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The role of neurotensin receptor 1 on fear memory in mice

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ABSTRACT: Neurotensin (NT) is commonly found in the central nervous system and the gastrointestinal tract and known to play role as a neurotransmitter/neuromodulator/neurohormone. We aimed to investigate the effect of the neurotensin receptor agonist PD149163 and the neurotensin receptor antagonist SR142948 on contextual and cued fear conditioning. In total 174 male Balb/c mice were divided into 29 groups. In the behavioral experiments, the NT receptor agonist PD149163 (0.25, 1, 4 mg/kg) or NT receptor antagonist SR142948 (0.1, 1, 3 mg/kg) were administered to subjects before conditioning on the first day (acquisition phase) or before the test performed 24 h after conditioning (retrieval). Rota-rod test, contextual and cued fear conditioning test were performed in all groups. It was observed that the neurotensin receptor agonist PD149163 had no effect on learning in the cued fear conditioning test, but enhanced retrieval dose-dependently. The neurotensin receptor antagonist SR142948 did not affect the learning and retrieval processes in cued fear conditioning. In the contextual fear conditioning test, neither the neurotensin receptor agonist PD149163 nor the neurotensin receptor antagonist SR142948 had any effect on the learning or retrieval of fear conditioning. Our results show that neurotensin receptor agonist PD149163 increases amygdala dependent cued fear conditioning responses dose-dependently.

KEYWORDS: Neurotensin; hippocampus; amygdala; fear conditioning; fear memory.

1. INTRODUCTION

Neurotensin (NT), a tridecapeptide, was first obtained from bovine hypothalamus by Carraway and Leeman [1] and was thought to act as neurotransmitter / neuromodulator / neurohormone. NT is commonly found in the central nervous system and gastrointestinal tract [2].

The effects of NT are mediated; NT receptor 1 (NTS1), NT receptor 2 (NTS2) and NT receptor 3 (NTS3). NTS1 and NTS2 are seven-transmembrane G-protein coupled receptors and both are widely distributed in the central nervous system. In addition to these two receptors, NTS3 (Sortilin 1) is a single transmembrane domain receptor which is not coupled with G-protein [3-5].

The main neurotransmitters taking part in the regulation of fear memory are glutamate and GABA. In, addition to these neurotransmitters, dopamine, noradrenaline, adrenaline, serotonin and histamine play role in this process [6-9]. Amygdala, hippocampus, stria terminalis, periaquaductal gray area and hypothalamus are involved in the formation of fear memory and physiological responses to fear [10, 11]. Central amygdala and lateral hypothalamus contain neurotensinergic neurons, located together with glutamate, GABA and substance P [12]. Also, it has been shown that dopamine regulates fear-mediated learning and other behavioral responses via dopamine receptor 1 (D1) and dopamine receptor 5 (D5) in the basolateral amygdala and hippocampus [7, 13, 14]. It is known that the NT system has relationship with dopamine receptor 2 (D2) in limbic and striatal areas [4]. The allosteric interaction between NT receptor and D2 is an antagonistic modulation, where the affinity of the agonist for D2 receptor is decreased [15, 16]. Today it is declared that NT may be an endogenous neuroleptic and play roles in diseases such as Huntington, Parkinson's and schizophrenia because of its interaction with dopaminergic system [4, 17, 18]. It is known that endogenous NT, which has an effect on dopamine and glutamate regulation, take a leading part in secretion of anterior pituitary hormones, motility of the gastrointestinal tract, thermoregulation, nociception, muscle relaxation, motor activity, fear, anxiety, memory and learning [4, 12, 15, 16, 19-21]. NT, has an anxiolytic effect through NTS1 in ventral pallidum[22]. Also NTS1 agonist, PD 149163, inhibits fear-potentiated startle in rats [23]. Prus et al [24] showed that acute PD149163 injection reduced conditioned footshock-induced ultrasonic vocalization in rats.

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Based on these data, we aimed to investigate whether NT plays a role in contextual fear conditioning and cued fear conditioning by applying different doses of selective, brain penetrating NTS1 agonist PD149163 and potent neurotensin (NT) receptor antagonist SR142948, which is not selective for subtypes of NT receptors.

2. RESULTS

2.1. Rota rod performance test

There was no significant difference in the time spent on rod between control and drug groups treated with PD149163 on C-FC groups (Figure 1A, p=0,4289 Kruskal-Wallis test, post-hoc Dunn) and Cu-FC groups (Figure 1C, p=0,1503 Kruskal-Wallis test, post-hoc Dunn). SR142948 had no effect on rota rod test in C-FC (Figure 1B, p=0,7469 Kruskal-Wallis test, post-hoc Dunn) and Cu-FC groups (Figure 1D, p=0,1210 Kruskal-Wallis test, post-hoc Dunn).

2.2. Effect of NT receptor agonist PD149163 on C-FC

No significant differences were found in total freezing duration between control and groups treated with PD149163 neither on the first (Figure 2A, p=0,3845 Kruskal-Wallis test, post-hoc Dunn) nor on the second testing day (Figure 2B, p=0,0611 Kruskal-Wallis test, post-hoc Dunn).

2.3. Effect of NT receptor antagonist SR142948 on C-FC

Similar to the NT receptor agonist, the NT receptor antagonist SR142948 did not change the total freezing time on the first and second test days (Figures 2C and D, relatively p=0,0741, p=06977 Kruskal-Wallis test, post-hoc Dunn).

2.4. Effect of NT receptor agonist PD149163 on Cu-FC

When PD149163 was administered before the test on the first day, the drug exerted no effect on the total freezing time (Figure 3A p=0,5465 Kruskal-Wallis test, post-hoc Dunn). On the second testing day, PD149163 increased total freezing duration significantly at the dose of 4mg/kg (Figure 3B, p=0,0078 Kruskal-Wallis test, *post-hoc* Dunn). It may be interpreted as agonist enhances recalling but not acquisition. The NT antagonist SR142948A did not inhibit this enhancement (Figure 4).

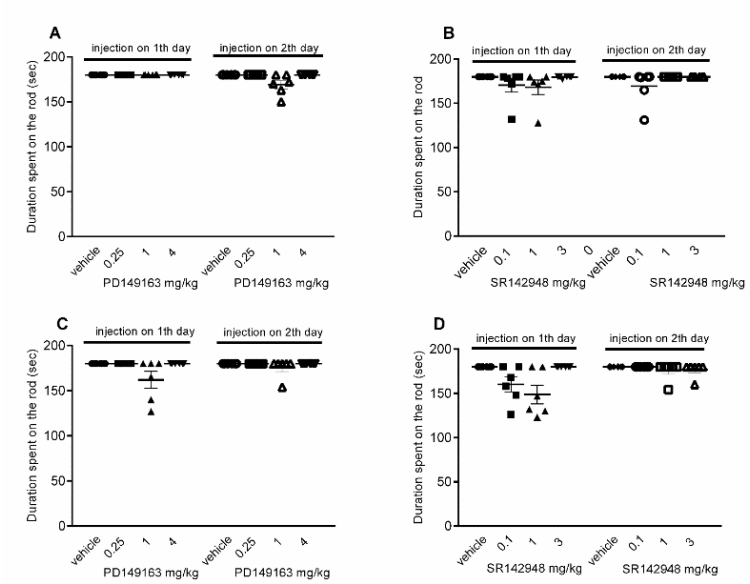

Figure 1. Duration spent on the rod A) Effects of PD149163 in C-FC groups. B) Effects of SR142948 in C-FC groups. C) Effects of PD149163 in Cu-FC groups. D) Effects of SR142948 in Cu-FC groups. (For each group n=6, Kruskal Wallis variance analysis. The vertical lines represent the S.E.M.).

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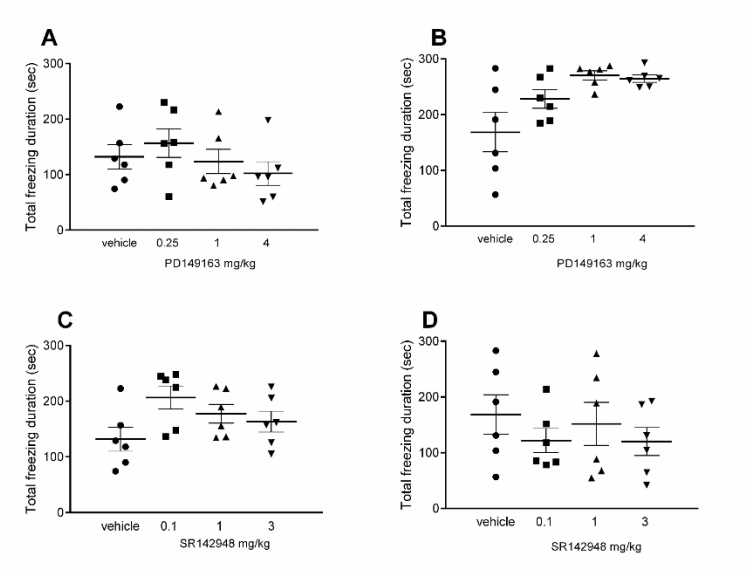

Figure 2. Total freezing duration in contextual fear memory test. A) Effects of PD149163 administered on day 1. B) Effects of PD149163 administered on day 2. C) Effects of SR142948 administered on day 1. D) Effects of SR142948 that administered on day 2. (For each group n=6, Kruskal Wallis variance analysis. The vertical lines represent the S.E.M.).

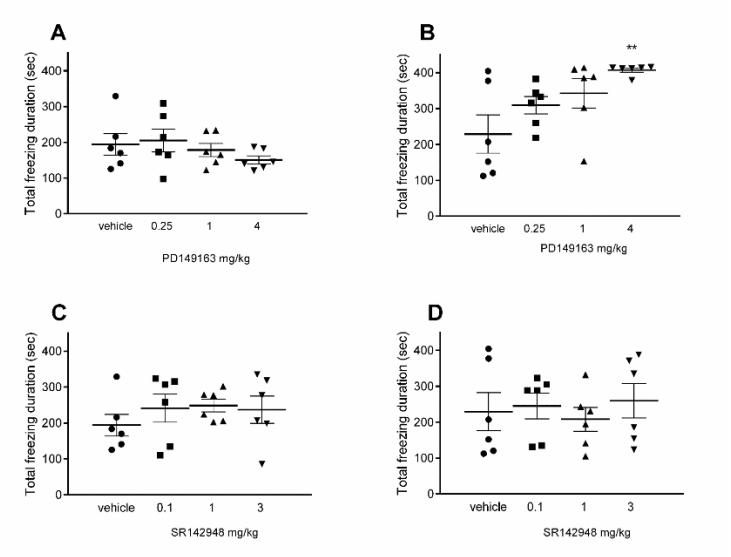

Figure 3. Total freezing duration in cued fear memory test. A) Effects of PD149163 administered on day 1. B) Effects of PD149163 administered on day 2. C) Effects of SR142948 administered on day 1. D) Effects of SR142948 administered on day 2. **:p<0,01, compared to vehicle group. (For each group n=6, Kruskal Wallis variance analysis, post hoc Dunn test. The vertical lines represent the S.E.M.).

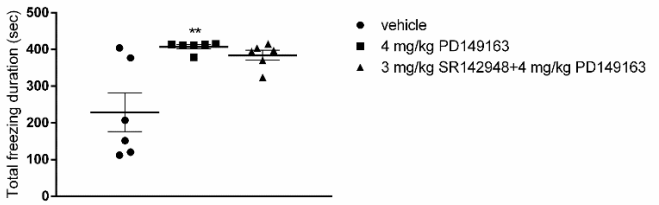

Figure 4. The effect of neurotensin antagonist SR142948 on the enhancement in total freezing duration in cued fear memory test observed after the neurotensin agonist PD149163. **:p<0,01, compared to vehicle group. (For each group n=6, Kruskal Wallis variance analysis. The vertical lines represent the S.E.M.).

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2.5. Effect of NT receptor antagonist SR142948 on Cu-FC

The NT receptor antagonist SR142948 had no effect on freezing duration neither on the first nor the second testing day (Figure 3C, 3D relatively p=0,6549, p=08303 Kruskal-Wallis test, post-hoc Dunn)

3. DISCUSSION

In our study, we aimed to investigate the acute effect of different doses of the NT receptor agonist PD149163 and the NT receptor antagonist SR142948 on contextual and cued fear conditioning. These behavioral experiments consisted of two days, where the first day can be considered as acquisition and the second day as retrieval. We showed that the NT receptor agonist PD149163 was ineffective in acquisition and retrieval processes in contextual fear conditioning, while retrieval was increased by PD149163 in cued fear conditioning at the dose of 4 mg/kg. The NT receptor antagonist SR142948 had no effect on the learning and recalling processes of either contextual or cued fear conditioning. It can be speculated that NT enhances the amygdala-dependent fear memory, it does not have a physiological role in learning and recall processes in both cued and contextual fear conditioning.

It is known that NT plays an important role in many physiological processes such as modulation of food intake, learning behaviors, and antinociception. Previous researches indicate that learning and memory activities are mediated especially by NTS1[25, 26]. Most of the central and peripheral effects of NT are blocked by NT receptor antagonist SR48692 [27, 28]. For example, it was declared that the rats administered SR48692 have more working memory deficits in spatial memory [25, 29]. Laszlo et al. [30] reported that microinjection of NT to central amygdala facilitates spatial learning and this effect is blocked by the NTS1 antagonist SR48692 in Morris water maze in rats. It was also shown that microinjection of the NT receptor agonist PD149163 into the entorhinal cortex facilitate spatial learning in the Barnes maze test in an Alzheimer's disease model of rats [26]. Azmi et al. [19] demonstrated that the NT agonist PD149163 improve scopolamine-induced deficits in a novel object discrimination task and the NT antagonist SR142948A inhibited this improvement dose-dependently.

Although NT and its receptors play critical roles in the regulation of fear memory and various emotional behaviors, the effect of NT on fear memory is not clear yet. Yamada et al. [31] reported that NTS1 *knock-out* mice have higher freezing rate compared to wild mice in C-FC. The result of this study seems to be inconsistent with our results. We could not find any statistically significant difference in SR142948 group compared to control in C-FC; but there is an increasing in freezing time in graphs. Maybe we could find a statistically significant result if we had more animals in groups. In addition, we showed that PD149163 improving retrieval in Cu-FC; it can be related to the difference between the mechanism retrieval and acquisition. Also, the difference between the processes of C-FC and Cu-FC can be reason for these results.

Toda et al. [32] showed that early maternal separation during postnatal period decreases mRNA levels in NTS1 gene in the amygdala but not in hippocampus. These animals also exerted an increased fear response when they reached adulthood. In addition, these researchers reported that microinjection of the NTS1 agonist PD149163 into the amygdala reduced fear responses and microinjection of the NTS1 antagonist SR48692 increased fear responses in normal animals.

Yamauchi et al. [29] demonstrated that Ntsr2-deficient mice have significantly reduced freezing response compared to normal mice in C-FC. In this study freezing response was evaluated at 1, and 24 hrs and 1, 3, and 6 weeks after conditioning. They indicated that the decrease in fear responses at 1 and 24 hours after the fear acquisition suggests that NTS2 had an effect on the consolidation of fear memory, whereas the decrease at 1, 3 and 6 weeks suggests that the deficiency of NTS2 modulated the extinction of fear memory.

4. CONCLUSION

Our results showed that the NT agonist increases recalling at the dose of 4 mg/kg in Cu-FC, but has no effect in C-FC. Animal species, route of administration and duration of administration may be the cause of contradictory findings. In conclusion, roles of NT and its receptor are not clear in fear memory; further studies are needed to elucidate this issue.

5. MATERIALS AND METHODS

5.1. Animals

Total 174 male Balb/c mice (Center of the Laboratory Animals, Trakya University) weighing 20-40 g (n = 6 for each group) were used in this experiment. Mice were maintained under controlled environment with a 12-12 h light/dark cycle at the temperature of 21±2 °C and had free access to food and water *ad libitum*. The experimental protocol of this study was approved by the local ethics committee of the Trakya University, Edirne, TURKEY.

5.2. Groups and drugs

Mice were randomly divided into 29 groups, each including 6 animals. In all groups, rota rod test was used to evaluate motor coordination. To clarify the role of NT in hippocampus contextual fear conditioning test (C-FC) was used in the first fourteen groups (Group 1-14) and cued fear conditioning test (Cu-FC) was performed in the rest (Groups 15-28) to evaluate NT role in amygdala in fear memory. Different doses of the NT receptor agonist PD149163 (0.25, 1, 4 mg / kg) and the NT receptor antagonist SR142948 (0,1, 1, 3 mg / kg) or vehicle were administered 30 min before the conditioning tests at first or second day. The group which SR142948 and PD149163 used together antagonist was injected 15 min before the agonist. In the groups that the acquisition will be tested, the drugs were injected on the 1st day however the injections were made on the 2nd day when the retrieval was tested.

The NT receptor agonist PD149163 and the NT receptor antagonist SR142948 were purchased from Sigma. All drugs were dissolved in distilled water and injected intraperitoneally in a volume of 0.1 ml/10 gr body weight. Drug doses were selected according to previous studies [33].

5.3. Behavioural tests

5.3.1. Rota rod performance test

A rota rod apparatus (Commat, Ankara, Turkey) was used to evaluate motor coordination in mice. The mouse was placed on a rotating bar and the time to fall of the animal from the rod was recorded. 180 sec. was accepted as the cut-off value and animals spending more than 120 seconds on the rota rod were included in the experiment.

5.3.2. Contextual fear conditioning test

The outer part of the fear conditioning apparatus was a matt black box with a lid (FCS 21200, Commat, Ankara, Turkey). There was a transparent box 27 x 27x 34 cm in the device. In the C-FC, the inner transparent box was covered with a patterned paper, and it was illuminated by white light on the ceiling. C-FC was a 2-day behavioral test. On the first day, the animal was taken into the box 30 minutes before the test. Then, mice were exposed to a 7 min session, with a 1 s foot- shock (0.5 mA) presented at 2^{th}, 4^{th} and 6^{th} mins. On the second day of C-FC, animals were re-tested in the same box and monitored for 5 mins without foot-shock. Total freezing times were recorded using the FCS 21200-R system. The apparatus was cleaned with 10% ethanol and dried after every animal.

5.3.3. Cued fear conditioning test

The Cu-FC was made with same apparatus, which was used for C-FC. The transparent box was covered with two different patterned papers on the first and second days. Animals were monitored for 7 min, three foot-shocks (1 s, 0.5 mA) was applied at 2^{th}, 4^{th} and 6^{th} mins and 20 sec. 2000 Hz white noise was used as a cue before the foot shock on the 1^{th} day. On the second day 20 sec. 2000 Hz white noise was applied at 2^{th}, 4^{th} and 6^{th} mins without foot-shocks and the experiments were terminated at 7^{th} min. During all experiments, background noise level was 46 Db. Total freezing times were recorded using the FCS 21200-R system. The apparatus was cleaned with 10% ethanol and dried after every animal.

5.4. Statistical analysis

The time spent on rod and total freezing duration was compared using Kruskal-Wallis test, followed by Dunn test. In all statistical analysis, $P < 0.05$ was considered as significant. Values reported are expressed as mean ± SEM of six mice per group.

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Conflict of interest statement: The authors declared no conflict of interest

Ethics committee approval: All experiments conducted in this study were approved by local ethics committee of Trakya University with the approval protocol number of 2017/20 on 2017 and 2019/18 on 2019.

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