 4).

### Porsolt Swim Test Assessments

The statistical analyses of the differences among the groups in terms of the struggle and immobility times in the first 5 min of PST II were evaluated.

When the differences between the groups in immobility time in the first 5 min of PST II were analyzed, it was found that the sham group ($p<0.01$) had a significantly longer immobility time than the CART ($p<0.001$), NPY ($p<0.01$), NPY-CART ($p<0.01$), and CART-NPY ($p<0.01$) groups (Table 3, Figure 5).

**Table 1.** Immobilization times (s), ambulation scores (n), and rearing scores (n) (mean±standard error of mean (M±SEM)) in the experimental groups in the open field test

| | Immobilization time (s) | Ambulation score (n) | Rearing score (n) |
|---|---|---|---|
| SHAM | 23.00±10.90 | 109.50±25.03 | 11.10±1.10 |
| CART | 74.20±21.08 | 60.20±14.22 | 6.40±0.97 |
| NPY | 19.83±3.62 | 132.42±22.19 | 11.71±1.64 |
| NPY-CART | 63.28±13.04 | 41.25±11.17 | 4.50±0.82 |
| CART-NPY | 57.12±20.09 | 58.14±16.11 | 6.25±0.99 |

SEM: standard error of the mean; M: mean; CART: cocaine- and amphetamine-regulated transcript; NPY: neuropeptide Y

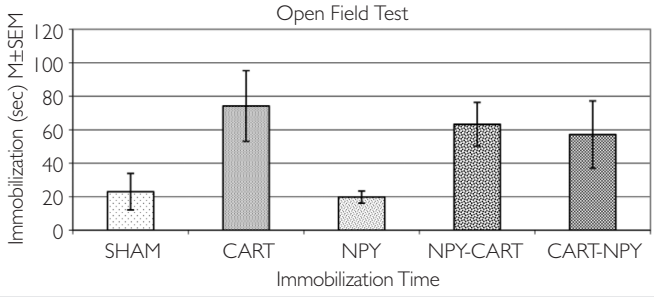


**Figure 1.** Differences in the immobilization time among the experimental groups
CART: cocaine- and amphetamine-regulated transcript; NPY: neuropeptide Y; SEM: standard error of the mean; M: mean; SHAM: cannula insertion with only phosphate buffered saline added

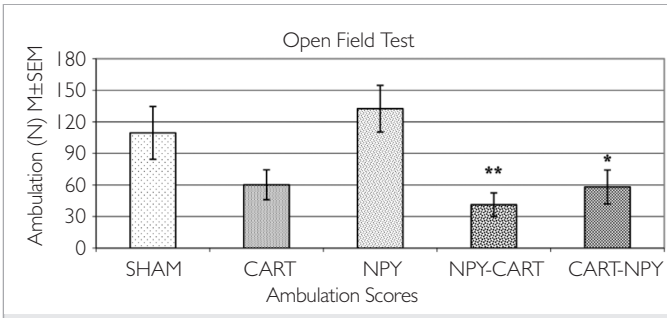


**Figure 2.** Differences in the ambulation scores among the experimental groups. Significance compared with the NPY group*: *p<0.05, **p<0.01.
CART: cocaine- and amphetamine-regulated transcript; NPY: neuropeptide Y; SEM: standard error of the mean; M: mean; SHAM: cannula insertion with only phosphate buffered saline added

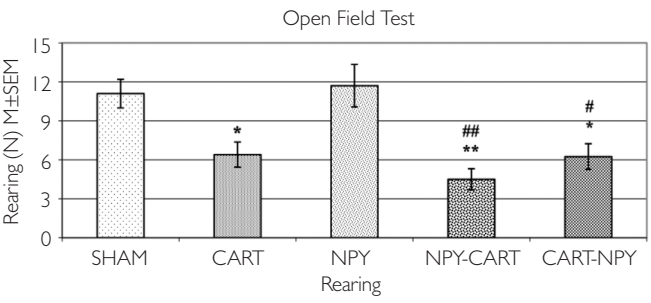


**Figure 3.** Differences in the rearing scores among the experimental groups. Significance compared with the NPY group* and significance compared with the sham group#: *p<0.05, **p<0.01, ##p<0.01, #p<0.05
CART: cocaine- and amphetamine-regulated transcript; NPY: neuropeptide Y; SEM: standard error of the mean; M: mean; SHAM: cannula insertion with only phosphate buffered saline added

**Table 2.** Number of entries into the open arms (n) and time spent in the open arms (s) (M±SEM) of the experimental groups in the elevated plus maze test

| | Entries into open arms (n) | Time spent in open arms (s) |
|---|---|---|
| SHAM | 2.20±0.20 | 23.80±3.90 |
| CART | 1.83±0.30 | 14.00±4.61 |
| NPY | 4.00±0.70 | 61.80±6.35 |
| NPY-CART | 1.25±0.16 | 19.67±1.68 |
| CART-NPY | 2.43±0.29 | 18.00±1.06 |

SEM: standard error of the mean; M: mean; CART: cocaine- and amphetamine-regulated transcript; NPY: neuropeptide Y

**Table 3.** Immobility time (s) and struggle time (s) (M±SEM) of the experimental groups in the first 5 min of the Porsolt swim test

| | First 5-min immobility (s) | First 5-min struggle (s) |
|---|---|---|
| SHAM | 134.42±5.86 | 20.28±3.16 |
| CART | 82.42±10.00 | 42.00±3.42 |
| NPY | 96.33±2.31 | 32.22±2.83 |
| NPY-CART | 95.44±8.04 | 30.88±4.72 |
| CART-NPY | 99.40±6.70 | 25.80±4.29 |

SEM: standard error of the mean; M: mean; CART: cocaine- and amphetamine-regulated transcript; NPY: neuropeptide Y

**Table 4.** Amounts of CART peptide measured in the experimental groups (M±SEM)

| | Amount of CART peptide (ng/mL) |
|---|---|
| SHAM | 6.51±0.05 |
| CART | 6.33±0.25 |
| NPY | 6.26±0.19 |
| NPY-CART | 6.37±0.12 |
| CART-NPY | 6.57±0.14 |

SEM: standard error of the mean; M: mean; CART: cocaine- and amphetamine-regulated transcript; NPY: neuropeptide Y

**Table 5.** Amounts of NPY measured in the experimental groups (M±SEM)

| | Amount of NPY (ng/mL) |
|---|---|
| SHAM | 6.00±0.18 |
| CART | 6.03±0.29 |
| NPY | 6.07±0.30 |
| NPY-CART | 6.33±0.45 |
| CART-NPY | 9.36±0.14 |

SEM: standard error of the mean; M: mean; CART: cocaine- and amphetamine-regulated transcript; NPY: neuropeptide Y

When the differences between the groups with respect to struggle time in the first 5 min of PST II were analyzed, we found that the CART group had a significantly longer struggle time than the sham ($p<0.01$) and CART-NPY ($p<0.05$) groups (Table 3, Figure 6).

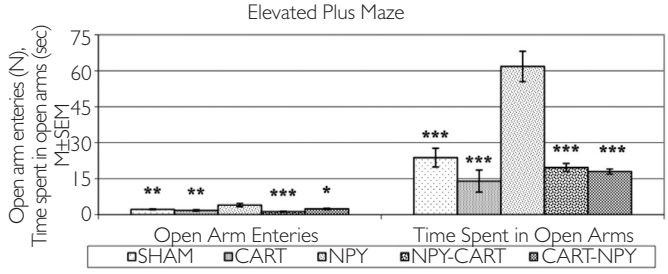


**Figure 4.** Differences in the number of entries into the open arms and time spent in the open arms among the groups. Significance compared with the NPY group*: ***p<0.001, **p<0.01, *p<0.05

CART: cocaine- and amphetamine-regulated transcript; NPY: neuropeptide Y; SEM: standard error of the mean; M: mean; SHAM: cannula insertion with only phosphate buffered saline added

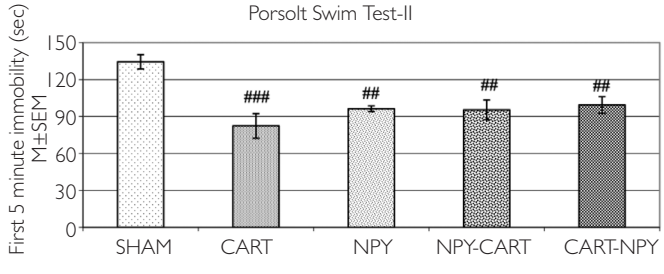


**Figure 5.** Differences among the experimental groups in terms of immobility time in the first 5 min of the PST II. Significance compared with the sham group#: (##p<0.01, ###p<0.001)

CART: cocaine- and amphetamine-regulated transcript; NPY: neuropeptide Y; SEM: standard error of the mean; M: mean; SHAM: cannula insertion with only phosphate buffered saline added

### Determination of Amounts of Peptides in Groups

### Determination of Amount of CART Peptide

No significant difference was observed among the groups in terms of the amounts of CART peptide (Table 4, Figure 7).

### Determination of Amount of NPY

The amount of NPY in the CART-NPY group was significantly higher when compared with the other groups ($p<0.001$) (Table 5, Figure 8).

## DISCUSSION

In our study, we examined the interaction of the ICV administration of NPY and CART peptide on behavior using the open field, elevated plus maze, and Porsolt swim tests. In addition, the amounts of NPY and CART peptide in the brain tissues of rats were measured.

The increases in ambulation and rearing in the open field test in the NPY group and the time spent on and number of entries into the open arms in the elevated plus maze test demonstrated that NPY has anxiolytic properties, which is consistent with previous studies (38,39,40). The results of studies on the paraventricular nucleus, basolateral amygdala (BLA), and central amygdala demonstrated that NPY exerts its anxiolytic effect mostly via the Y1 receptor and also that the ICV administration of NPY or Y1 receptor agonists cause anxiolytic effects in experimental anxiety models (41,42,43). Increased stimulation of NPYergic neurons leads to the activation of presynaptic Y2 receptors and has a reciprocal effect on Y1 receptors, and thus, has overall anxiogenic and depressant effects (44,45).

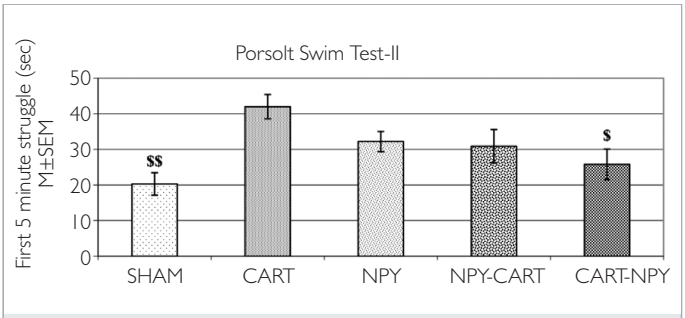


**Figure 6.** Differences among the experimental groups in terms of struggle time in the first 5 min of the PST II. Significance compared with the CART group[$]: ([$$]p<0.01, [$]p<0.05)

CART: cocaine- and amphetamine-regulated transcript; NPY: neuropeptide Y; SEM: standard error of the mean; M: mean; SHAM: cannula insertion with only phosphate buffered saline added

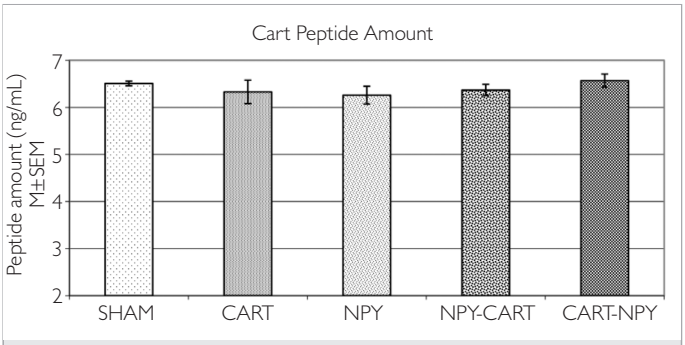


**Figure 7.** Differences in the amount of CART peptide among the experimental groups

CART: cocaine- and amphetamine-regulated transcript; NPY: neuropeptide Y; SEM: standard error of the mean; M: mean; SHAM: cannula insertion with only phosphate buffered saline added

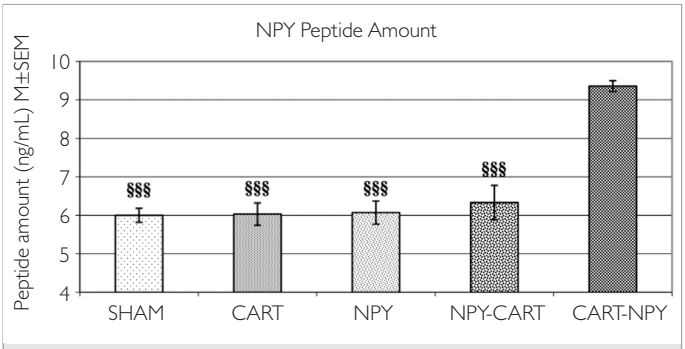


**Figure 8.** Differences in the amount of NPY among the experimental groups. Significance compared with the CART-NPY group[§]: ([§§§]p<0.001)

CART: cocaine- and amphetamine-regulated transcript; NPY: neuropeptide Y; SEM: standard error of the mean; M: mean; SHAM: cannula insertion with only phosphate buffered saline added

The role of Y1 receptors in the anxiolytic effect of NPY has been mostly investigated in the amygdala nuclei (46). NPY inhibits the pyramidal cells in BLA via Y1 receptors (47,48,49).

The excitability of the pyramidal cells of BLA is correlated with anxiety behaviors, where an increase in the excitability is anxiogenic and a decrease is anxiolytic (50,51,52).

There are other studies that revealed that the interaction with GABAergic interneurons has a role in the anxiolytic effect of NPY on BLA. Anxiety-like behavior emerges as a result of GABAergic suppression in BLA (52,53). Studies of neuroanatomy revealed that NPY and GABA coexist in the neurons of the amygdala complex. The presence of NPY receptors in GABAergic interneurons may result in the modulation of these neurons by NPY. Furthermore, the behavioral and neuroendocrine responses induced by NPY via Y1 showed that NPY may directly modulate the activity of GABAergic neurons (53). As a result, NPY may exert its anxiolytic effect by inhibiting pyramidal cells and by stimulating GABAergic transmission via Y1 receptors in BLA.

The increase that we observed in the CART group when compared with the NPY group in terms of time spent on and number of entries into the open arms and the decrease in the extent of rearing and ambulation in the open field test confirm the anxiogenic effects of CART peptide, which is consistent with past studies (19,22,54,55,56).

There are studies that demonstrate that the anxiogenic effect of CART peptide is caused by its interaction with CRH. The anxiogenic effect of CRH is well known and is exerted via CRH-R1 and CRH-R2 receptors. In a recent study in which an elevated plus maze experimental setup was used on CRH-R1 knockout mice, the number of entries into the open arms and the time spent in the open arms were found to be less. These results suggest that CRH mainly exerts its anxiogenic effect via CRH1 receptors (57,58,59).

There is a close relationship between CRH neurons and CART peptide. CART peptide is in a synaptic relationship with CRH neurons and stimulates CRH release (60,61,62). In contrast to NPY, CRH excites the pyramidal cells of BLA (49). Moreover, GABAergic projections and CRH are colocalized in the amygdala, and CRH suppresses the release of GABA (50,51). GABAergic suppression in BLA causes anxiety-like behavior (52).

All these findings suggest that CART peptide may exert its anxiogenic effects by means of CRH.

Studies on the effects of the interaction of CART peptide and NPY on feeding behavior have focused on their amounts in the hypothalamus (63,64). However, the effect of the interaction of these peptide on behavior has not been studied. In our study, the fourth and fifth groups had these two peptides consecutively administered to investigate their effects on behavior. The similar anxiety-like behaviors in the CART-NPY and NPY-CART groups in the open field and elevated plus maze tests make it possible to conclude that the anxiogenic CART peptide suppresses the anxiolytic effects of NPY. These results can be linked to a possible interaction of NPY and CART peptide with CRH and GABA. The increased activation of GABAergic neurons caused by NPY may have been suppressed by the increase in CRH release in response to the anxiogenic CART peptide. In other words, the inhibitory effect of CRH on GABA may have outweighed the stimulatory effect of NPY on the release of GABA via postsynaptic Y1 receptors. Another possibility is that increased activation of NPYergic neurons to compensate for the ICV administration of CART peptide can stimulate anxiogenic presynaptic Y2 receptors. This may explain the significant increase in the amount of NPY in the brains of the CART-NPY group of rats.

As a result, the anxiogenic effect of CART peptide may be suppressing the anxiolytic effect of NPY in their competitive interaction in the central nervous system. Elaborate studies are required to further understand the mechanisms of this suppression.

In our study, as a third tool we used the Porsolt test, which is a forced stressful swim test, to evaluate behavioral despair by means of a learned

helplessness paradigm. Immobility in the Porsolt swim test has often been regarded as an animal model of despair or depression. However, there is a debate that suggests that immobility during the Porsolt swim test is not a failure to cope but instead reflects an emotional learning style, accepting that the current situation is unavoidable and unchangeable under stressful conditions and staying immobile under stress (65).

When we compared the immobilization times of the NPY, CART, NPY-CART, and CART-NPY groups to that of the sham group on the second day of the Porsolt swim test, we observed that the immobilization times of these groups had significantly decreased. When we compared the struggle times on the second day of our study, we observed that the CART group had a significantly longer struggle time compared with the sham group, whereas the other groups did not show a significant difference. Furthermore, the CART-NPY group's struggle time was significantly lower compared with that of the CART group.

When we evaluated the results of the CART group in the Porsolt swim test, the immobilization time of the animals was reduced compared with the sham group, whereas the struggle time was increased. These data suggest that the CART group rats did not show learned helplessness behavior.

Several studies have previously correlated the antidepressant and anxiogenic properties of CART peptide with animals that do not show learned helplessness behavior (15,60). When we analyzed the open field and elevated plus maze tests in our study, CART peptide showed an anxiogenic effect. Therefore, the reason why the CART group of animals did not show learned helplessness behavior may be explained by the anxiogenic effects of CART. As is known, anxiety impairs the learning process regardless of whether this is emotional or declarative (37,66).

In our study, the immobilization time of the NPY group animals was reduced but the struggle time did not change. However, in previous studies, the ICV administration of NPY has reduced the immobilization time and increased the struggle time of rats when the Porsolt swim test was administered (40,67). Our Porsolt swim test results may suggest that the dosage of NPY that we administered did not cause an increase in the struggle time of the NPY group but inhibited the increase in the struggle time that we observed in the CART group.

In our study, another finding is the significant increase in the amount of NPY in the brains of the CART-NPY group when compared with the other groups. This probable compensatory increase in the amount of NPY may have played a role in neutralizing the stimulatory effect of CART peptide on the struggle time in the Porsolt swim test but was not yet enough to inhibit the anxiogenic effects of CART peptide on behavior in the open field and elevated plus maze tests.

In summary, in the open field and elevated plus maze tests, our results suggest that the ICV administration of NPY is anxiolytic whereas that of CART peptide is anxiogenic. Moreover, in behavioral tests anxiogenic CART peptide has a suppressing effect on the anxiolytic NPY.

In the Porsolt swim test, NPY reduced the struggle time that had been increased by the administration of CART.

Further studies are required to reveal the mechanisms of NPY/CART peptide interactions. The main difficulty is that whereas NPY receptors and their functions are well studied, the putative receptor target for CART peptide has not yet been identified; however, some in vitro studies suggest that CART peptide binds to a specific G protein-coupled receptor (24,68). To further understand the effects of these two peptides on physiological mechanisms, firstly CART receptors should be identified and then their interaction with NPY receptors should be studied.

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